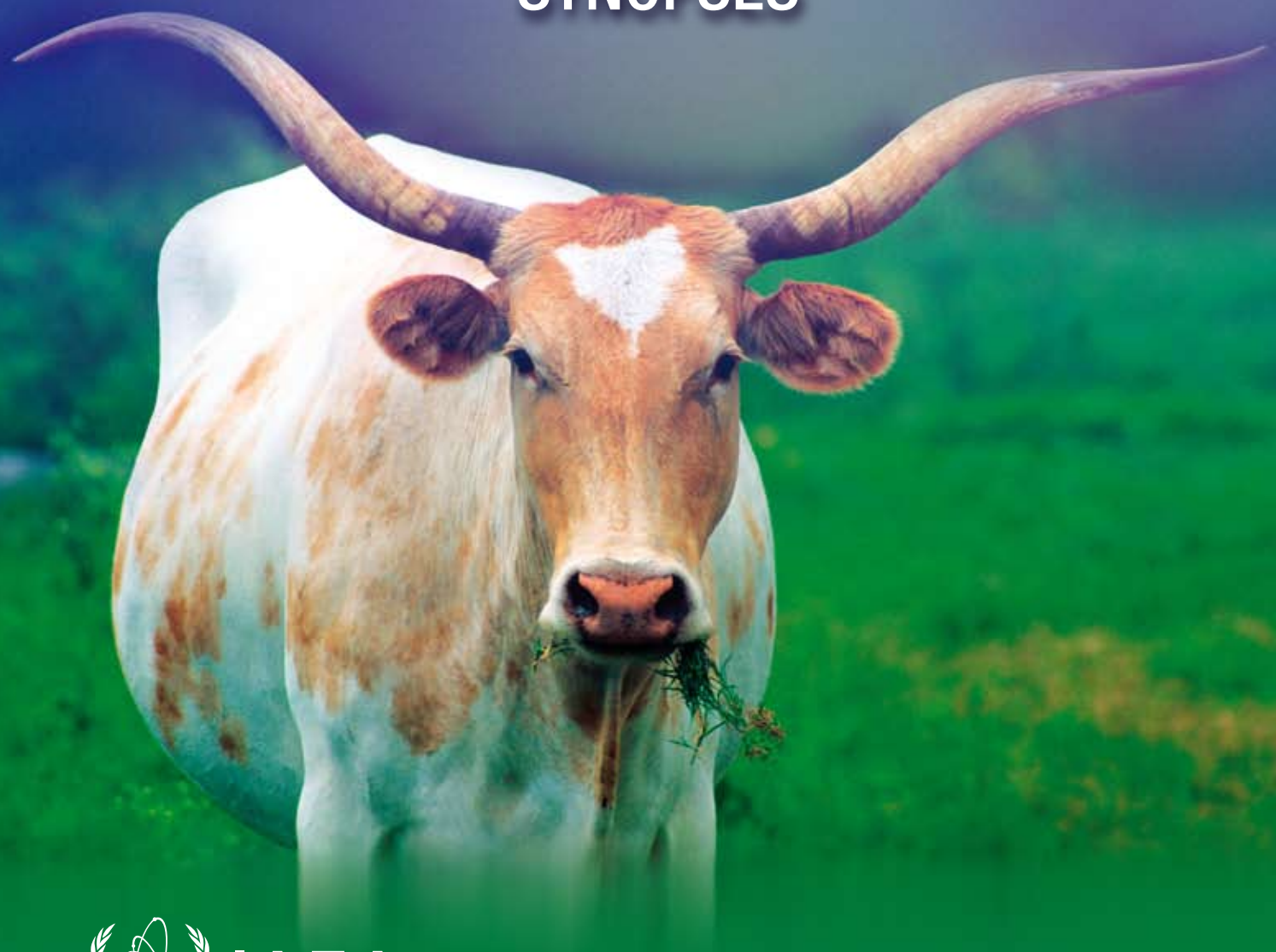
FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health

8–11 June 2009
Vienna, Austria

SYNOPSES



IAEA
International Atomic Energy Agency
Atoms for Peace

Food and Agriculture Organization
of the United Nations

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PLENARY SESSION

HISTORIC ROLE OF NUCLEAR TECHNIQUES IN SOLVING PROBLEMS OF ANIMAL PRODUCTION AND HEALTH

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In reflecting on the role nuclear techniques have played in resolving the needs of developing country livestock keepers I have taken a slightly different approach to previous presentations in this area [1]. I will view developments to date from the perspective of the user and beneficiary of such new knowledge. The paper will also attempt to put the work of the Animal Production and Health Section (APHS) into historical context, thus giving its future strategy a place in the development marathon.

Efforts to assess the value of previous investments in nuclear techniques for the benefit of livestock farmers have been few and far between. Most of the earlier work focused on the contribution to new knowledge - the elaboration of metabolic pathways, unravelling the complexity of disease transmission or understanding the endocrine system feedbacks in relation to reproductive and metabolic hormones. Hence the products originally had more intellectual than economic/practical values. It is only relatively recently (last 20-30 years) that the more applied consequences of such knowledge have been used to help resolve some of the difficult problems facing third-world animal agriculture.

This paper elaborates some of the past and present successes and future challenges in:

Nutrition and feeding – from the use of isotopes (deuterium) in the investigation of metabolism in the rat in the 1930s to the use of stable (N15) and radio-isotopes (P32 and S35) in assessing new diet formulations and feeding standards for livestock in marginal environments. New 'political' targets in Africa to increase livestock productivity by 6% per annum provide new challenges to the livestock feed sector – for which the foregoing technologies are uniquely placed to address.

Reproduction – from the use of competitive protein-binding assays (based on 3H) for various steroid and protein hormones to the use of rapid (I125 based) radioimmunoassay kits for quantifying progesterone levels – and thereby to detect oestrus and diagnose pregnancy in farm animals and improve the efficiency of AI and traditional pregnancy diagnosis and animal husbandry practices.

Animal Health/Disease diagnosis – from the use of (P32-based) DNA probes to the development of ELISA technologies for a range of key livestock diseases – Rinderpest, Foot and Mouth disease, PPR, Newcastle Disease etc. Recent collaborative work with international diagnostic researchers has also produced kits, which detect the difference between vaccinated and naturally infected animals. These kits are being proposed as candidates for commercial application. Also a range of exciting new technologies, including genetic modification (GM) and RNA Interference will need to be assessed for their potential. The control of disease vectors which affect livestock - such as tsetse flies and New-World screwworm- also need a mention since the Sterile Insect Technique is uniquely placed to eradicate both pests.

Based on the outputs of the FAO/IAEA's Coordinated Research Programmes over the last 30 years, the APHS has generated an excellent series of publications. There is also a unique series of laboratory training manuals. The professional quality of these publications is extremely high and their benefit to third world livestock systems 'potentially' enormous.

However, despite the acknowledged benefits derived from the use of the foregoing nuclear techniques in animal agriculture, these publications remain largely unknown outside those directly involved in nuclear agriculture and there is little evidence that in-country livestock keepers have

benefited significantly from the investment. Yet such techniques are sorely required when one considers annual losses of 50 million cattle and water buffalo and 100 million sheep each year from parasitic and infectious diseases let alone the many more subject to morbidity due to inadequacy of feed during the more frequent droughts and floods that increasingly characterise climate change.

Several reasons come to mind for this failure: a natural reserve by researchers to market and promote the results of publicly–funded research findings outside the research community; the jaundiced view of society to things 'nuclear' brought about by nuclear accidents/fictional films etc; the perception that it is 'someone else's' responsibility to inform the world at large about such findings. Unfortunately, there has been only a modicum of proactive marketing of FAO\IAEA results. Contrast this with marketing in the commercial sector. For instance, in the drug industry, the millions of dollars used in developing a new drug is often matched with equally high marketing budgets – and they are at a loss when they see the paltry sums used for marketing the products of public-sector funded research. There is a desperate need therefore to take IAEA research and transform it into discreet and customized products for specific audiences – field practitioners, policy makers, the private sector, the media to name but a few. This requires professional marketing staff with sizeable budgets. Without this investment, these jewels in the IAEA crown will largely remain as dusty 'shelf' based products – and the much-vaunted need by Member States to illustrate value for money will not be realized.

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DECLINE IN AVAILABLE WORLD RESOURCES – IMPLICATIONS FOR LIVESTOCK PRODUCTION SYSTEMS

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The world is faced with three simultaneous and interrelated/interactive crises that if not responded to will create chaos, i.e. climate change, peak oil (the end of inexpensive energy) and global resource depletion. There is an urgent need to respond to these challenges in order to produce and deliver food to maintain the present world population, let alone the increased population predicted by 2020 of 8 to10 billion people.

The primary resource depletion is fossil fuel energy. The world has been using more fossil energy then is being discovered and it appears that the reserves of energy that can be cheaply mined are now at peak production (half these resources have been combusted) [1]. As oil reserves are depleted, prices will rise continuously with the extra demand, now coming through the increased wealth in many emerging economies. Inexpensive oil allowed food to be produced cheaply but this has changed greatly as oil prices rise creating the potential for major disruptions in food availability and even famine.

The dependency of the industrialized countries on oil to drive agricultural production, particularly in countries that cannot meet their domestic requirement, has seen 'panic development' of bio-ethanol and bio-diesel. The production of bio-fuels and its effect on land use and grain availability is blamed for at least 30% of the price hike that has occurred in world food prices. Cereal grain availability for industrial livestock production (pig, poultry and feedlot beef) will be highly restricted and the short fall in meat that will result can only be replaced by expanding the production of the ruminant fed on crop by-products. Ruminants are the logical animals for future meat and milk production but other herbivores are likely to be used more extensively with time, particularly the rabbit. There is a greater need to intensify ruminant production on crop residues applying better methods of treatment to improve digestibility and to prioritize protein supplementation that increase feed conversion efficiency, building on such demonstration trials as those of Dolberg and Finlayson [2] to increase beef production in China.

Ruminants produce 80 million tonnes of methane annually mostly by animals fed low quality feeds. Improving these feeds reduces methane production per unit of product and utilization of nitrate to replace urea in low protein diets can reduce methane production per animal to almost zero [3] removing the down side of increasing enteric methane production, a major contributor to global warming, as production from ruminants increase.

Water the other major resource required for agriculture has also been depleted [4]. Many of the world's large river systems are being drained for urban and industrial water supplies or for irrigated crops before they reach their previous destination. Glacial melt is altering the timing of water availability, particularly where it supplies irrigation water in the dry season**.** The Ghengetic plains are in grave danger of losing their third rice crop in a year putting 200 million people at risk. Ground water has been exploited using cheap fuel, but many of the large aquifers are now too deep to be economically mined for irrigation, reducing some of the major areas of crop production. Bio-fuel production on the Texas high plain is putting enormous strains on water from the aquifer. Yet bio-fuels produce a huge demand in terms of water pollution. The advent of peak oil will clearly return vast areas of irrigated cropland to rain fed cropping, pasture or desert with major loss of food productivity.

Global warming cannot be ignored in any discussion on future agriculture**.** Increasing sea levels will undoubtedly remove considerable areas of fertile delta and weather patterns will certainly change, leading to at times more intense drought and or flooding rains. Warming also carries with it the risk

of decreased crop production as recent research has demonstrated that rice yields decrease by 10% for every degree Celsius increase in nighttime temperatures.

Each of the major global crises has the potential to lower world crop production by direct or various flow on effects. It is suggested that we must now enter a stage in the world where grain based animal production will become increasingly expensive. The animal production industries based on herbivores will need extensive development exploiting a wide range of waste by-products of agriculture or will have to be produced from land not dedicated to food or bio-fuel production.

Oil depletion, pressure to produce bio-fuels, soil fertility decline, the high cost of fertilizers, decreasing water availability and the loss of arable land to erosion and non agricultural purposes, coupled with likely overall decreases in crop production from global warming appear to be interacting such that it will be difficult to produce staples and animal products to meet world demand for an expanding population.

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SESSION 1: INTERACTIONS AMONG NUTRITION, REPRODUCTION AND GENOTYPE

ANIMAL NUTRITION IN A SYSTEMS CONTEXT – THE WAY FORWARD

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Secondary production (i.e. milk, meat, wool and eggs) in animal production systems is a function of complex interactions between animal potential and the environmental conditions (biotic and abiotic). A major factor limiting secondary production is animal nutrition. Obviously, in the absence of food, the animal will stop producing and eventually die; consequently, the investment in it, to that point, is lost. Supplying only enough nutrients to maintain the animal results in no productive output, and thus the marginal cost of production is infinite, i.e. animal input costs are incurred but no return is harvested. Provision of nutrients in excess of maintenance allows the animal to become productive thus generating a return on the investment. Animals differ in their nutrient requirements according to their inherent genetic potential and the desired level of production. There are multiple combinations of dietary ingredients that can meet an animal's nutrient requirements, which create variation in dietary costs when food resources are finite in supply.

Optimization algorithms can be utilized to solve for maximum production or economic return given a set of constraints. For animals, these constraints include nutrient requirements and the availability and accessibility of food supplies. Temporal fluctuations of abiotic environmental conditions may directly impact key components of the primary production systems. For example seasonal drought diminishes and changes the seasonal pattern of herbage growth, altering or limiting the nutrient availability from local sources such as pasture [1]. Thus, it is important that animal performance models are capable of accurately predicting secondary production responses to varying and dynamic feed inputs.

The accuracy and precision of current nutrient requirement models for animals has improved over time [2]. Although static in form, these models can and have been utilized to predict secondary production from a set of inputs. However, prediction errors are relatively large. Additionally, since these models are not dynamic, they are not well suited to simulate changes in body weight over time, which is important if cycles of nutrient restriction and surplus occur within the system.

At least 2 dynamic animal models of lactation have been developed in the last 25 years [3, 4]. These models show promise in terms of providing more accurate predictions of the relationships between dietary nutrients and animal performance. Evaluations of the model of Baldwin [3] after some modification indicate that predictions of milk yield and body weight changes are reasonably well predicted [5]. Additionally, since it is a dynamic model, it has the ability to predict body weight changes over time and consider the impact of those changes on current and future secondary production.

Neil et al., [6] integrated Baldwin's model into a whole farm systems model (WFM). This particular WFM has a grass sub model [7] predicting grass availability based on daily climatic and soil conditions. The cow model consumes growing grass based on nutrient needs and herbage availability and quality, which can be supplemented or complemented with other feeds. Cows within a herd are represented as discrete entities with each initialized to represent the different ages, breeds, and other characteristics that are unique to individual animals in the herd. Historical climate data from different regions and years are used to drive the grass sub model. Management strategies that are simulated include feeding management, stocking rate, and frequency of paddock defoliations. This allows the effects of a wide range of management policies [8] on outputs of primary and secondary production, environmental impacts, and economic performances of the farm system to be evaluated. It also allows optimization of sustainable management decisions. If

model accuracy is adequate, these models can be applied to a wide range of production systems and used at the regional or national level to develop strategic policies for optimizing secondary production while achieving sustainability goals.

Two modelling exercises demonstrate the utility and value of a WFM systems approach. The above WFM was used to evaluate the nitrogen discharges and economic consequences of implementing different nitrogen taxes in heterogeneous farming systems in terms of soil physical variables and production structures [9]. The model is also currently being used to explore the efficacy and profitability of mitigation strategies for greenhouse gas emissions on pastoral dairy farms in New Zealand. New Zealand's commitment to the "Kyoto Protocol" requires agriculture, including dairy farmers, to reduce greenhouse gas emissions by 20% compared to no-mitigation by 2012. In this exercise the cumulative impacts of currently available, practical, on-farm strategies are evaluated in terms of greenhouse gas mitigation and profitability as influenced by climate variability.

These applications demonstrate the utility and value of building and utilizing models of animal performance within a dynamic whole farm system context. However, to be of use, prediction accuracy must be good or inappropriate conclusions may be derived.

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HIGH MILK PRODUCTION IN DAIRY COWS: A DANGER OR A CHALLENGE FOR METABOLISM, FERTILITY AND SUSTAINABILITY

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Considerable improvements in the genetic merit for milk in the dairy herd management have led to a tremendous increase in milk yield. This process of milk yield maximization has been paralleled by a worrisome decrease in reproductive performances and a significantly higher incidence of metabolic and infectious diseases during the early post-partum period. Disappointing fertility results are a worldwide concern in modern dairy industry as it is a major factor contributing to a reduced number of calvings per lifetime, number of days spent producing milk and decreased longevity. In Belgium, the average calving interval increased from 390 to 420 d during the last 10 years. In the USA the number of AI per conception increased from 1.7 to over 3. Pregnancy rates to first service in the UK have reached an absolute nadir of 40% and pregnancy rates are declining at approximately 1% per annum.

After calving, the process of getting pregnant again in dairy cows starts with clearance and involution of the uterus followed by resumption of ovarian activity. This should result in the completion of the growth of a healthy follicle, enclosing a competent oocyte, and ultimately in oestrus, ovulation, fertilization and uterine attachment by a viable embryo. Adequate hormone levels are essential to maintain this early pregnancy. Any disturbances of these balanced and fine tuned biological and mechanical events leads to failing reproduction. This “sub-fertility syndrome” is a complicated and multi-factorial problem. Based on almost unchanged heifer fertility parameters we learned that the reproductive processes of modern dairy cattle are essentially normal when lactation demands are not imposed. Modern dairy cows prioritize milk production at the expense of sustained reproductive efficiency. From a biological point of view, it makes sense for mammals in early lactation to favour milk production over fertility, which is referred to as nutrient prioritization. The constant strive for higher milk yields exploited this fylogenetic prioritization leading to a high metabolic pressure on reproduction and general health.

During the transition period, cows are unable to compensate for the increased energy demands (gravid uterus and increasing milk production) by increasing feed intake, and this result in a status of negative energy balance. The consequential catabolic status is characterised by low insulin, and glucose concentrations and reduced insulin sensitivity, leading to a massive mobilisation of the body reserves. Dairy cows can lose up to 10% of their body weight during the first weeks post partum. It has been shown that the degree and duration of energy deficit during this early post partum period is positively correlated with the number of days to first oestrus. We demonstrated that such a metabolic condition can directly hamper growth and function of the ovarian follicle. After a whole series of in vivo and in vitro experiments, we now have sufficient evidence that also oocyte and embryo quality is jeopardized under such metabolic conditions. Especially the elevated free fatty acid and the low glucose concentrations are toxic for the granulosa cells and the oocyte. These scientific findings are supported by field observations, showing disappointing conception rates and an increasingly high incidence of early embryonic mortality. Fertilisation of oocytes from high genetic merit dairy cows resulted in significantly lower blastocyst yields *in vitro*, irrespective of milk production as such. Furthermore, a higher proportion of non-viable embryos were flushed from lactating cows compared with non-lactating cows. Taken together, the female gamete in high producing dairy cows is in danger due to genetic and direct metabolic but probably also due to epigenetic effects.

In the USA, only 50 to 60% of dairy cows survived beyond their second lactation. The increased incidence of metabolic and infectious diseases is also responsible for the high culling rates. The

high metabolic pressure, changes in diets, growing herd sizes and altered housing conditions are responsible for the higher susceptibility of these animals. In Belgium, cows are on average culled after 3.2 lactations, while one of the best farmers in the country was able to reach 7.2 lactations at the moment of culling combined with an average lifetime production of 80,140 kg of milk. These observations substantiate that only optimal and professional herd management can overcome many of the adverse effects described above. Europe is aware of these problems as demonstrated in their seventh framework programme for food, agriculture, fisheries and biotechnology. Call KBBE-2007-1-3-01 wants to promote scientific work focusing on "Breeding tools for improved livestock products" in order to improve sustainability in European dairy herds.

Yield maximization per animal remains preferable from an economical and environmental point of view. However, only an outstanding herd management can (partially) compensate for the enormous pressure on these animals and should be able to safeguard fertility, animal health, welfare and sustainability of modern dairy herds.

IMPROVING REPRODUCTIVE SUCCESS AND FERTILITY PRESERVATION BY OVARIAN TREATMENTS IN DIFFERENT SPECIES

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Optimization of ovarian treatments in combination with cryopreservation of ovarian tissue, follicles and oocyte are valuable strategies to improve the reproductive process, to create diversity and also to save rare species in extinction. The mouse model has been used by several researchers to unravel basic ovarian physiology by combining molecular genetics (knock-out animals), in vitro culture of gametes and the subsequent IVF, embryo culture and embryo transfer techniques. Until today, it is only in the mouse model that healthy live offspring has been generated from primordial follicles (the Eppig Laboratory, Jackson Laboratory, Bar Harbor in Maine, USA). Especially in the ovine and bovine models, reproductive research in the last decades has been moving forward the field in assisted reproduction.

Today in vitro production in bovine has become a routine technique with widespread applications in countries relying on breeding programmes for an efficient milk and meat production. There is a consensus among reproductive scientists however, to state that our actual techniques for the culture and maturation of oocytes are still inefficient (when compared to using in vivo ovulated oocytes). The hormonal treatments that are actually used to pre-treat animals before oocyte retrieval are suboptimal and do not rescue as many oocytes as one would expect. Although many hundreds of thousands of oocytes are present in juvenile animals, very few are prone to full development for as yet unknown reasons. Interference with the process of follicle selection influenced by several external (seasonality, pasture, environment) and internal factors (disease, stress) can contribute to the variability in oocyte quality. Currently, there are biologically purified and recombinant proteins available for interfering with the animal's reproductive cycle. By using combinations of hormone treatments the synchronisation of follicles can be improved. A better synchrony in the cohort provides oocytes of an equal maturation state and homogenises outcome. A challenging new concept is that it might be possible to optimize oocyte quality by culturing (and "treating") the oocyte-cumulus complex in vitro instead of the ovary in vivo. Several factors produced and secreted by the oocyte itself are essential to condition the cumulus-corona cells in producing essential metabolites that are transferred back into the oocyte by specialised communication (paracrine, gap junctional). It is possible today to produce these factors by recombinant technology and to use them in culture media.

Another technique that could greatly contribute to the genetic diversity is gamete banking. Ovarian tissue from valuable animals can be conserved (i.e. primordial follicles) by programmed freezing. Recent progress has been documented by using vitrification in combination with high concentrations of cryoprotectants. Although first results in the human model show that by vitrification the oocytes survive better the freezing than with slow freezing, it still needs to be proven a safe method a long-term basis. Current molecular techniques permit us to be more proactive in predicting safety. Several small animal models could learn us whether interference with the natural cycle by hormone treatment, collection of immature gametes and long-term culture or in vitro maturation might affect the imprinting of susceptible genes in oocytes. Each species has its particularities in terms of reproductive physiology; hence going back to fundamental research will often be needed instead of simply extrapolating the results from another species published in literature. It is therefore essential that in the different parts of the world research infrastructures are brought closer to the natural habitats of economically and ecologically important species. As an example we take the case of the South American Camelids, for which it

is expected that research efforts in the field of assisted reproduction will lead to the improvement of the economical situation of a significant part of the population in the Peruvian Andes.

Rural alpaca breeders in the Andes have low quality basic education and have a lack of information regarding the economic potential of the Alpaca fibre. Actually there is an inadequate selection and handling of top quality animals. The selection of animals, based on phenotype, leads to a further progressive deterioration of fibre quality (= lower competitiveness and price). A prerequisite for success is to strengthen the links between research and production communities. The scientific approach towards a selection of animals (based on genotype) in combination with the modern techniques for handling of gametes and embryos could ensure the preservation of highly valuable animals for high quality fibre. An additional problem that has been discovered in this species is the very high embryonic mortality rate in the Andes. Research is done to find the possible origins of this peculiar problem.

Making use of the sophisticated knowledge from assisted reproduction techniques (ART) targeted ART of "identified" genetically pure alpacas will allow to obtain a higher quantity of the most valuable embryos, which could be transferred to lower genetic value females. This strategy could lead to a more rapid increase in the percentage of animals with good quality fibre. This is a great challenge because -in general- the knowledge of reproduction physiology of South American Camelids is poor and there are as yet no species-specific diagnostic reagents or pharmacological compounds developed. Dedicated Camelids Research programmes on male and female gametes need to be supported by building appropriate facilities for in vitro cell culture, assisted reproduction techniques, molecular biology, and immunoassay techniques.

The high technicity of the current reproductive techniques and the complexity of the reproductive system in different species require a profound training in reproductive biology. Reproductive biology has become an important part in the curriculum of veterinary sciences. The precise understanding of the metabolic regulation changes of cells and tissues cultured in vitro is essential to know the precise boundaries within which the gametic culturist needs to operate in order to ensure normal healthy offspring.

ORAL PRESENTATIONS

ANIMAL NUTRITION AND OPTIMIZED UTILIZATION OF LOCALLY AVAILABLE RESOURCES

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Rice straw is a major source for ruminant in many tropical countries, especially during dry season. Rice straw is the most abundant among crop residues. Actually, rice straw is the most important roughage in Myanmar for ruminant feeding. Like other fibrous residues, it is a poor quality feed. The major cause of low productivity of livestock in tropical regions is the inadequate and poor quality of feed. The nutritional limitations of rice straw may be overcome by supplementation with concentrates, urea or green forage. Supplementation of rice straw with concentrate would improve the utilization of rice straw. Supplementation of by-product, which may increase intake and/or digestion, and/or utilization of the basal diet are the condition directly related to microbial activity, which is required to optimize rumen digestion. The microbes within the rumen grow efficiently when ammonia nitrogen in the rumen is adequate.

In Myanmar, sesame meal is one of the common feed supplements for the draft cattle and crossbred dairy cows fed rice straw. Sesame meal is highly degradable (88.7%) in the rumen. Therefore, degradation of protein is a considerable factor when the protein sources are supplemented. Several processing treatments (heat, tannin, formaldehyde, etc.) have been used to increase the proportion of dietary protein, which is not degraded in the rumen. Protections of highly degradable feed protein by the heat treatment and formaldehyde have already been reported. However, little information is available about the effect of tannin included in tree foliages for the protein protection.

Conventionally, tree foliages have been fed together with agricultural by-products, mainly crop-residues, containing low levels of nitrogen to enhance rumen microbial fermentation and hence the animal productivity. Tanniferous trees and shrubs are important in animal production because they can provide significant protein supplements. Forages containing tannin are *Leucaena leucocephala, Ziziphus mauritiana, Albizia chinensis, Manihot esculenta, Terminalia oblongata*, etc. Tree legume forages offer a cheap alternative to concentrate diets in ruminant nutrition.

Among tanniferous trees and shrubs, *Leucaena leucocephala* and *Ziziphus mauritiana* are common feedstuffs for the goats in Myanmar. *Leucaena leucocephala* is now widely spread through most tropical and sub-tropical regions of the world and provides an important source of feed for ruminant livestock. *Leucaena leucocephala* is a valuable, high quality fodder tree for the tropics. The crude protein content of *Leucaena leucocephala* is 28.8% in Myanmar. Due to its high crude protein content, this forage can replace commercial protein feed resources in rations or be used as a supplementary diet.

Tannins are generally regarded as inhibitory to the growth of microorganisms but the mechanisms involved are poorly understood. Petroleum ether, chloroform, methanol, etc, are used to extract tannins sequentially from the plant materials. However, detannification methods are very expensive and cannot be applicable in developing countries including Myanmar. Since tannins are widely distributed in tropical feedstuffs, if tree forages containing tannin could be used as a source to reduce protein degradability, protein economy would be expected for the ruminal production in developing countries.

Therefore, experiment was undertaken to investigate the effect of tannin included in *Leucaena leucocephala* and *Ziziphus mauritiana* on protein degradation of sesame meal by nylon bag method in a fistulated bull and the effect of *Leucaena leucocephala* and *Ziziphus mauritiana* as sources of tannin on nutrients digestibility and their influence on nitrogen utilization in goats.

In first experiment, the lower crude protein disappearance and degradation constants of the treatments included *Leucaena leucocephala* and *Ziziphus mauritiana* would elucidate that tannin containing in these tree foliages interfered the protein degradation in the rumen of a fistulated bull. These results are shown in Figure 1, 2, 3 and Table I. In second experiment, 25% of *Leucaena leucocephala* in the ration tended to increase the nitrogen retention without decrease in DM and OM digestibility. Therefore, *Leucaena leucocephala* could be used as a partial replacement of sesame meal and also as a source of tannin for protein protection in the rumen. These results are shown in Figure 4 and 5.

In accordance with the results obtained from experiments, supplementation of rice straw with Leucaena forage would improve the utilization of rice straw as well as tanniferous tree such as *Leucaena leucocephala* could be used as one of the dietary component as a source of tannin to protect the highly degradable of feed protein in the supplementation of rice straw with concentrate would improve the utilization of rice straw.

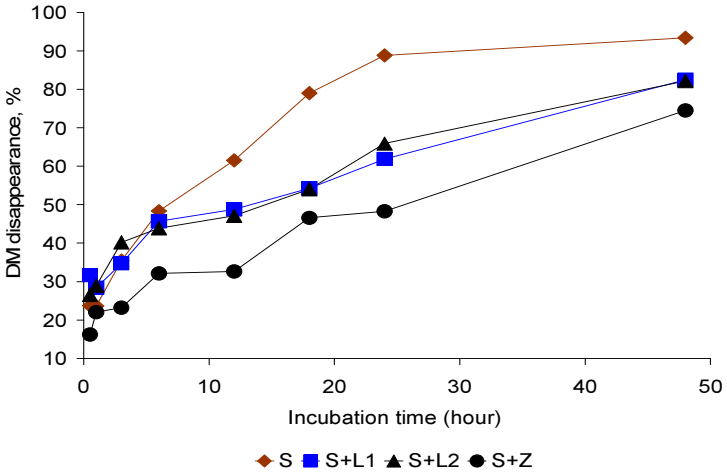

FIG 1. Disappearance of dry matter (%) of sesame meal supplemented with Leucaena leucocephala and Ziziphus mauritiana in the rumen of a fistulated bull

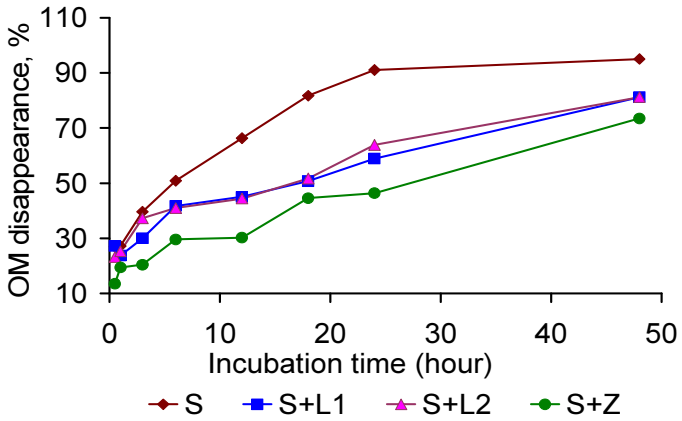

FIG 2. Disappearance of organic matter (%) of sesame meal supplemented with Leucaena leucocephala and Ziziphus mauritiana in the rumen of a fistulated bull

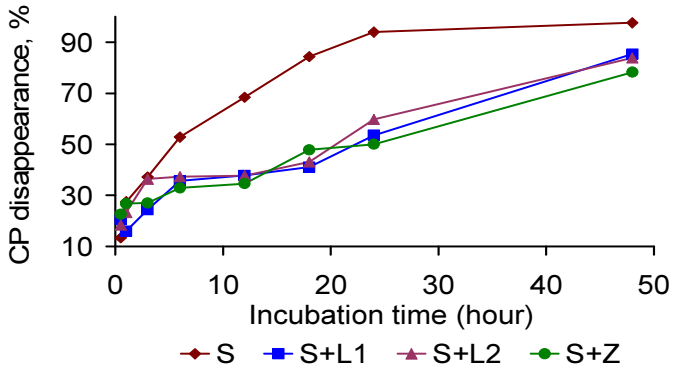

FIG 3. Disappearance of crude protein (%) of sesame meal supplemented with Leucaena leucocephala and Ziziphus mauritiana in the rumen of a fistulated bull)

TABLE I. DEGRADATION CONSTANTS OF RESPECTIVE DIET IN THE RUMEN OF A FISTULATED BULL

Description	S	S + L_1	S + L_2	S + Z
DM [1)]				
a, %	5.0	16.0	12.0	2.0
b, %	88.0	54.0	60.0	56.0
c, h^{-1}	0.085	0.084	0.085	0.090
a + b, %	93.0	70.0	72.0	58.0
OM [1)]				
a, %	7.0	10.0	9.0	1.0
b, %	87.0	58.0	61.0	57.0
c, h^{-1}	0.088	0.088	0.090	0.088
a + b, %	94.0	68.0	70.0	58.0
CP [1)]				
a, %	5.0	1.0	9.0	10.0
b, %	93.0	63.0	58.0	51.0
c, h^{-1}	0.091	0.087	0.088	0.087
a + b, %	98.0	64.0	67.0	61.0

S = Sesame meal
S + L_1 = Sesame meal + *Leucaena leucocephala* 25% in the diet
S + L_2 = Sesame meal + *Leucaena leucocephala* 50% in the diet
S + Z = Sesame meal + *Ziziphus mauritiana* 50% in the diet
a = rapidly degradable fraction
b = slowly degradable fraction
a + b = potentially degradable fraction
c = rate of degradation
[1)] As described in Table I.
Exponential equation: $P = A + B\ (1\text{-}e^{-kdt})$

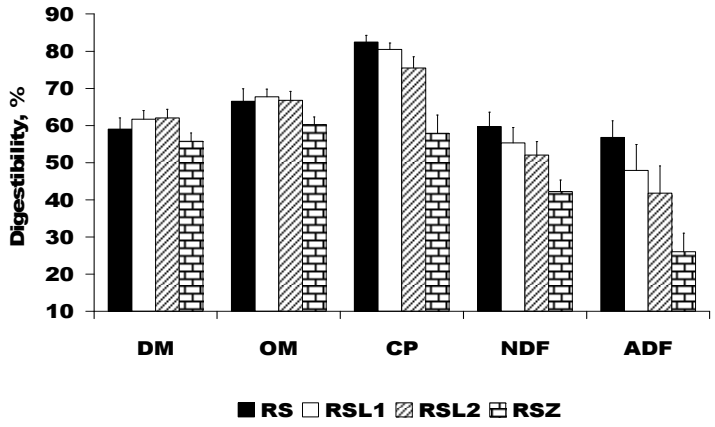

FIG 4. Digestibility of nutrients (%)

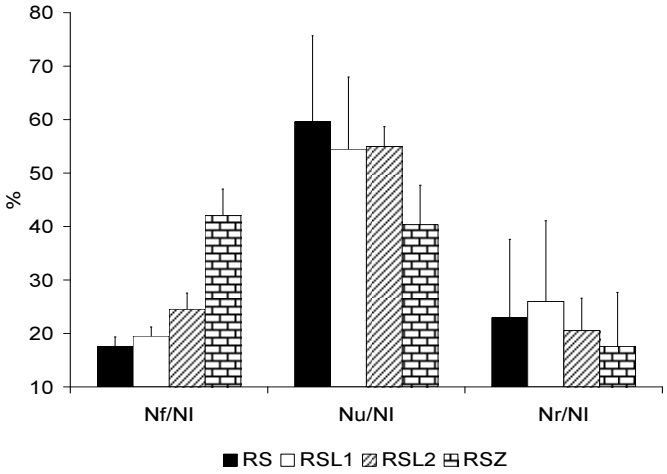

FIG 5. Nitrogen utilization of goats fed respective diet

EFFECT OF SUPPLEMENTING UREA-TREATED SORGHUM STOVER WITH SESAME CAKE OR FISHMEAL ON THE PERFORMANCE OF CATTLE

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On-farm feeding trials were carried out to examine the effect of supplementing urea treated sorghum stover (UTSS) with sesame cake (SC) or fishmeal (FM) on the performance of fattening cattle. Nine Barka cattle were divided into three groups of three cattle in each treatment. The trials were conducted in Zoba Gash Barka, South Western lowlands of Eritrea. All the animals were fed on UTSS for an adaptation period of 15 d. The control diet consisted of UTSS fed *ad libitum* for both species of animals. The second and third treatments consisted of UTSS fed *ad libitum* supplemented daily with 576 g/head of SC and 432 g/head of FM, respectively. The experimental period lasted for 90 d. Feed intakes and body weights were recorded regularly. The group supplemented with SC had the highest significant ($P < 0.05$) total DMI (6.133 kg/head/d) followed by cattle supplemented with FM (5.81 kg/head/d) and the control (5.78 kg/head/d), which were not significantly different ($P > 0.05$) from each other. The body weight gain for cattle fed urea treated sorghum stover alone was 559 g/head/d. This increased to 650 g/head/d with FM and 741 g/head/d with SC supplementation. In cattle, BWG was not significantly different ($P > 0.05$) between all the treatments. The feed conversion in cattle was 8.28 and 8.93 for SC and FM, respectively. Urea treated stover alone has the lowest cost of feed per Kg of BWG, which was the most economical (16.83 Nfa) feed than the other two treatments. It can be concluded that feeding UTSS alone or supplementing with small amounts of sesame cake can increase the live weight of cattle at a reasonable cost.

EFFECTS OF GAMMA IRRADIATION ON RUMINAL PROTEIN DEGRADATION AND INTESTINAL DIGESTIBILITY OF COTTONSEED MEAL

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The effects of gamma irradiation on ruminal degradability and intestinal digestibility of crude protein and the fate of true proteins of cottonseed meal were investigated by using in situ and electrophoresis techniques.

The dry matter of cottonseed meal (CSM) was determined by freeze-drying 1 g sample in duplicate. Based on this value, sufficient water was added to increase the moisture content of 2.5 kg of CSM to 250 g/kg. Gamma irradiation was carried out in a cobalt-60 irradiator at 20°C. The dose rate determined by Fricke dosimetry was 0.37 Gy/s [1]. Three polyethylene packages of samples were irradiated in a gamma cell for total doses of 25, 50 and 75 kGy in the presence of air. Prior to sealing in plastic bags, samples were allowed to air equilibrate for 2 h, then refrigerated (4 °C).

Duplicate nylon bags of untreated or irradiated CSM were suspended in the rumen of four non-lactating Holstein cows for up to 48 h, and *in situ* resulting data were fitted to non-linear degradation model to calculate degradation parameters of crude protein [2]. Proteins of untreated and treated CSM bag residues were fractionated by gel electrophoresis. Intestinal digestibility of crude protein was measured using the mobile nylon bag technique.

As shown in Fig. 1, gamma irradiation of CSM resulted in decreasing of crude protein disappearance in the rumen. This is beneficial effect for high producing ruminants, which need high amount of escaped protein from rumen to intestine. Fitting disappearance data to non-linear degradation model showed that the wash out fraction and degradation rate of crude protein decreased linearly ($P < 0.01$), but the potentially degradable fraction increased quadratically ($P < 0.01$) with increases in irradiation doses. As a consequence, the effective degradability of crude protein decreased linearly ($P < 0.01$) as doses increased. Intestinal digestibility of ruminally undegraded crude protein increased linearly ($P < 0.01$) as irradiation doses increased.

Three major protein components were observed: globulin 9S, globulin 5S and albumin 2S. Electrophoretic results (Fig. 2) indicated that globulin 9S in untreated cottonseed meal (whereas globulin 9S, globulin 5S and albumin 2S in gamma irradiated cottonseed meal) make the bulk of escaped protein. In gamma irradiated CSM, there were cross-linked products of the degraded protein molecules that could not penetrate the running gels. Gamma irradiation of protein will result in denaturation of protein and probably transform the proteins to a more resistant structure. The results of this study indicated that gamma irradiation of cottonseed meal appeared to be an effective means of increasing digestible rumen undegradable protein content.

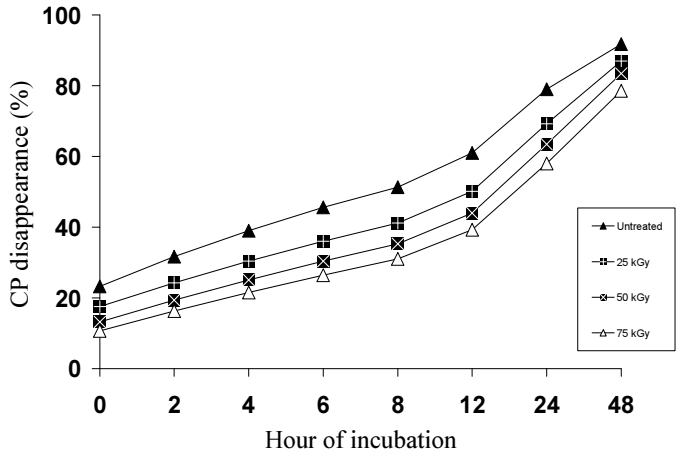

FIG. 1. Crude protein disappearance of untreated and gamma irradiated cottonseed meal in the rumen at different incubation times.

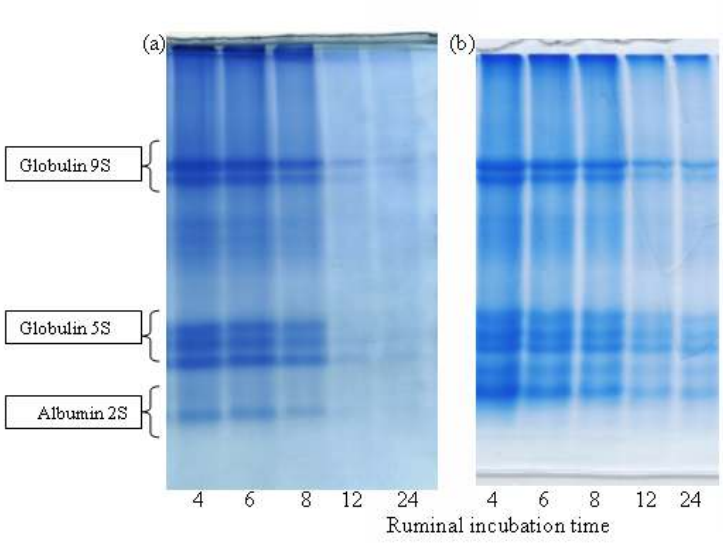

FIG. 2. Protein subunits patterns of untreated (a) and 50-kGy gamma irradiated cottonseed meal (b) incubated in the rumen at different incubation times.

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THE EFFECT OF SUPPLEMENTATION LEVEL OF CONCENTRATE FEEDING (25 VS. 75%) ON RICE STRAW TREATED WITH URINE ON ONGOLE CROSSBRED CATTLE PRODUCTIVITY AND METHANE EMISSION

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The study to increase productivity while reducing methane emission on beef cattle was carried out by using eight Ongole Crossbred cattle (age 1.5 year, live-weight 240 kg). They were fed rice straw treated with urine (1 L urine for 1 kg DM rice straw) and concentrate feeding composed of wheat bran (CP 15%) and beer cake (CP 24%) at a ratio of 50:50. The cattle were grouped into two for feeding treatments. First group of cattle was allowed concentrate feeding at 25% (C25) of estimated dry matter intake (DMI) at 3% of body weight, while the other group was given concentrate feeding at 75% (C75) of estimated DMI. Daily intake of dry matter (DMI), methane production and live-weight gain (LWG) were measured. Methane measurement was done using Facemask method, which was performed for 10 minutes at 3-hour intervals over two days.

The results showed that, higher-level concentrate (C75) resulted in higher daily live weight gain (LWG) (P = 0.0145) than that in C25, even tough the DMI of both treatments were similar. The better LWG in C75 than of C25 is caused by the better nutrients intake in C75, especially in protein and Total Digestible Nutrients (TDN) (P = 0.0017). Protein intake in C75 was higher (P = 0.0002) than that in C25, being 1.19 and 0.80 kg/d, respectively. Similarly, TDN intake in C75 was higher (P = 0.0017) than that in C25, being 5.77 and 4.39 kg/d, respectively. The protein intake was playing an important role in growth and development of the Ongole Crossbred cattle, while TDN fulfilled an energy requirement of cattle. This result also showed that Ongole Crossbred cattle have a high potential as beef cattle by LWG over 1 kg/d. This obtained LW was higher than that previously reported [1], being 0.62 kg/d by 70% concentrate (composed of wheat bran and rice bran at 50:50). In addition, many studies reported the low productivity of Ongole Crossbred cattle, ranged at 0.4-0.6 kg/d.

Daily total methane production (L/d) in C75 and C25 was similar, being 219.3 and 240.4 L/d, respectively. This was an unexpected result. Normally, methane production will lower in higher quality feeding regimes. This phenomenon might be explained by an effect of rice straw, the roughage supplemented with. Rice straw is highly fibre fibrous feed and low digestibility; therefore the retention time in gastrointestinal tract will be longer, and within this period the fermentation process resulting methane. This reason might be different at high quality concentrate supplementation that might activate microbial fermentation in straw in which resulting a shorter retention time. However, this shorter retention time did not resulting in lower methane production, because when rumen distension lowers it will sign animal to eat, and therefore, the total daily DMI in both treatments was similar (6.72 and 7.59 kg/d). This result agreed with the statement of Shibata et al., [2] that methane production is highly correlated with DMI. The significant different of LWG, however, if taken into calculation of methane production per kg LWG found the C75 was lower (P = 0.0186) than that of C25, being 205.8 vs. 967.2 L/kg LWG, respectively.

The present study showed that supplementation of high quality concentrate in the low quality diet such as rice straw, did not lower the methane production quantitatively, but if the animal product such as live weight gain taking in the consideration, the significant mitigation of methane was clearly observed. The results of the present study are similar to our previous study [3] by using soybean pulp to supplement Napier grass hay. This study showed that high quality concentrate feeding could relatively maintain methane production at promising level by increasing the production, and suggested that combination of rice straw treated with urine and wheat bran and

beer cake – both was by-product of food industries could lead to a significant productivity and mitigation of methane from cattle or other ruminants in tropical climates.

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CASSAVA BASED DIETS FOR SUSTAINABLE RUMINANT PRODUCTION

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Cassava (*Manihot esculenta*, Crantz) is an annual crop grown widely in the tropical regions of Africa, Asia and Latin America. It thrives in sandy-loam soils with low organic matter and in climate with low rainfall and high temperature. Cassava could also response more with manure fertilization. Cassava tubers contain high levels of energy and minimal levels of crude protein, have been used as readily fermentable energy in ruminant rations, and have been used extensively as a feed for livestock [7, 8].

Recent attempts have been made to develop new products using cassava chips as an energy source with urea as non-protein nitrogen (NPN). Two new cassava based products have been developed: cassarea and cassaya. Cassarea was formulated to contain the following ingredients: 57.1% Cassava chips + 9.9% urea and 3% tallow (Cassarea I, 30% CP); 83.6% Cassava chips + 13.4% urea and 3% tallow (Cassarea II, 40% CP); 80.2% Cassava chips + 16.8% urea and 3% tallow (Cassarea III, 50% CP). Cassarea was tested for rumen degradability using the nylon bag technique and was found to have a 46.2 to 56.7% effective DM degradability. Further investigations with Cassarea II (40% CP) showed that it could be used to replace SBM in the rations of lactating cows, but supplementation with a rumen by-pass protein such as cottonseed meal would be recommended. Cassaya (30% CP) is a product formulated using chopped whole cassava crop hay (85%) + soybean meal (5%) + cassava chips (5%) + urea (2%) + tallow (2%) + sulphur (1%), mixing with water, pressed through a pelleting machine and sun-dried to at least 85% DM. The use of Cassaya in lactating dairy cows as a protein source proved to be efficient in promoting rumen fermentation, improved milk yield and composition and providing an increased economical return.

Moreover, cassava hay (CH) has been applied in ruminant nutrition as a high-quality protein supplement for dairy cattle, beef and buffalo production [8]. CH consists of whole crop of cassava harvested at 2-4 months of growth. The stems with leaves are chopped into 3 to 5-cm lengths and then sun-dried for 2 to 3 d to attain DM of about 80 to 90%. Cassava hay contains a high level of protein (25% of DM) and a strategic amount of condensed tannins (CT) (4% of DM) and appreciable amount of essential minerals (e.g. Mg, K). In comparison with SBM, CH has a higher concentration of RUP [9], which is beneficial since it can supply total AA for absorption in the lower gut. The AA profiles of CH were relatively comparable with SBM while methionine in CH was higher [8]. CT was generally higher in matured cassava leaf but was lower in CH harvested at younger stage. Reed [6] reported that if CT in the feeds exceeded 6% of DM, it would reduce feed intake and overall digestibility. However, if CT is only 2 to 4% of DM, they would help to protect protein from rumen digestion, thereby increasing total by-pass protein.

Feeding trials with different class of animals is shown in Table I. The data revealed that CH enhanced rumen fermentation and increased milk yield and composition [1, 3, 4, 9, 10 and 11]. Furthermore, supplementation with CH to dairy cows could markedly reduce concentrate requirements. In addition, CH supplementation in dairy cattle could increase milk thiocyanate and thus, possibly enhance milk quality and storage, especially in smallholder-dairy farming. CT contained in cassava hay has also been shown to have potential for reducing gastrointestinal nematodes and therefore, acts as an anthelmintic agent [2, 5].

In conclusion, CH is therefore an excellent source of feeds for ruminants. The whole crop of cassava can be formulated as a sole resource of nutritious diets for productive ruminants. Therefore, cassava has great potential to increase the productivity and profitability of sustainable livestock production especially under food-feed-system.

TABLE I. SUMMARY OF TRIALS USING CASSAVA BASED-DIETS FOR RUMINANT PRODUCTION

Item	Sources of Data							
	[10]	[4]	[3]	[1]	[8]	[5]	[2]	[11]
Exp. animals	Dairy	Dairy	Dairy	Dairy	Dairy	Buffalo +Beef cattle	Buffalo & Beef cattle	Beef cattle
Supplements	CH	HQFB-CH	CH	HHCC-CH	SBM-CH	CH	CH	CH
Level suppl., kg/d	1.7	Conc. use (-30%)	2	1	Conc. use (-20%)	1	1	*ad-lib*
Rumen ecology								
pH	6.8	5.69		6.9	6.7			6.9
NH_3-N, mg/dl	17.0	9.14		18.1	14.4			14.7
Total VFA, mmol/l					73.8			86.5
C2, mol/100 mol					73			66.7
C3					17.6			20.2
C4					9.5			13.0
BUN, mg/dl					13.6			8.8
Milk production								
Milk yield, kg/d	15.4	9.4	8.0	7.4	8.1			
Milk fat, %	4.6	4.1	5.0	4.3	4.2			
Lactose, %	-	5.0		4.6	4.7			
Milk protein, %	5.3	3.3	3.9	3.0	3.1			
Solids- not- fat, %	8.4	8.0	8.6	8..3	8.6			
Total solids, %	12.6	13.0	13.6	12.5	14.3			
Faecal parasitic egg count								
% Reduction	-					31.0	58/45	

HQFB = High-quality feed block, HHCC = High degradability-high level cassava chips, SBM = Soy bean meal, CH = Cassava hay

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EFFECTS OF NUTRITIONAL SUPPLEMENTATION AND GENOTYPE ON MILK PRODUCTION AND FERTILITY OF LACTATING DAIRY CATTLE UNDER TROPICAL CONDITIONS

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Nutrition has a profound influence on milk production and reproductive performance of ruminants. Insufficient intake of energy, protein and minerals is associated with suboptimal reproductive performance resulting in delayed ovulation and reduced conception rates. Lactating cows are in a state of negative energy balance because energy required for milk production and body functions exceed energy ingested. However, the relationships of nutritional dynamics and reproduction are not well understood. The objective of this study was to determine effects of nutrition on milk production and fertility in lactating cattle.

Forty in-calf cows comprising of 20 Friesian and Sahiwal were selected and upon calving they were randomly assigned to 5 dietary groups consisting of concentrate supplementation at the rate of 0, 1, 2, 3 and 4 kg fed twice per day after grazing for 24 weeks post partum. Each treatment comprised of 8 cows of 4 Friesian and 4 Sahiwal. Pastures and concentrates were analyzed using proximate analysis to determine nutrient composition. Milk and blood samples were collected bi-weekly for determination of composition using infrared spectroscopy and progesterone using radioimmunoassay respectively. Cow parameters recorded included milk yield and composition (fat, protein, solids not fat and density). Reproductive data included days from calving to luteal activity as indicated by progesterone profiles (>3nm/L), days to 1st insemination and conception to first service.

Data was analyzed by SAS using GLM procedure. Breed, treatment, breed by treatment, parity, and weight of cow significantly affected milk yield. Heavier cows produced more milk with a mean increase of 0.2 kg for each increase in weight. Mean milk yields were different ($P < 0.05$) at 69.7 ± 1.75 and 47.9 ± 1.4 L for Friesian and Sahiwal cows respectively. Treatment 4 had the highest mean milk yield per week ($P < 0.05$) in both breeds averaging 88 and 55 L for Friesians and Sahiwal respectively where the supplementation of 3 kg of concentrate provided adequate nutrients to tap the cow potentials.

There were breed differences for days to peak milk production and peak milk yield. Friesians and Sahiwal cows averaged 64.8 ±1.65 and 37.9 ± 1.07 d to reach peak milk yield, while peak milk yields were 90.75 ± 0.76 and 69.88 ± 0.49 L respectively. This shows that cows with high milk peak take longer to reach the peak. There were interactions between breed and treatment for d to peak and peak milk production but the patterns were not clear for the various supplementation levels. Differences for %fat, protein, SNF and density of milk between the two breeds were observed as indicated in the Table I. These could be due to differences in feed conversion and rumen function dependent on volatile fatty acids and microbial proteins produced.

Fat to protein ratios show that Sahiwal were in more energy deficit than Friesians. The density of Sahiwal milk was higher ($P < 0.05$) than that of Friesian because of higher total solids in Sahiwal milk causing larger specific gravity. Breed effects on milk components far outweigh treatment effects.

TABLE I. LEAST SQUARE MEANS AND SEM FOR MILK COMPONENTS OF COWS

Breed	% Fat	% Protein	% SNF	Density	Fat/Protein ratio
Friesian	3.55 ± 0.22^{a}	3.07 ± 0.03^{a}	8.12 ± 0.08^{a}	26.8 ± 0.04^{a}	1.17 ± 0.33^{a}
Sahiwal	4.52 ± 0.14^{b}	3.22 ± 0.02^{b}	8.50 ± 0.05^{b}	27.4 ± 0.26^{b}	1.40 ± 0.21^{b}

^{a,b} different superscripts within columns differ significantly ($P < 0.05$)

Sahiwal exhibited better reproductive performance than Friesians as indicated in Table II. It was observed that 18% of in-calf cows lost their foetus before term and 25% of them never showed heat 120 d postpartum. Of these, 15% never showed any luteal activity, while 10% had silent heat. Sahiwal came into heat and started cycling earlier ($P < 0.05$) than Friesians (Table II). Friesians had more ($P < 0.05$) cows conceiving at 1st insemination and showing luteal activity than the Sahiwal.

TABLE II. REPRODUCTIVE PARAMETERS OF THE TWO BREEDS

	Breed	
Reproductive characteristics	Sahiwal (n = 20)	Friesian (n = 20)
Cows Calving %	85	80
Foetal loss %	15	20
Non return to heat 6 months post partum %	18	31
Mean ± SEM of days to 1st heat (d)	72.6 ± 1.7	96.9 ± 5.4
Mean ± days to start of luteal activity	55.5 ± 1.2	74.6 ± 1.9
Cows showing heat after parturition %	82	69
Cows conceiving at 1st insemination %	21	54
Cows showing luteal activity after 120 d %	88	81

There was within breed and between treatment differences ($P < 0.05$) for d to 1st heat and start of luteal activity as seen in Table III. However the outcomes were quite varied and did not show specific patterns for treatment effects in both breeds.

TABLE III. MEAN DAYS TO 1ST HEAT AND START OF LUTEAL ACTIVITY FOR VARIOUS TREATMENTS

	Mean d to 1st heat		Days to start of luteal activity (> 3ng/L)	
Treatment	Sahiwal	Friesian	Sahiwal	Friesian
1	61.5 ± 0.1	53.3 ± 3.9	53.0 ±1.6	49.3 ± 3.1
2	83.9 ± 0.8	51.5 ± 2.5	29.3 ± 2.0	63.4 ± 3.4
3	35.7 ± 0.3	116.1 ± 9.1	60.7 ± 3.2	72.2 ± 2.9
4	109.1 ± 3.9	42.4 ± 2.8	66.8 ± 2.3	58.7 ± 0.8
5	60.4 ± 2.4	243.3 ± 4.4	58.5 ± 2.1	101.6 ± 3.3

It can be concluded that breed effects were more important than the nutritional effects on milk production, composition and reproductive performance.

INDIGENOUS CATTLE IN SRI LANKA: PRODUCTION SYSTEMS AND GENETIC DIVERSITY

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The production status, farming systems and genetic diversity of indigenous cattle in Sri Lanka were evaluated using six geographically distinct populations in Sri Lanka, which is a small island located below the southern tip of Indian subcontinent. The indigenous cattle population of the country is considered as a non-descript type mixture of genotypes [1], and represent more than the half of total cattle population of 1.2 million heads [2].

Six distinct indigenous populations (NE, NC, So, No, TK and Th) were investigated for morphological and genetic differences. The respective farming systems were also evaluated to complete the requirement in developing conservation and utilization strategies. The sampling was carried out based on the non-existence of artificial insemination facilities to assure the target populations are indigenous. The six populations were assumed genetically isolated from each other in the absence of nomadic pattern of rearing and regular cattle migration. The farming systems were analyzed using a pre-tested structured questionnaire by single visits to each location. Single visits were practiced, as there is no variation in farming system according to the period of the year. Morphometric measurements were taken during the visit and the genetic variation was assessed within and between five populations using 15 autosomal and two Y-specific microsatellite markers. The farming system analysis revealed that indigenous cattle are reared as a traditional practice in all the regions of the country under limited or no input situations. Since the low productivity masks its real contribution to the rural livelihood, the level of utilization was confounded within the attributes of respective farming systems. The contribution of indigenous cattle to total tangible income ranged from 0% to 90% in different regions reflecting the high variation in the purpose of keeping indigenous cattle [3]. Integration with crop, especially with paddy was the common feature in systems across the regions. Morphometric measurements identified the specific phenotypic characteristics resulted by geographical isolation and selective breeding. Though vary according to the regional preferences, the compact body, narrow face, small horns and humps with shades of brown and black coat colour described the indigenous cattle phenotype in general.

The diversity analysis based on microsatellite genotyping indicated that indigenous cattle in Sri Lanka has a high genetic diversity with average number of alleles per locus ranging from 7.9 to 8.5. Average heterozygosity of different regions varied within a narrow range (0.72 ± 0.04 to 0.76 ± 0.03). The genetic distances (DA) between regions were low (ranged between 0.085 and 0.066) suggesting a similar mixture of genotypes across regions despite the geographical isolation. However, two genetic clusters were visible though no relationship of those clusters with the geographical distribution of different regions could be observed. Introgression of taurine cattle was evidenced in one of the cattle populations (NC) as suggested by the Y-specific microsatellite analysis.

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COMPARATIVE GENOMICS FOR PREDICTION OF THE RELATIVE LOCATION OF EST IN THE GOAT GENOME

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Worldwide the goat is an important agricultural species that is highly adaptable to many environmental conditions, and goat production is a rapidly growing industry within the U.S. A better understanding of the goat genome could lead to new discoveries based on the genetic diversity and environmental adaptations important to ruminant health and production. An effort is underway to increase our understanding of the goat genome and develop a radiation hybrid (RH) map for stronger comparative genomic analyses. Correlations among the bovine, sheep, human, and the goat maps have been made previously and are useful for comparative genomic approaches to understanding phenotypes. Recently an embryo/uterine cDNA library was sequenced and about 12,800 ESTs added to the public database. In this study, comparative analyses among goat, sheep, and cattle maps were used to predict the location of the assembled EST contigs (n = 1920) and singlets (n = 4400) in the goat genome. Prediction of goat EST locations was determined through comparisons with the sheep map using the bovine map as a backbone. A virtual goat map was then developed by using the markers on the most recent published genetic map for the goat as a base. These markers were localized on the sheep genetic and virtual genome map and bovine sequence map when possible. Alignments of ESTs were predicted based on the relative location of mapped goat markers on the bovine sequence and refined by comparisons with the sheep maps. The predicted map attempts to localize the relative genomic position of the unique contigs and singlets developed from the available ESTs. Additionally, the degree of conservation among goat, cow, sheep, human, mouse, and rat genomes has been indicated on the map. The predicted map, or virtual goat genome, will be a crucial resource for comparative genomic analyses and for the determination of EST and microsatellite markers during development of a goat RH map.

A NEW METHOD FOR IMPROVING THE DAIRY PRODUCTION SECTOR IN A DEVELOPING COUNTRY: THE CASE OF CAMEROON

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Milk production in Cameroon was estimated at 184 000 tons [3]. Yet the demand of milk products is far above production and 24% of national consumption is imported. Due to urbanization and population growth, milk production needs to double by the year 2020 if it is to meet the demand [4]. Therefore, efforts have been made to increase dairy production [1]. However the efforts of non-governmental organizations, research and government institutions failed to significantly boost domestic dairy production because farmers did not see the economic gain associated with potential biological improvements [5].

A hypothesis was then devised whereby an integration of interventions at the level of farmers associating nutrition, health, reproduction and management would bring more economic benefits to smallholder farmers and improve dairy production. This involved reviewing dairy research done in Cameroon, carrying out a participatory rural appraisal and an economic opportunity survey at selected dairy farms, setting up various interventions on farms, investigating post-partum return to oestrus, evaluating milk quality and the impact of integrated interventions. This paper intends to critically evaluate the results of different studies and present the integrated method developed in this research. It also aims to set up guidelines for a successful sustainable improvement of dairy production in Cameroon.

The study involved reviewing dairy research done in Cameroon. A participatory rural appraisal and an economic opportunity survey were carried out in selected dairy farms. On-farm interventions were set up, investigating cow reproduction, evaluating milk quality, setting up an artificial insemination centre and the impact of integrated interventions. Guidelines for improvement of the dairy sector were drawn. These series of experiments were designed to evaluate the impact of interventions carried out holistically.

In small-scale dairy systems the uptake and use of research results by wider communities of farmers, organization and livestock extension services has often been less than expected. This in turn resulted in interventions for supplementary feeding, or for improving reproductive performance that did not demonstrate an economic benefit to the farmers. One of the reasons is that they focused only on one constraint or one discipline at a time, and other concurrent production problems were limiting the economic benefits.

This study has developed an integrated method in improving dairy production in Cameroon and has found that marketing and milk production per cow per day were the most limiting factors of dairy improvement. Interventions were carried out to solve these constraints and others. Farmers adopting interventions had returns of 193% and 232% with or without opportunity costs proving the positive impact of interventions using the integrated method. The integrated method in solving these constraints will bring much improvement and clear economic benefits to smallholder farmers, proving its effectiveness in ensuring improvement of dairy systems in Cameroon. These interventions need to be spread to more farms in the country. This method needs to be adopted for further dairy production improvement by the creation of multidisciplinary intervention teams and the training of integrated intervention specialists in the dairy sector [2].

The application of integrated interventions in dairying requires the synergistic action from the government, researchers, non-governmental organizations and farmers. It requires expertise from

many different fields and calls for the need to create integrated action teems in each administrative region. Each team will be multidisciplinary constituted of an extension agent, an animal nutritionist, a veterinarian, a socio-economist, a dairy technologist and a reproduction scientist. It is quite likely that there be a lack of such specialists in each subdivision. In which case there can be a creation of intervention teams covering special areas of the country. It is not that these intervention teams will replace the private sector but they will guide local authorities in the extension of research results and in actions needed for regulation, advice and support the private sector.

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MILK PRODUCTION AND REPRODUCTION PERFORMANCE OF MURRAH BUFFALO OF TAMIL NADU, INDIA

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The data pertaining to variable production and reproduction traits (1980 lactation records of 698 Murrah buffaloes) distributed over a period of 28 years (1979 to 2006) were collected from Central Cattle Breeding Farm, Alamadhi, Tamil Nadu, India. To utilise all available data the entire duration of the study was grouped into seven periods and each year was classified into four seasons. In addition, parity effect was also considered. First six parities were considered and parties six and above were lumped together as sixth parity. LSMLMW and MIXMDL PC-2 Version computer programme [1] was used to study the effect of various non-genetic factors.

The overall least-squares means for different production traits viz. peak yield, days to attain peak yield, 305-day milk yield, lactation length, lactation milk yield and milk yield per day of lactation were 8.87 ± 0.05 kg, 53.4 ± 0.8 d, 1804.9 ± 14.7 kg, 297.8 ± 1.9 d, 1855.6 ± 16.1 kg and 6.16 ± 0.04 kg respectively. The overall least-squares means for reproduction traits such as service period, calving interval and dry period were 225.0 ± 5.5, 532.8 ± 5.5 and 230.2 ± 4.9 d respectively.

Period of calving had highly significant effect on all the traits studied except lactation length, where it had only significant effect. The highly significant ($P < 0.01$) influence of period of calving on lactation length observed corroborated with the previous finding on Murrah buffaloes [2]. The significant differences in milk production and reproduction performances among different periods may be attributed to differences in management, selection of bulls and environmental conditions such as the ambient temperature, humidity, rainfall, etc.

Season of calving had significant ($P < 0.05$) effect on peak yield and lactation milk yield and highly significant ($P < 0.05$) effect on days to attain peak yield, 305-day milk yield, milk yield per day of lactation and all the reproduction traits studied. The winter and summer calvers produced maximum yield than monsoon calvers owing to two succeeding favourable seasons, i.e., south-west and north-east monsoon seasons. During these periods the climate was conducive and there was abundant availability of good quality fodders. Whereas, the monsoon calvers produced the least, as they suffered from hot humid stress and availability of poor quality fodder during major part of the lactation period. The significant effect of season of calving on lactation milk yield observed is in accordance with earlier reports on Murrah buffaloes [3, 4]. The lowest calving interval was observed in southwest monsoon calvers and they differed significantly ($P < 0.05$) with winter and summer calvers. There was no significant difference in calving interval between winter and summer calvers and southwest and northeast monsoon calvers.

Parity had highly significant effect ($P < 0.01$) on all the traits studied. The 305-day and lactation milk yields increased up to third parity, which was stabilized at fourth lactation (Figure 1) and declined thereafter. Pair wise comparison revealed that the lactation milk yield was lowest in first parity and differed significantly ($P < 0.05$) from other parities. There was an initial sharp reduction (3.09%) in lactation length in the second parity followed by gradual decline later. The reduction in lactation length between first and second parities was statistically significant ($P < 0.05$). The significant influence of calving sequence on calving interval and longer first calving interval than the rest found in the present study concurred with the other reports on Murrah buffaloes [2, 3].

In general, the reproduction traits such as service period, calving interval and dry period were slightly higher than those observed in its home tract and hence better breeding management and introduction of genetic evaluation programmes are needed for further improvement of these traits.

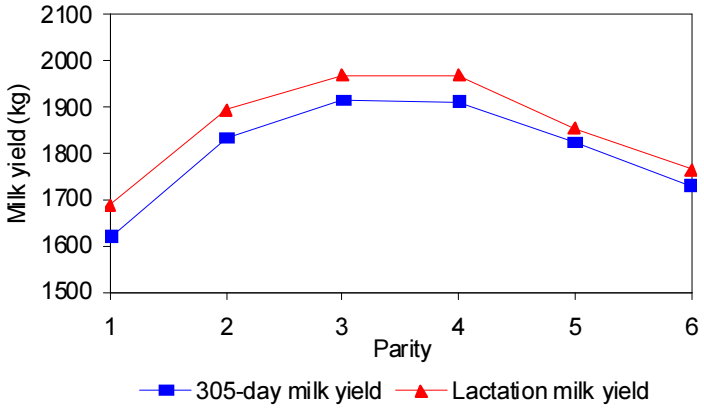

FIG 1. Effect of parity on 305-day milk and lactation milk yields

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MATCHING GENOTYPE WITH THE ENVIRONMENT USING AN INDIGENOUS CATTLE BREED: INTRODUCTION OF BORANA CATTLE FROM SOUTHERN ETHIOPIA INTO THE LOWLANDS OF NORTH-WESTERN ETHIOPIA

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Pastoral, agro-pastoralism and transhumance cattle production systems are important determinants of livelihoods in the semi-arid areas of north-western, southern and eastern parts of Ethiopia [1, 4]. The highlands are important for mixed crop-livestock enterprise, while the arid to semi-arid lowlands, that occupy 61% of the land area, are dominated by livestock production. The livestock species and breeds in these production systems have been traditionally selected, over millennia, to adapt to the challenges of the agro-ecologies.

This initiative was undertaken in the arid to semi-arid lowlands of Metema district, which shares a 60 Km border with the Sudan, in North Gondar Zone of Amhara Region. The total area of the district is 440,000 ha, and 72% is covered with forest and rangeland, while 23.6% is cultivated. The cattle population is estimated at 136,910. Sesame-livestock followed by cotton-livestock production are the dominant farming systems [4]. Although the Gumuz people are native in the district, most of the land is occupied by settlers from the highlands of Amhara and Tigray Regions. As a result, the dominant cattle population is the highland Zebu (mainly Fogera cattle breed crossed with other highland Zebu) brought by the highlanders. Rutana and Felata cattle breeds constitute a smaller proportion of the total cattle population.

As a result, there is a mismatch between the cattle genotype and the environment. The major problems associated with cattle production are diseases and biting flies, water shortage, heat stress, long distance to watering points and grazing areas. Cattle production is therefore, characterized by high pre-weaning calf mortality (35-40%), slow growth rates, low fertility and calving rates, low milk yield and carcass weight. Breeding is entirely based on natural mating, and farmers' selection is based on milk yield, body conformation and colour; with considerations to disease resistance, heat tolerance and draft power potential. Table I presents the productive and reproductive performances of highland Zebu cows in the lowland agro-ecology [4].

There is an evolving market-oriented cattle-fattening system in the district due to the increased domestic demand for meat and also the expanding export opportunity of live animals to the Sudan and other neighbouring countries. As a result, farmers are demanding for more adapted and productive animals. In response to this challenge, the Improving Productivity and Market Success (IPMS) project of ILRI examined the performance of a number of indigenous lowland breeds and decided to introduce and test the most promising indigenous Borana cattle breed in Metema. The Borana cattle breed is found in the semi-arid lowland areas of Borana in Ethiopia and the adjoining areas of Kenya. The production system is a pastoral and semi-pastoral that makes use of marginal resources in the area [3]. The Borana cattle is known for its heat and drought tolerance, good walking capacity, faster growth rate, higher fertility and superior meat production potential [2].

With an overall aim of enhancing a market-oriented cattle production system under a tropical environment, the IPMS project introduced pure Borana bulls for natural mating with highland Zebu cows. In addition, over 400 highland Zebu cows were hormonally oestrus synchronized and artificially inseminated with Borana semen. This paper explains the new approach, the processes involved and the results achieved so far in an attempt to match genotype with the environment through introduction of the indigenous Borana cattle into the lowlands of north western Ethiopia.

TABLE I. PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF HIGHLAND ZEBU COWS IN THE LOWLANDS OF METEMA DISTRICT

Traits	Farming system				Overall	
	Cotton-livestock		Sesame-livestock			
	HH	Mean	HH	Mean	HH	Mean
Age at first calving, yrs	90	4.7 ± 0.06	177	4.4 ± 0.04	267	4.5 ± 0.05
Daily milk yield, L	90	1.6 ± 0.06	177	2.0 ± 0.05	267	1.8 ±.05
Lactation length, mo	90	6.7 ± 0.28	173	5.5 ± 0.14	263	5.9 ± 0.14
Lactation yield, L	90	341.9 ± 23.30	173	314.7 ± 9.83	263	328.3 ±16.56
Calving interval, mo	89	19.3 ± 0.52	173	17.3 ± 0.37	262	18.3 ± 0.44
Weaning age, mo	89	9.5 ± 0.55	173	10.1 ± 0.32	262	9.8 ± 0.43
Life time calf crop, hds	90	6.8 ± 0.24	169	7.9 ± 0.13	259	7.3 ± 0.18
Pre-weaning calf mortality, %	89	35.0	173	40.0	262	37.5

HH = households

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LIVESTOCK FOR REBUILDING COMMUNITIES, ENHANCING LIVELIHOODS AND PROTECTING THE ENVIRONMENT

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A. "The shock, grief, distrust and desolation I went through in the past can only be understood by God." These are the words that Gorreti Kibikiriwo uses when asked about her life before receiving support from Heifer International. Gorreti, age 52, lives with seven orphaned children in Masaka District, Uganda. She lost her husband in 1989 to HIV/AIDS. Since then, the life of the entire family that solely depended upon the late husband faced the realities of dependency. Heifer International provided her a dairy cow and several trainings. The heifer has caused great changes in the family in terms of benefits. These include: improved family nutrition, increased income, soil fertility, family cohesiveness, diversification to other enterprises, and renovation of her house and the establishment of a kitchen garden, among others. Gorreti summarized the entire trek to self-help family development as "a business, an asset, a wonderful project'', because it addressed her genuine need and above all, a source of inspiration.

B. After Hurricane Mitch in 1998, the poverty index rose almost 3% in Honduras. Small-scale rural families suffered from damage to the economy, including risks to their food security, unemployment and migration from the rural areas to the cities.

In response to the devastation, Heifer International started the "Integrated Farms with Gender Focus" project to improve food security and income generation. A total of 1,981 families have received animals of different species. The project has contributed to the improvement in the frequency and diversity of food consumption. Besides consuming corn and beans, the families now have animal products such as milk, eggs, meat, etc.

C. When the Tsunami of December 2004 hit several countries around the Indian Ocean, Heifer International immediately started new projects in Thailand, India, Sri Lanka, and Indonesia. Strong and collaborative community groups were formed. Following training, they were involved in decisions to select appropriate livestock for their local markets and feed resources. The recipients of Heifer animals have already become donors. A total of 3,875 families received animals and training from local extension workers and Heifer International's attempt to rebuild communities has been very successful.

D. In Kosovo, one of the Heifer International's projects helped farmers of three villages with 52 pregnant cattle. The project also provided 10 saplings per family aiming to improve the environment on their farms. Farmers handed over the saplings to the elementary school students in the Shipashnica e Poshtme village. The students planted trees for a better environment and learned horticultural techniques. Now, the Heifer International Cornerstone "Improving the Environment" is practiced not only by Heifer International's beneficiaries, but by the entire community as well.

Building on its valuable experience of working with farm communities, Heifer International uses livestock and trainings as tools to end hunger and poverty and to care for the earth. Heifer International strongly believes that development is not only about distributing inputs but about empowering individuals, building communities, producing deeper level impact and transformation of lives of families from receiver to giver, crossing the border of selfishness to sharing, and community development. By addressing the inter-related causes of poverty like social discrimination, poor health, and communal conflicts through a model of holistic development, Heifer International has brought lasting social and economic empowerment to the lives of more than 10 million families around the world.

MILK PRODUCTION POTENTIAL, QUALITY OF RAW MILK AND REPRODUCTIVE EFFICIENCY OF DROMEDARY CAMELS (*CAMELUS DROMEDARIUS*)

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Old world camelids (Dromedary and Bactrian camels) are important source of milk in rural areas of many arid countries. However, extensive production system cannot guarantee constant quality and quantity raw milk for the market. Development of the world first large scale-camel milking farm in Dubai draws attention to camels as potential source for high quality milk and meat in developing countries. This presentation summarizes the development and results of the project.

Animals: Intensive management and production require concentration of camels. Camels arrived to the farm from different countries of the region with variable age, condition and lactation stage resulting in great range in individual milk production. In practice, history of the purchased animals (health status, previous production) is not available. Despite the negative preconception, camels can be trained successfully to adopt the machine milking technology. Training takes 1-4 weeks depending on the background of the animal. So far we have trained more than 1400 camels for machine milking.

Infectious disease control, general and udder health: Due to lack of history, it is extremely important to establish and maintain strict quarantine. Infectious diseases (Brucellosis, Tuberculosis, Surra, FMD etc.) are monitored with serological tests. The main problem is that most of the tests are not validated for camels and kits from different manufacturers may give controversial results. The prevalence of Brucellosis exceeded 30% in purchased animals from certain regions. Udder health is also monitored carefully. In previously hand milked, multiparous camels, teats are frequently enlarged/deformed or the udder is infected with pathogen bacteria (Str. agalactiae, Str. bovis, Corynebacterium amycolatum, Staph. aureus, etc.). In a recent survey, 21.8% (54 camels) of 248 new lactating animals were infected. These camels should be either rejected or treated before starting machine milking.

Adaptation of milking technology, milk production, husbandry and management: first, milking parlour had to be designed that is comfortable and suitable for different sizes of camels. We have developed a 2 x 24 herringbone parlour. Commercially available, standard milking equipments is used but adapted to the physiology of dromedaries. Milking liners and claw were selected carefully or developed because of the anatomical features of camel udder. There is a great variation in size of teats between individuals (length: 7.1 ± 2.22 cm, mean ± SD, 2.93-16.0 cm). In addition, teats undergo significant increase in size (length by 50%) and volume (by 170%) during milk letdown. Camels have limited cistern volume so initiation of milk letdown reflex is crucial. Traditionally it is induced by the suckling effect of the calf, but this is impossible to do in the parlour. This fact highlights the importance of well-trained milkers who can carry out efficient manual udder and teat stimulation for a long time (mean ± SD: 123.2 ± 84.4 sec) and fast and also precise milking (mean ± SD: 126.9 ± 41.1 sec) [2]. At present, the average daily milk production per camel is 7.2 ± 0.14 kg (± SEM), mean length of lactation is 340 ± 7.9 d (± SEM) and mean total production per lactation is 2,467 ± 79.4 kg (± SEM). So far, the highest and lowest production were: lactation average 14.8 kg and 2.9 kg milk/d; total production 8002 kg and 919 kg; length of lactation 541 and 312 d, respectively. Dams and calves are kept in adjacent paddocks throughout lactation and are allowed together after each milking. Every day they go for a one-hour walk. Staff is trained to take care of the animals in a gentle way to provide low-stress environment. In return, camels are tame, easy to handle and cooperate well during all procedures (milking, nail cutting, rectal examination, blood collection, etc.).

Food safety management system and quality of raw camel milk: In the UAE, all food-producing establishment must implement minimum the HACCP system. We aimed to develop ISO 22000:2005 Food Safety Management System, which is in the final stage of certification. There is no camel milk standard and many studies are required to establish the normal range and acceptable limits for chemical and microbiological content, SCC, etc. in raw camel milk. The total viable count (TVC) of our bulk tank milk is 4,340 ± 86 cfu/ml (mean ± SEM, Figure 1) and the somatic cell count (SCC) is 388,826 ± 5255 cells/ml (mean ±SEM, Figure 2).

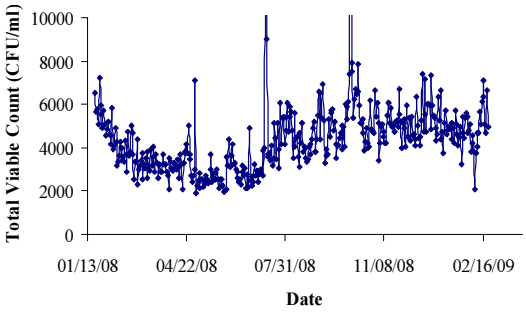

FIG 1. TVC (cfu/ml) of bulk camel milk

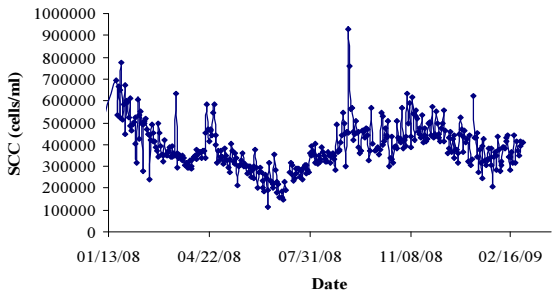

FIG 2. SCC (cells/ml) of bulk camel milk

Reproductive management to maximize breeding efficiency: During 2 breeding seasons (2006-2007, 2007-2008), 483 dromedaries were included in the breeding program using 10 males. The overall per-cycle pregnancy rate was 41.7% and the end of season pregnancy rate was 86.5%. Per-cycle pregnancy rate was influenced by season, male, breed, lactation and environment. The incidence of early embryonic death (EED) was 8.3% (38 of 456 pregnancies). It occurred at an average of 55.6 ± 2.8 d (± SEM, 30-97 d). So far, the overall foetal loss rate is 5.7% (20 of 354). The life-birth rate in the first season was 80.5% (165 of 205). We have demonstrated that by using appropriate reproductive management program, dromedaries can be as fertile as any other domestic species. In traditional breeding systems the life-birth rate does not exceed 40% [1].

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POSTER PRESENTATIONS

EFFECT OF SELENIUM VITAMIN E SUPPLEMENT ON THE PERFORMANCE OF SHEEP IN CENTRAL IRAN

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Selenium (Se) is an essential trace element in animal nutrition involved in the defense against the toxicity of reactive oxygen species and the oxidation state of cells, where its deprivation reduces activities of the Se-dependent enzymes. The signs in animals depend upon vitamin E status and appear only when both nutrients are limiting where they vary according to species. Estimating actual selenium intake is often difficult [1] due to the specific housing and feeding conditions of sheep husbandry, which is mostly in extensive system in most part of the world and in Iran as well. Indirect indices such as Se concentration in blood, glutathione peroxidase activity and production are used to evaluate Se status [4]. Correct levels of selenium supplementation at different growth stages of sheep are essential to maximize productivity, but few studies have been conducted to determine the composition of mineral supplements for sheep in Iran. This study was designed to monitor the health status of sheep grazing in a dry land zone and to test the response of ewes to selenium vitamin E injection.

Three flocks each with 120 sheep, representative as sheep husbandry system of a district zone in Markazi province in central Iran, were considered. Sheep keeping in this area is mostly based on the extensive system depends on poor rangeland, crop residues and stubbles with supplement feeding of barley grain during cool winter when the ewes are at late stage of pregnancy. In each of the selected flock, 60 ewes were identified and divided in two groups including 1) control and 2) treatment, where the ewes in treatment group received selenium-vitamin E (each dose contained 0.5 mg sodium selenate and 50 mg vitamin E) supplement by subcutaneous injections. Each ewe injected by one dose of the supplement 2 weeks before mating and again 2 weeks before lambing. Blood samples were collected, one month post injection that was occurrence to autumn and spring seasons, and analysed for glutathione peroxidase activity (GSH-px). The reproductive parameters were recorded and lamb body weight measured at birth day and weaning time as well.

Results of the enzyme activity (Table I) showed that the treatment had significant effect on the GSH-px at different seasons for flock I and II where the injection of Se-vitamin E increased the enzyme activity comparing to the control but no significant differences were found between the control and treatment in flock II. There were also significant variations of GSH-px among the flocks where the highest amount was found in flock III and the lowest amount in flock I. It may be a realistic explanation that no response was found in flock III as a result of injection. This finding may be indicated the probability of selenium deficiency in the flock and correction of deficiencies by supplementation. These results support the finding of other workers [3] where they reported an increase in erythrocyte glutathione peroxidase activity (139.5 vs. 86.3 U/ml) in dairy ewes by subcutaneous injection of vitamin E and Se. Furthermore supplementation had positive effects on the reproductive performance in all flocks where the lambing rate and final lambs obtained per 100 sheep significantly ($P < 0.05$) increased in the treatment ewes than the control (Table II). However no differences were found between the treatment and control for the live weight of lambs at birth or weaning times. There was a positive relation between glutathione peroxidase activity and reproductive performance in ewes. It is suggested that reduced fertility and increased mortality of lambs may be related to congenital muscular dystrophy [2], arising from Se deficiency.

TABLE I. EFFECT OF TREATMENT ON THE GSH-PX ACTIVITY IN BLOOD (UNIT/MG HAEMOGLOBIN)

Flocks	Season	treatments		significance	
		control	supplement	[1]T	[2]F
1	Autumn	42.8 ± 5.5	97.8 ± 10.2	**	**
	Spring	40.2 ± 6.5	91.5 ± 19.3	**	
II	Autumn	63.5 ± 9.7	100.3 ±13.9	**	**
	Spring	50.2 ± 7.8	86.4 ± 12.1	**	
III	Autumn	104.8 ± 8.8	113.3 ± 7.5	ns	**
	Spring	98.1 ± 14.1	99.6 ± 21.6	ns	

[1] Treatment [2] Flock ** = ($P < 0.01$) ns = non significant

TABLE II. EFFECT OF TREATMENT ON THE REPRODUCTIVE PERFORMANCE OF DIFFERENT FLOCKS

Variables	Flocks					
	I		II		III	
	treatments		treatments		treatments	
	Control	Suppl.	Control	Suppl.	Control	Suppl.
Pregnancy rate %	85.7 [b]	96.4 [a]	82.8 [b]	92.9 [a]	85.7 [b]	96 [a]
Non pregnant %	14.3 [a]	3.6 [b]	17.2 [a]	7.1 [b]	14.3 [a]	4.0 [b]
Abortion %	17.4	14.8	20.8	19.2	16.7	12.5
[1]Lambing rate %	73.9	74.1	79.2	80.8	83.3	87.5
[2]Lambing rate %	8.7	11.1	0.0	0.0	14.3	4.0
[3]Total lambing %	82.6	85.2	79.2	80.8	83.3	87.5
Lamb mortality %	15.8 [a]	8.7 [b]	26.3 [a]	14.3 [b]	14.3	14.3
Total lambs/100 sheep	70.8 [b]	82.1 [a]	65.6 [b]	75.1 [a]	71.4 [b]	84.0 [a]
Total weaned lambs/100 sheep	59.6 [b]	75.0 [a]	48.3 [b]	64.3 [a]	61.2 [b]	72.0 [a]
Birth weight (kg)	3.37	3.71	3.7	3.24	3.25	3.13
Weaning weight (kg)	22.72	23.5	21.94	22.54	20.72	20.29

Values with different superscripts within rows for each flock are different ($P < 0.05$).
1 = single, 2 = twins, 3 = from 100 pregnant sheep

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SUPPLEMENT FEEDING IN LATE GESTATION TO IMPROVE THE PERFORMANCE OF EWES IN A DRY AREA

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Nutritionally, late gestation is an extremely critical production phase in the ewe flock. This is the period of the majority of foetal growth and the period when the majority of the ewe's mammary system develops [3]. Restriction, during foetal life, will exhibit suboptimal development of the small and large intestine, deposit less bone, less muscle and fatter to weaning, and may resulted lower birth weights and weaning weights of lambs [1]. It is essential that a specialized feeding program be used pre-lambing to support the nutrient requirements in the ewe flock. Supplement feeding pregnant ewes with molasses and cottonseed meal resulted in a higher weight of lambs at birth day, 3 months old and 6 month old [4]

In this study, the effect of supplementary feeding on the performance of pregnant ewes, grazing in a dry area of Borazjan, located at northern of Bushehr province in south Iran, was considered. In a completely randomize design, thirty pregnant ewes with average body weight of 43.2 ± 3.4 Kg, were tested for supplementary feeding with 2 treatment groups against a control group. The supplementary feeding started from 120 d of gestation and prolonged till parturition. All animals were grazed in dry land pasture and cereal crops residues all the day times but the treatment groups received concentrate supplement mixed up of: I) wheat bran +sugar cane molasses +cotton seed meal +vitamins and II) wheat bran +sugar cane molasses +urea +vitamins to provide extra macro and micro nutrients with different portion of rumen degradable and undegradable protein between treatments as described in Table I. The lambing rate was recorded and the live weight of lambs was measured at birthday, 3 and 6-month age.

Results showed that the lambing rate, based on the number of lambs per groups, were similar in both treatment groups and the control as well. As it is shown in Table II, lams born from the ewes received supplement feed, had significantly ($P < 0.05$) higher birth weight comparing to the control but no differences were found between the treatment groups. At 3-month age, the lambs born from the ewes in treatment I showed significantly ($P < 0.05$) higher live weight than those of the control and treatment II. Similar trend was observed for the 6-month age lambs.

The average daily live weight gain from birth to 3 month was significantly ($P <0.05$) affected by the treatment I but there were no difference between treatment II and the control group. Similar status was found for daily live weight gain of the lambs during 3-6 month and birth to 6 month age where only treatment I resulted a higher daily gain comparing to the control and treatment II. Approximately 50 percent of the foetal growth occurs at last month of gestation. Nutrient restrictions during this period may result in lighter lambs at birth, increased postnatal lamb losses, and lower levels of milk production [2]. Therefore it can be concluded that feeding supplement in treatment I, compensate the nutrients deficiencies to obtain normal lambs weight but no result found when fed supplement II. As it is shown in Table I, the major difference between the two treatments is related to the protein source of supplement where it was provided with cotton seed meal in treatment I but urea in treatment II which resulted in a higher rumen degradable but negligible un-degradable or by pass protein source in treatment II comparing to the treatment I. It can be concluded that source of protein supplements is important to provide a reproduction response in pregnant ewes in dry area.

TABLE I. AMOUNT OF FEEDS AND NUTRIENTS PROVIDED BY SUPPLEMENTS (PER ANIMAL/DAY)

Feed ingredients	Treatments	
	I	II
Wheat bran (g)	100	100
Sugar beet molasses (g)	10	200
Cotton seed meal (g)	200	0.00
Urea (g)	0.0	25.0
Vitamin A supplement (g)	1.1	1.1
Vitamin E supplement (g)	7.2	7.2
Vitamin B supplement (g)	2.0	2.0
Nutrients intake		
Feed (g) DM basis	286.0	286.5
ME (MJ)	3.00	3.10
FME (MJ)	2.51	2.92
MP (g)	51.51	51.35
RDP (g)	51.12	85.40
ERDP (g)	45.57	60.00
RUP (g)	31.68	6.60
CP (g)	82.88	92.00
Vitamin A (IU)	5500	5500
Vitamin E (IU)	32	32
Vitamin B_{12} (mg)	0.04	0.04

DM: dry mater, ME: metabolisable energy, FME: fermentable metabolisable energy,
MP: metabolisable protein, RDP: rumen degradable protein,
ERDP: effective rumen degradable protein, RUP: rumen un-degradable protein,
CP: crude protein, IU: international unit.

TABLE II. EFFECT OF THE TREATMENTS ON THE LIVE WEIGHT OF LAMBS AT DIFFERENT TIMES

Live Weight (kg)	Treatments			SEM
	Control	I	II	
Birth	2.87 [b]	3.97 [a]	3.50 [a]	0.091
3 month	15.27 [b]	18.10 [a]	14.20 [b]	0.49
6 month	28.44 [b]	33.70 [a]	27.84 [b]	0.79
Average daily gain (g)				
Birth to 3 month	136 [ab]	157 [a]	119 [b]	0.09
3 month 6 month	135 [b]	173 [a]	149 [b]	0.49
Birth to 6 month	141 [b]	165 [a]	135 [b]	0.79

Means within a row with different superscript letters are significantly different ($P < 0.05$).
SEM = Standard error of means.

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ROLE OF CATTLE AND LOCAL FEED RESOURCES ON THE SUSTAINABILITY OF A COCONUT CATTLE INTEGRATED SYSTEM

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In this paper, results of a two year experiment conducted with cross-bred cattle grazing natural herbage under coconut with the objective of alleviating feed shortage and improving the quality by feeding tree fodder and a low cost concentrate with critical nutrients are discussed.

The experiment was conducted in a coconut plantation at Kotawila, Matara district (WIZ) of the Southern Province of Sri Lanka. There were four treatments, coconut only with out fertilizer (T1); coconut only + fertilizer (recommended levels) (T2); coconut + tethered cross-bred heifers (165 kg ± 25) grazed natural herbage + urea treated straw during dry period (T3); coconut + tethered cross-bred heifers grazed natural herbage +tree fodder (2 kg/d fresh) + concentrate supplement (250 g/d) +urea treated straw during dry season (T4) arranged in a randomized block design with 3 replicates with a stocking rate of 2 heifers / 0.4 ha. The concentrate supplement contained Rice bran 400 g, Molasses 400 g, urea 100 g and minerals 80 g per kg with minimum amount of water to dissolve as a paste.

Herbage dry matter yields in all treatments were positively related to the seasonal rainfall. The highest and lowest average bi-monthly dry matter (DM) yields were 2296 kg/ha/yr for T2 and 1496 kg/ha/yr for T3 respectively. The herbage yields of grazed treatments were marginally sufficient to meet the feed requirements of grazing cattle during the wet season. Botanical composition of herbage increased with grazing due to improved ground cover. In grazing treatments horizontal species such as *Axonopus affinis, A. compressus and Puraria phasiolides* were dominant while vertical species such as *Veronica cinera* and *Lantana camara w*ere dominant in ungrazed plots. The differences in dry matter yield between T3 and T4 increased towards the latter stage of the experimental period, probably due to low grazing pressure by animals in T4 receiving supplementation. Similarly, herbage nitrogen content increased in T4 but decreased in T3 due to overgrazing by animals with out supplementation. Depletion of soil and herbage nitrogen in T3 stimulated conservation of nitrogen through recirculation within the animal. It was also estimated that each coconut palm received 141 kg of fresh dung /year in T3 and 146 kg/year in T4 along with 66.6 kg/urine /year in T3 and 69.6 kg/yr in T4. The dung and urine could totally replace nitrogen and phosphorous fertilizers applied to coconuts. Also it could reduce the potassium fertilizer applied to coconut by 85% in T3 and 88% in T4 and magnesium fertilizer applied by 85% in T3 and 88% by T4.

There was a marked increase ($P < 0.05$) in live weight gains of cattle (Table I) recording 688 g/d for heifers in T4 and 349 g/d for heifers in T3. Heifers fed supplements were in oestrus significantly earlier and at a higher body weight than those fed on natural herbage only. Thus fairly evenly matched initial ages and live weights of T3 (145.5 ± 2.4) and T4 (144.2 ± 2.9) groups, respectively differed significantly in favour of T4 at first oestrus. Heifer fed supplements calved significantly earlier than the heifers fed only natural herbage.

An additional benefit of the integrated system was the improvement ($P < 0.05$) of coconut and copra yield per palm in grazed plots over monoculture plots, especially in T4 plots with animals receiving supplements. Soil nitrogen content also increased ($P < 0.05$) in grazed plots (T3 - 0.964% and T4 -1.004%) plots as compared to monoculture plots (T1-0.839%, T2-0.859%) demonstrating further benefits on cattle integration.

Results suggest that supplementation of tree fodder and low cost concentrate to heifer's grazed natural herbage under coconut alleviated seasonal feed shortages and improved cattle and coconut performance, which contributed to sustainability of the integrated system. Further investigations, would show the actual benefits with the passage of time.

TABLE I. LIVE WEIGHT AND WEIGHT GAIN OF ANIMALS REARED WITH OUT SUPPLEMENTS (T3) AND WITH SUPPLEMENTS (T4) DURING THE GROWTH PHASE. FIGURES ARE FOR ONE YEAR AND MEAN OF 12 ANIMALS.

Live weight	T3	T4	Significance
Initial (kg)	145.5	144.2	$P > 0.05$
Final (kg)	162.9	170.3	$P < 0.05$
Average daily gain (g/d)	349	688	$P < 0.05$
Total yearly gain (kg/ha)	125.6	247.8	$P < 0.05$

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THE EFFECT OF POLYETHYLENE GLYCOL AND WOOD ASH ON THE DETANNIFICATION OF SORGHUM EVALUATED BY AN *IN VITRO* GAS PRODUCTION PROFILE AND ORGANIC MATTER DEGRADATION

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The objective of this work was to evaluate the effect of polyethylene glycol (PEG, MW 4000) and wood ash on the detannification of sorghum grains. In the first experiment, different sorghum genotypes (14) were evaluated using tannin bioassay [1] based on incubation of feeds with and without PEG in a semi-automatic in vitro gas production technique [2]. From this study, genotype 9929030 was selected for detannification because it contained the maximum level of biological active tannins. The results from this experiment indicated the effect of PEG on the reduction of tannin effects; a consequent increase in the volume of gas produced with PEG during the fermentation (Figure 1) indicated reduction of tannin effect by PEG. In addition, the results of the parallelism test demonstrated that the curves were different and not parallel. Organic matter degradability was also higher in presence of PEG (33.4% vs. 24.3%).

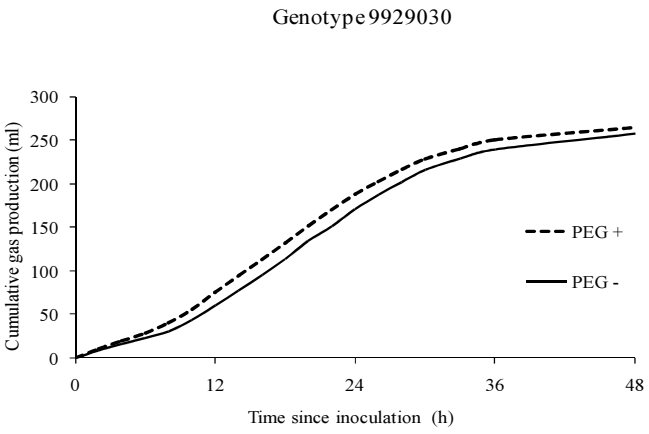

FIG. 1. Gas production profile for genotype 992930, with PEG (PEG +) and without PEG (PEG -)

The second experiment was conducted to evaluate the effect of wood ash as a tannin-neutralizing agent. The wood ash was obtained from the burning of the stems of Eucalyptus sp (T1) and Bauhinia spp (T2), and milled (1 mm). In addition, two methods of adding ash to the substrate (sorghum grain 9929030) were tested. In method one (M1), wood ash was added to milled sorghum grains and placed inside a gas bottle used for the *in vitro* fermentation. In method two (M2), wood ash was mixed with water and whole grains for 3 h, dried and milled (1 mm). In both methods, three concentrations of wood ash/grain were tested, 0 (C1), 100 (C2) and 200 (C3) mg of wood ash / g of substrate. Fermentation was conducted in a semi-automatic *in vitro* gas production technique for up to 96 h [2]. The results demonstrated that wood ash increased gas production volume (Figure 2) and organic matter degradation (Table I) and the effect was concentration dependent. Gas production volume and organic matter degradation were also higher when using wood ash obtained from Bauhinia tree. However, these effects were not observed when method 2 was applied (Table I).

Therefore, the results of this study showed that the use of wood ash, especially from Bauhinia tree specie, increased fermentation and organic matter degradation and may be an option to reduce the effect of tannins in sorghum grains.

However, the use of wood ash should also be tested for tannin-rich legume forages. The use of ash as a mineral source in the nutritional balance of the diet also needs evaluation. In addition, the use of ash should also be tested as a feed supplementation block in place of PEG, generally used to inactivate tannins since wood ash is easily available at almost no cost.

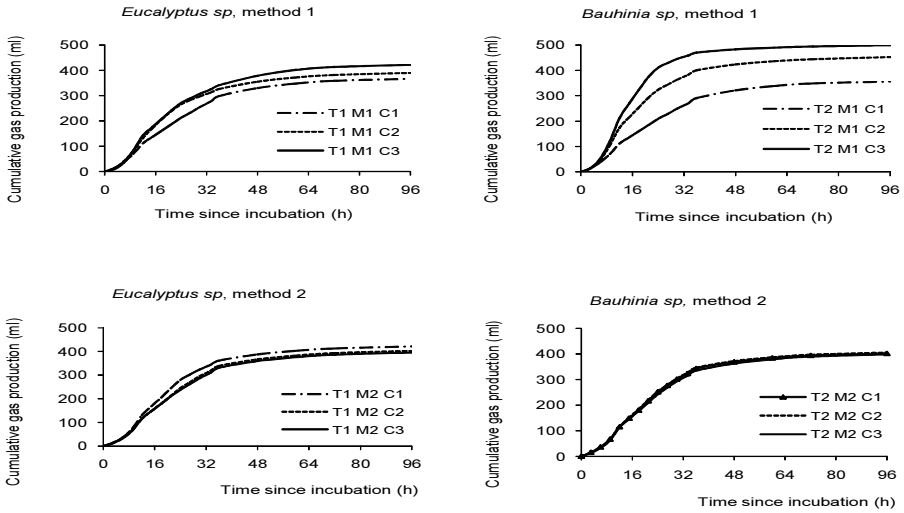

FIG. 2. Gas production profiles

TABLE I. ORGANIC MATTER DEGRADATION (%), 0% (CONTROL), 10%, 20% (100 OR 200 MG OF ASH / G SUBSTRATE RESPECTIVELY), EUCALYPTUS SP (T1) AND BAUHINIA SP (T2)

[redacted]

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IMPACT OF USING SODIUM OR CALCIUM SALTS OF FATTY ACIDS AS SOURCES OF ENERGY IN BUFFALO RATIONS DURING LATE GESTATION

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Added fat is generally favourable especially for late pregnant buffalo to foetal development, mammary adipose tissue and subsequent milk yield. Soapstock is produced from seeds oil refining processes as a by-product potentially harmful to the environment but can use it as dietary fat source. The aim of this study were to study impact of adding either Na-SFA or Ca-SFA as a energy source instead of corn grains in buffalo rations on rumen activities and performance of late pregnant buffalo.

Thirty pregnant buffaloes expected to calve within 60-75 day were divided into three balanced groups. First group received the control ration consisted of concentrate diet (75% concentrate feed mixture with 25% yellow corn) plus berseem hay and rice straw. In the second and third rations, yellow corn was replaced with either Na-SFA or Ca-SFA. Chemical composition of Na-SFA, Ca-SFA and the experimental rations are presented in Tables I and II. Content of AEE in Ca-SFA was lower than that of Na-SFA, while TFA's in Ca-SFA was higher.

Incubation of teased rations in the rumen showed reduction in DM, OM and CP disappearances, also ED and PD with ration containing Na-SFA. Undegradable values increased with adding Na-SFA compared to adding Ca-SFA or control diet. As a result of foaming and physical coating of the fibre with added Na-SFA has been proposed as a possible theory for the sometimes observed depressed DM, OM and CP disappearances [1].

Digestion coefficient of DM, OM, CP and WCS were decreased with feeding ration containing Na-SFA compared to that containing Ca-SFA, while no significant differences were found between ration containing Ca-SFA and control one. These results might be due to the effect of LCFA in Na-SFA, which reflects on rumen fermentation, and consequently affect fibre digestibility [2]. Nutritive values as TDN and DCP were decreased ($P < 0.05$) with the ration contained Na-SFA compared to Ca-SFA. Feed intake was not affected with feed rations containing Na-SFA or Ca-SFA. Body weight was higher with feeding ration containing Ca-SFA or Na-SFA than that of the control. pH values, propionic acid and FFA's in the rumen were significantly ($P < 0.05$) higher when feeding ration containing Na-SFA compared to that containing Ca-SFA or control, while, significantly decreased TVFA's, acetic, Ac/Pr and NH_3-N. These results might be due to release of FFA's in the rumen when feeding Na-SFA decreased both NH_3-N and TVFA's. Fatty acids, especially unsaturated fatty acids, are antimicrobial and interfere with normal function of the ruminal microbes [3]. Adding Na-SFA in the ration decreased glucose and total protein concentration in blood compared to Ca-SFA or control. Concentration of albumin, globulin and their ratio were not affected with feeding rations containing either Na-SFA or Ca-SFA. TL, triglyceride and FFA's were increased ($P < 0.05$) with feeding ration containing fat compare to control.

TABLE I. CHEMICAL COMPOSITION OF NA-SFA AND CA-SFA (% ON DM BASIS)

Item	DM	OM	AEE	TFA's[a]	OL[b]	Ash	CE Mcal/kg
Na-SFA[c]	61.73	92.60	81.00	68.95	12.05	7.40	6.562
Ca-SFA[d]	94.78	81.39	78.22	76.96	1.27	18.61	7.402

[a] Total fatty acids; [b] Other lipids; [c] Sodium salts of fatty acids; [d] Calcium salts of fatty acids

TABLE II. FORMULATION AND CHEMICAL COMPOSITION OF DIFFERENT RATIONS AND ROUGHAGES ON DM BASIS

Item	Control	Na-SFA	Ca-SFA	BH[a]	RS[b]
Chemical composition (%)					
DM	89.98	87.85	90.20	88.00	90.00
OM	87.67	86.93	86.20	86.00	83.50
CP	10.09	9.77	9.91	13.70	3.51
AEE	2.32	7.78	6.96	1.80	1.30
TFA	1.63	6.26	6.22	1.01	0.63
OL	0.69	1.52	0.74	0.79	0.67
Ash	12.33	13.07	13.80	14.00	16.50
Cell wall constituents (%)					
NDF	44.04	43.67	43.82	51.24	68.43
ADF	34.55	34.20	34.32	39.87	54.39
Cellulose	30.96	30.62	30.72	36.75	48.32
Hemicellulose	9.49	9.47	9.51	11.37	14.04
GE (Mcal)	3.884	4.028	4.060	3.856	3.647

[a] Berseem hay; [b] Rice straw.

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ENHANCING OFF-SEASON FERTILITY IN DAIRY BUFFALOES THROUGH MONENSIN SUPPLEMENTATION

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'Sub-oestrus' forms one of the major reproductive disorders in dairy buffaloes, with incidence varying between 15 and 80% [1] causing huge economic losses to the dairy industry. Heat stress and malnutrition have been implicated among the major causative factors. The present study aimed at improving fertility of sub-oestrus dairy buffaloes through a composite application of synchronized breeding and modulation of rumen microbial environment.

Ninety sub-oestrus, pleuriparous, otherwise healthy lactating Murrah buffaloes reared under semi-intensive system formed the subject of this study. They were fed ad libitum seasonal green fodder, supplemented with about 5 kg home made concentrate ration and from 1 to 2 kg wheat straw during August through March and about 15 to 20 kg green fodder supplemented with about 6 kg concentrate ration and 4 kg wheat straw in April through July. Oestrus was detected visually by the farmers. These buffaloes, 60-180 d postpartum, had failed to exhibit overt oestrus and remained un-bred. However, clinical evaluation confirmed them as cycling for having functional corpus luteum, and hence were designated as suffering from 'sub-oestrus'. To manage the menace of sub-oestrus, the buffaloes were subjected to synchronized breeding using 'ovusynch protocol' which included 5 ml Receptal (20 µg Buserelin, Intervet, Holland) on day 0, 2 ml Vetmate (500 µg cloprostenol, Vet Farma, India) on day 7 and 2.5 ml Receptal on day 9, injected intramuscularly. Fixed time artificial insemination was done 16 and 40 hours after the last injection [2]. Some of the buffaloes which returning to oestrus about 15-21 d later was served naturally. The pregnancy was diagnosed 90 d later through rectal palpation of genitalia.

In the first phase, buffaloes were synchronized and bred either in the favourable breeding season (October through March; n = 40,) or in the unfavourable season (April through July; n = 40). The first service and the overall conception rates were 30 and 37.5 % as compared 55 and 65 %, respectively. Assuming that poor quality and low quantity of the fodder availability could be a major limiting factor to optimal fertility in the unfavourable period, the second phase of the study incorporated oral supplementation of 200 mg monensin (an ionophore) per head per day (n = 10) for a period of 30 d before and during the application of synchronized breeding. The first service and the overall conception rates increased substantially to 50 and 80%, respectively. Average follicular diameter 36 hours after the cloprostenol injection in the buffaloes that conceived (n = 5) or remained un-conceived (n = 5) were similar (9.99 ± 0.33 vs. 8.45 ± 0.56 mm; $P > 0.05$). Supplementation of monensin did not affect ($P > 0.05$) the quality of the milk produced in terms of concentration of milk urea nitrogen (10.67 ± 0.49 Vs 12.16 ± 0.61 mg/dl), fat (8.84 ± 0.46 vs. 7.83 ± 0.58%), solid not fat (9.83 ± 0.18 Vs 10.02 ± 0.20%), density (31.14 ± 0.69 Vs 32.79 ± 0.74%), protein (4.67 ± 0.08 vs. 4.76 ± 0.09%) and lactose (4.42 ± 0.08 vs. 4.51 ±0 .09%). It is concluded that supplementary feeding of monensin substantially improved fertility in sub-oestrus buffaloes following their management through ovusynch synchronization and fixed time insemination during the unfavourable breeding months.

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THE PHYSIOLOGICAL MECHANISM OF LOW PURINE DERIVATIVE EXCRETION IN URINE OF BUFFALOES

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Three cattle calves (Bos Taurus) and three buffalo calves (Bos bubalus) were weaned after receiving colostrum and reared by bottle-feeding of milk. During the first month the animal did not have access to solid food. Urinary purine derivative (PD), concentration, basal PD excretion and glomerular filtrate rate (GFR) were determined during fasting and feeding. After one month the animals were given access to solid feed (urea-treated rice straw 80% and molasses 20%) to stimulate rumen development [2]. At three months of age, while the solid food was given, urinary PD, basal PD excretion and GFR were again determined.

Urinary PD excretion both during fasting and milk feeding did not differ significantly between buffaloes to cattle during the period of milk feeding ($P > 0.05$), but there were highly significant differences between cattle and buffaloes after 3 months of age and two months of access to solid feed ($P < 0.01$) [3]. The GFR was lower in buffaloes than cattle on both milk fed and solid feed periods (Table I).

It is suggested that the lower GFR found in buffaloes may be the reason for the differences as PD stay longer in the blood to give more time for recycling to the rumen when the rumen is developed and are then metabolized by bacteria. Whether permeability of PD from blood to rumen is an additional factor is not known [1, 3].

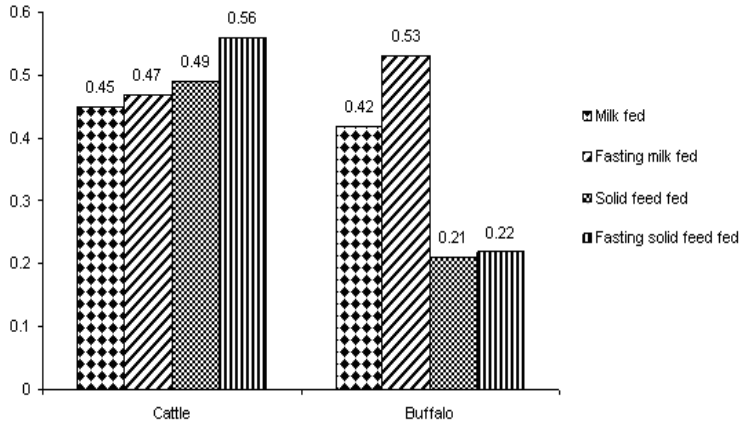

FIG 1. PD excretion from cattle and buffaloes

TABLE 1. GFR OF BUFFALO AND CATTLE IN BEFORE AND AFTER RUMEN DEVELOPMENT

	Cattle	Buffalo	SEM
Before rumen development (lít/KgW$^{0.75}$/d)	3.2	2.3**	0.1
Rumen development (lít/KgW$^{0.75}$/d)	2.1	1.6***	0.1

** $P < 0.01$; ***$P < 0.001$

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CHANGES IN THE CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION OF MAIZE STOVER DEGRADED WITH TWO EDIBLE MUSHROOMS: *PLEUROTUS SAJOR CAJU* AND *PLEUROTUS PULMONARIUS*

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Maize Stover was degraded using two white rot fungi: *Pleurotus sajor caju* and *Pleurotus pulmonarius,* in a solid-state fermentation. In vitro gas production of the resulting substrates was carried out in 24hr incubation [1] and, the metabolisable energy, organic matter digestibility (OMD) and short chain fatty acid were predicted. The chemical composition and the crude fibre fractions were also determined [2].

The result of the experiment shows that the crude protein (CP) increased from 3.6% in the control (UM) to 12.17% for *Pleurotus sajor caju* (PSM) and 19.63% for *Pleurotus pulmonarius* (PPM).This agrees with the report of Farkas [3]. The crude fibre (CF) decreased ($P < 0.05$) from 31.84% in UM to 18.14% and 25.24% in PSM and PPM respectively. The neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemi-cellulose also followed the same trend. The final gas produced (a+ b) ml, were not different ($P > 0.05$) while the rate of gas production (c h $^{-1}$) was highest in the control (UM) at 0.31ml/200mg DM. Short chain fatty acid and metabolisable energy were not different ($P > 0.05$). However, organic matter digestibility increased ($P < 0.05$) from 28.32% (UM) to 34.68% and 37.35% in PSM and PPM respectively. The methane (CH_4) produced also differed significantly.

The outcome of this study shows that fungal degradation of maize stover enhanced the crude protein contents while fibre fractions were reduced, this suggest it that it can be used as protein supplement in the diet of ruminants. Further in vivo work is warranted to confirm this.

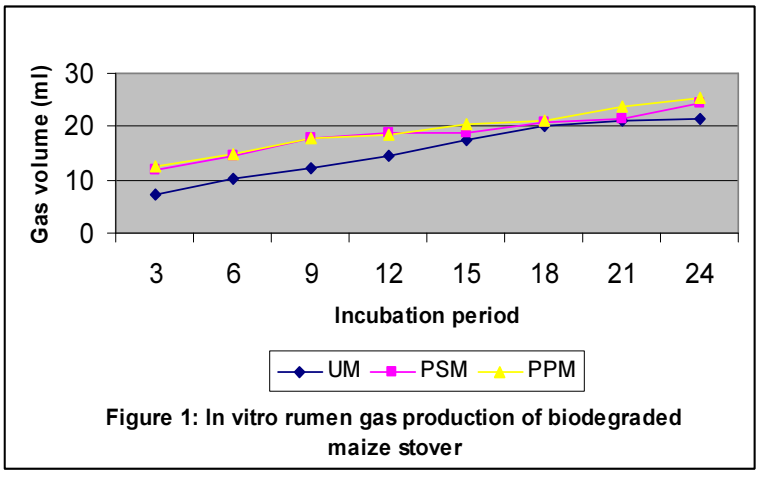

Figure 1: In vitro rumen gas production of biodegraded maize stover

TABLE I. PROXIMATE COMPOSITION (G/100G DM) OF BIODEGRADED MAIZE STOVER

	UM	PSM	PPM	SEM
Dry Matter	88.75[a]	86.47[c]	87.47[b]	± 0.00
Crude Protein	3.69[c]	12.17[b]	19.63[a]	± 0.03
Crude fibre	31.84[a]	18.14[c]	25.24[b]	± 0.00
Ether extract	0.82[c]	1.67[b]	1.82[a]	± 0.00
Ash	9.47[c]	9.63[b]	10.29[a]	± 0.02
Nitrogen free extract	54.16[a]	55.35[c]	43.32[b]	± 1.71
ME (MJ/kg DM)	4655.0[a]	3739.21[c]	4060.82[b]	± 1.24

[a,b,c] means on the same column with different superscripts are significantly varied ($P < 0.05$), UM = undegraded maize stover (control), PPM = *Pleurotus plumonarius* degraded maize straw, PSM = *Pleurotus sajor caju* degraded maize straw

TABLE II. CRUDE FIBRE FRACTION (G/100G DM) OF BIODEGRADED MAIZE STOVER

	UM	PSM	PPM	SEM
NDF	67.55[a]	62.52[c]	63.73[b]	± 0.00
ADF	46.53[c]	41.77[b]	44.26[a]	± 0.03
ADL	13.62[a]	11.54[c]	12.63[b]	± 0.00
Cellulose	32.91[c]	30.23[b]	31.63[a]	± 0.00
Hemicellulose	21.32[a]	20.75[c]	19.51[b]	± 0.01

[a,b,c] means on the same column with different superscripts are significantly varied ($P < 0.05$), UM = undegraded maize stover (control), PPM = *Pleurotus plumonarius* degraded maize straw, PSM = *Pleurotus sajor caju* degraded maize stover, NDF =neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin.

TABLE III. SHORT CHAIN FATTY ACID (MOL) ORGANIC MATTER DIGESTIBILITY (%), AND METABOLISABLE ENERGY (MJ/KG DM) OF TREATED MAIZE STOVER

	UM	PSM	PPM	SEM
SCFA	0.34	0.42	0.45	± 0.04
OMD	28.31[b]	34.68[a]	37.35[b]	± 0.42
ME	3.87	4.61	4.56	± 0.48

[a, b] means on the same column with different superscripts are significantly varied ($P < 0.05$), UM = undegraded maize stover (control), PPM = *Pleurotus plumonarius* degraded maize straw, PSM = *Pleurotus sajor caju* degraded maize stover, SCFA=short chain fatty acid, OMD=organic matter digestibility, ME = metabolisable energy.

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PRODUCTIVE AND REPRODUCTIVE EFFICIENCY OF DIFFERENT GENOTYPES OF GOAT IN BANGLADESH INFLUENCED BY NUTRITIONAL STATUS

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This experiment is being conducted in flood fed area in the western part and hilly area in the eastern part of Bangladesh covering rural and urban areas with Black Bengal and crossbred (Black Bengal X Shirohi) goats to find out the effect of nutrition on growth and reproductive performances.

Pure Black Bengal goats and crossbred goats were selected from the rural area of two regions. Purity of the goats was ascertained through history of the animals as well as microsatellites test [1]. Black Bengal goats were reared under normal grazing condition without any supplementation in both the regions. Crossbred goats were maintained in the urban area of the western part and kept on grazing as well as supplementation (green leaves and concentrates). All the experimental goats were dewormed and vaccinated against PPR regularly. The experiment started in January 2007 and still is continuing. Litter size, kidding interval, birth weight, weaning weight and yearling weight are the parameters that are being recoded. The results presented here represent the data from January 2007 to August 2008. The birth weight, weaning weight, yearling weight, litter size and kidding interval for different genotypes in different regions have been presented in Table1.

TABLE1. GROWTH AND REPRODUCTIVE PERFORMANCES OF BLACK BENGAL AND CROSSBRED GOATS

Parameter studied	Black Bengal goat		Crossbred goat
	Flood fed rural area	Hilly rural Area	Flood fed urban area
Birth weight (kg)	1.20 ± 0.40 (n = 31)	1.37 ± 0.36 (n = 40)	2.57 ± 0.62 (n = 28)
Weaning weight (kg)	4.94 ± 0.48[c] (n = 14)	8.40 ± 1.30[b] (n = 15)	12.46 ± 3.16[a] (n = 21)
Yearling weight (kg)	14.58 ± 1.57[c]	21.40 ± 4.23[b]	29.72 ± 4.49[a]
Litter size	1.78 + 0.56 (n = 19)	1.91 + 0.70 (n = 45)	2.27 ± 0.79 (n = 11)
Kidding interval (d)	198.25 ± 34.11 (n = 12)	192.90 ± 37.60 (n = 20)	213.90 ± (n = 14)

Within the Black Bengal goats, birth weight, weaning weight and yearling weight were more in group maintained in hilly area than those reared in flood fed area. There was no significant difference of birth weight between two groups; however weaning weight and yearling weight of goats reared in hilly region were significantly higher ($P < 0.01$) than those of goats reared in flood fed area. Reproductive parameters like litter size and kidding interval performances were also better in goats reared in hilly area. The flood fed area is inundated by floodwater every year; paddy is cultivated throughout the year and has very limited grazing land with high human population density. Animals in this area always suffer from malnutrition due to lack of sufficient forages. The hilly area remains free of flood, covered by dense forest and has a vast grazing land with low human population density. Animals in this area get sufficient forage naturally. The better performances in growth and reproduction in goats reared in hilly area is certainly for better

nutrition that occurs naturally. On the hand, crossbred maintained urban area had better performances over Black Bengal goats. This may be for heterosis effect as well as supplementation of feeds.

The preliminary study of this experiment indicates that nutrition as well as genotypes has significant influence in the production and reproduction performances of goats in Bangladesh. In fact, this is the first work on Black Bengal goats in the hilly area and crossbred goats of Bangladesh. Further experiment has been planned for confirming this fact.

Reference:

[1] Progress report of CRP 13005 on "Phylogenetic studies of Bangladeshi goats (Capra hircus) based on morphological variation, blood protein and DNA (microsatellite) polymorphisms

THE CORRELATION BETWEEN MILK AND BLOOD UREA NITROGEN IN HIGH AND LOW YIELDING DAIRY COWS

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Excess rumen degradable protein in the diet leads to high ammonia production, which elevates rumen pH and rate of ammonia absorption, raising urea-nitrogen concentration in blood. Urea, which is a normal constituent of milk, diffuses quickly from blood into milk [1]. An urea-nitrogen concentration higher than 20 mg/dL of milk suggests excess of protein supply in the diet, which can decrease production, cause fertility problems [2], and increase nitrogen excretion into the environment [3]. The measurement of urea nitrogen in blood and milk has been proposed as a tool to monitor protein nutrition [4].

A study was carried out in two dairy farms (Olocuilta and Los Conacastes) in the central region of El Salvador. Sixty Holstein cows were grouped according to milk yield and days in milk: high yielding (HY, 30 to 90 d in milk) and low yielding cows (LY, >180 d in milk). The objective of the study was to evaluate the effect of the milk yield and time after feeding on milk and blood urea-nitrogen concentration, and to establish a correlation between these two parameters. Dietary crude protein was analyzed and a protein balance was done for each group using the NRC (2001) Dairy Cattle Program. Blood and milk samples were taken after feeding at 30 min, 1, 2 and 4 hours. Blood samples were centrifuged and sera were then stored at -20°C until analyzed. Blood urea-nitrogen (BUN) concentration was determined using a commercially available kit (HUMAN®, Wiesbaden, Germany). Milk urea-nitrogen (MUN) was also determined with the use of a spectofotometric procedure (MERCK®, Damstad, Germany). Blood and milk urea-nitrogen concentrations were analyzed using repeated measures analysis and the MIXED procedure of SAS. To determine the correlation between BUN and MUN, the GLM procedure of SAS was used (SAS Institute, Version 9.1.3, 2006).

In Olocuilta HY cows presented the highest BUN and MUN concentrations. BUN least squares concentration was 12.77 mg/dL and 13.98 mg/dL for the LY and HY cows, respectively; while MUN average concentration was 12.30 mg/dL and 14.82 mg/dL for the LY and HY cows, respectively, (Figure 1). BUN and MUN concentrations were similar at 30 min, one and two hours post feeding but at four hours post feeding BUN concentration decreased and was significantly lower than that of MUN ($P < 0.05$).

On the other hand, in Conacastes the highest values were found for the LY group. BUN least square concentration was 11.22 mg/dL and 9.12 mg/dL for the LY and HY cows, respectively; while MUN average concentration was 10.18 mg/dL and 8.83 mg/dL for the LY and HY cows, respectively. The reason for these different results seems to be related protein balance. For instance in Los Conacastes farm, protein balance was negative in the HY group (-88 gr/d) while in Oloculita farm the balance was positive (Table I).

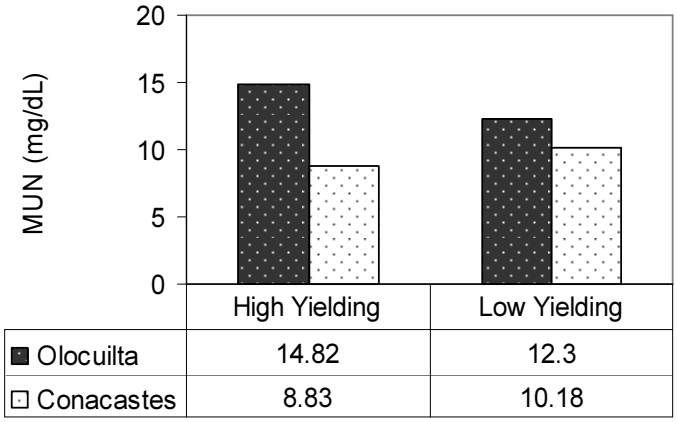

FIG 1. Milk urea nitrogen (mg/dL) for high and low yielding dairy cows on two different farms.

TABLE I. MILK PRODUCTION, NUTRITIONAL COMPOSITION OF THE DIET AND PROTEIN BALANCE TWO DIFFERENT FARMS

	Low yielding group		High yielding group	
	Olocuilta	Los Conacastes	Olocuilta	Los Conacastes
Milk yield, kg/d	16.50	11.40	22.25	19.00
NDF (% DM)	42.30	42.20	41.40	44.30
NE_l (Mcal/kg DM)	1.48	1.55	1.47	1.57
Crude protein (% DM)	15.90	13.80	16.40	13.70
Crude protein balance (g/d)	428	401	537	- 88

The correlation between BUN and MUN for Olocuilta farm had regression coefficient of 0.84, and correlation (r^2) of 0.7543. For Los Conacastes they were of 1.04 and 0.9017, respectively. It should be noticed that the BUN and MUN are correlated better at the 30 minutes, one hour and two hours post feeding and the correlation decreased at the four hours post feeding because a drop in BUN concentration.

Based on the current study, it can be concluded that BUN and MUN concentrations are not related directly with milk yield but with the protein balance. There is a high correlation between BUN and MUN concentration; hence, any of these parameters can be used to monitor protein nutrition in dairy farms.

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IMPLICIT PRICES OF TRAITS OF INDIGENOUS CATTLE – IMPLICATIONS FOR SUSTAINABLE LIVESTOCK PRODUCTION

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Both revealed and stated preference approaches were employed to determine the values attached to the different features of indigenous cattle in central Ethiopia. For the revealed preference analysis a hedonic model was employed to examine the determinants of cattle prices in the primary rural markets of central Ethiopia. Transaction level data of cattle farmers and farmer-traders were used in the analyses. Data collected in rural markets to identify cattle price determinants result in estimates with standard errors that are mostly heteroscedastic. We employed SHM estimations to account for heteroscedastic errors.

The empirical estimation of revealed preferences showed that market place, seasonal differences, sex and function based classification of cattle, body size, and age were very important factors influencing the market prices cattle sellers receive. The significance of the characteristics of animals in influencing prices paid for the animals reveals the importance of the preferences for traits in the decision-making process related to buying and selling of cattle. These preferences at the farmers and farmer-traders levels are the ones that matter most in shaping up the diversity of animals kept at farm level. This diversity of the cattle genetic resources is essential for generating or identifying best-suited breeds of cattle in the context of the livelihood objectives of the target community. Thus, the cattle breeding strategies and activities should duly consider the preferences expressed through the prices paid for animals in such markets, where the cattle keepers are the main sellers and buyers.

For the stated preference analysis the study employed choice experiments (CE) and random parameters logit to elicit and analyze cattle trait preferences of buyers in the semi-subsistence livelihood systems of rural central Ethiopia. The results of the cows CE revealed that in areas where livestock serve multitude of purposes and where the production and marketing system is semi-subsistence, cows have other functions more important than milk production. Fertility, disease resistance and strength of the calves they bear are as much or more important than milk. The breed concept, which is very much associated in Ethiopia with the area where the animal is brought from, was found to be less important as such and it appears that farmers are interested in obtaining animals from the district or locations in which they live in. This is essentially because cattle buyers, who are mostly farmers, are more concerned about adaptability and therefore give high value to the fact that they know the pedigree of the cattle they buy.

The results of the CE for bulls indicate that cattle buyers assign high values for good traction potential, disease resistance, calf vigour, and for places of origin when choosing bulls in the market. The preferences cattle buyers have for these attributes do vary essentially due to differences in occupation, education and age. The primary objective of the rural community to produce sufficient food for the family for each year was manifested through the value assigned to traction potential which is more than twice that of disease resistance. These results are consistent with the basic reasons why animals are kept in the area, but appear to be incoherent with the government funded interventions of livestock development. Given the importance of livestock, bulls in particular, for the livelihoods of the communities in rural Ethiopia, such consistent valuation of the traits show that the objectives of the agrarian life are quite clear among the community – farmers, farmer traders, traders, and others – that production and marketing decisions are made on broader considerations than just milk and meat production.

The government of Ethiopia needs to revise the structure of the livestock improvement programs still running and needs to make note of the important details that influence the production, marketing and utilization of livestock products. The smallholder community in this part of Ethiopia depends on semi subsistence agriculture and so livestock development interventions should focus on reproductive and adaptive traits that stabilize the herd structure, rather than focusing on traits that are only important for commercial purposes. It can also be observed that improving these traits of cattle owned by smallholder farmers in the area will facilitate adoption of the innovations or improvements instead of bringing over cattle from unknown sources and obviously with low adaptability.

SUSTAINABLE MILK PRODUCTION IN MAURITIUS: NEW STRATEGIC DIRECTIONS TO REVAMP THE DAIRY SECTOR

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Mauritius is totally dependent on importation for its milk and milk products. The present local milk production amounts to 4.0M litres of milk annually (400 MT dry equivalent) which represent only 2% of the total local requirements for milk (21, 700MT). Milk and milk products imports cost the country around Rs 2 billion (1 U$ ~ Rs30) annually. The recent successive increases in prices of imported milk have impacted negatively on the food import bills and the retail price of powdered milk has also increased drastically. The dairy sector in Mauritius is mainly characterised by backyard producers operating on a low input- low output system (1). Over the years, the number of cattle and farmers has declined steadily from 9600 in 2000 to 5800 head in 2006 while the number of farmers has also declined from 2500 to 1700 for the same period. In the past, a wide range of initiatives has been taken to develop the domestic milk sector, but milk production has not increased.

Under the present circumstances, it is imperative to revamp the local milk production sector. In this context, the Government has drafted developmental policies for boosting the milk sector. However, such policy needs to be implemented through a coherent framework of actions. This study attempts to firstly to gain a better understanding of the existing potentials and constraints of the smallholder dairy industry and secondly to propose an approach for successful implementation of the policies.

In an extensive questionnaire based survey with the dairy producers and interviews with key informants in the sector, the main factors identified for the decline of the dairy herd population and productivity are increasing cost of production, ageing of cow breeders, better job opportunities offered to the younger and educated generation in other sectors of the economy, distorted economic policy, poor genetic potential of the herd, limited land availability for cultivating pasture, seasonal scarcity of fodder and grass, low quality of concentrates, inefficient marketing facilities scheme and reproductive problems mainly due to inefficient artificial insemination service. The survey has also shown that the dairy producers are socially, economically and technically diverse. A new category of dairy producers is now investing in medium and large sized scale dairying production systems. One of the medium sized farms is now producing about 1000 litres of milk daily.

A holistic approach is being proposed to increase milk production over the next 10 years. The 3 main strategies being proposed are setting up and operation of 10-20 dairy units, equipped with appropriate housing systems and modern equipment, scattered around the island. These dairy units will be rented to selected farmers who have proven experience in dairy farming and those who have followed training courses in milk production. The second one proposes to restructure the existing medium sized dairy farms such as dairy cooperatives to increase their efficiency and effectiveness of producing milk of good quality. Finally, the last strategy is to build large-scale farms on land that would be released following the restructuring of the sugar cane industry.

The successful implementation of the strategies hinges on the following approaches; supply of dairy cattle stock of good genetic potential adaptable to the local conditions, development of appropriate infrastructure and logistics (e.g., housing), ensuring a regular supply of fodder and concentrates feeds, provision of more veterinary support to the dairy farmers, adoption of sound reproductive management of the herd [1], improvement of the technical education of farmers and farm staff through training, and the development of an organized system of milk collection and marketing schemes [2].

It is postulated that the adoption of these strategies will benefit the local dairy industry through increased herd size, and increased availability of fresh quality milk and the development of sustainable and environmental friendly dairy enterprises.

The data generated from the survey has been fitted into a model to estimate the total acreage of land, number of animals, total feed requirements and herd progression over the years for the implementation of the proposals. It is estimated that milk production can be increased to about 22 million litres of milk by 2015, which would represent a 10% self-sufficiency ratio in fresh milk.

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RADIOIMMUNOASSAY (RIA), RADIORECEPTORASSAY (RRA) AND ATOMIC ABSORPTION SPECTROSCOPY (AAS) APPLIED TO STUDIES ON ANIMAL NUTRITION AND HEALTH

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In 1990, our group began working in the development of a sensitive method to measure the active principle (1,25dihydroxy-vitamin D_3-glycoside) of *Solanum glaucophyllum*, a plant that grows wild in our country causing calcinosis of breeding cattle. RIA and RRA have been applied to determine this glycoside in the aqueous extracts of the plant leaves and the free vitamin D metabolite in animal plasma samples, respectively (see Table I and Figure 1). AAS was also used to determine calcium, together with phosphorus determined by colorimetric methods, in blood and tissues of experimental animals in order to study the relationship between the active principle kinetics and its effects [2, 3, 4].

TABLE I. PLASMATIC 1α,25$(OH)_2$VITAMIN D IN RABBITS TREATED BY ORAL OR SUBCUTANEOUS ROUTES WITH AQUEOUS EXTRACTS OF *S. GLAUCOPHYLLUM*. BLOOD SAMPLES WERE TAKEN 24 HOURS AFTER THE LAST ADMINISTRATION AND PLASMA SAMPLES WERE ANALYZED BY RRA AFTER SOLID PHASE EXTRACTION OF ITS LIPID EXTRACT.

DOSES	ANIMAL (Nº)	[1,25]D (pg/ml)	Mean ± SD
17 x 100 mg//kg BW *per os*	1	347	277± 100
	5	206	
17 x 100 mg/kg BW *sc*	3	169	260 ± 129
	4	351	
Control	6	25	15 ± 14
	2	5	

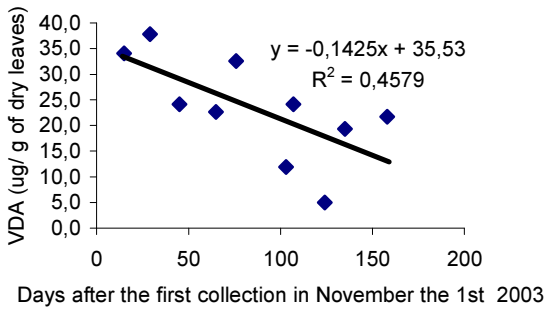

Fig 1. Time evolution of vitamin D activity (VDA) in two natural stands of S. galucophyllum measured by RIA on the aqueous extract of dry leaves, collected from November 2003 to may 2004, in Buenos Aires province, Argentina.

More recently, this plant has been proposed as a source of vitamin D activity (VDA) that might contribute with environment care improving calcium and phosphorus utilization by animals [1]. Our group is by now, as a first step, studying the effects of different diet levels of calcium (Ca) and phosphorus (P) [covering the range between commercial recommendations and half of NRC requirements (1994)], as well as different sources of those minerals, upon productive, nutritional, skeletal and biochemical parameters, in a series of experiments covering either a part or the entire breeding cycle of broilers. We think that the high levels of vitamin D_3 employed in commercial farms (4 times NRC recommendations) could enable birds fed on basal diets to enhance the synthesis of the active metabolite of the vitamin in order to overcome partially these minerals deficiency (see Figure 2).

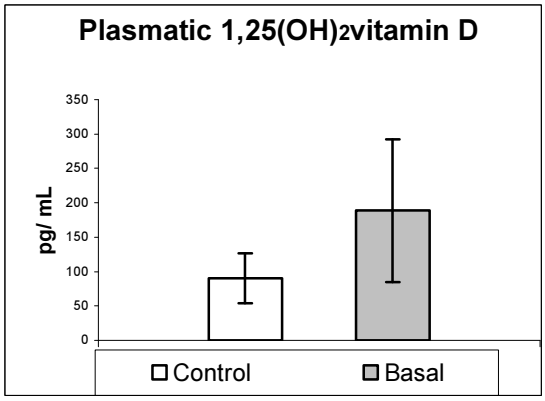

FIG 2. Broilers Cobb 500 of 21 d of age. Control: Ca= 0.95% and available P= 0.45% in feed; Basal: Ca= 0.77% and available P= 0.35% in feed. The metabolite was measured by RRA in the lipid plasma extract purified by solid phase extraction.

These methods of analysis have been applied successfully in our research projects contributing to the improvement of animal health and production and our approach has been considered adequate for the study of this additive and therefore has been required by the private industry of foreign countries.

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EFFECTS OF NUTRITIONAL SUPPLEMENTATION AND EXPOSURE TO BULLS ON RESUMPTION OF POST-PARTUM OVARIAN ACTIVITY IN BUNAJI (*BOS INDICUS*) CATTLE

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Over 90% of the population of zebu cattle in Nigeria belong to smallholder agro-pastoral farmers who adopt relatively inefficient production systems (Otchere and Nuru, 1988). Shortage of feed and poor quality of tropical pastures are a major constraint to livestock development, and this is often characterized by extended post-partum anoestrous periods. Biostimulation via pheromones can play an important role in mammalian behaviour and reproductive process (Rekwot et al., 2000).

A total of 51 pleuriparous post-partum cows belonging to agro pastoralists were involved in a 2 x 2 factorial experiment for a period of 180 d. The cows were assigned randomly at calving to four treatments: (1) grazing only (no supplementation) and exposure to bull (NSBE); (2) grazing only (no supplementation) and no exposure to bull (NSNE); (3) grazing plus feed supplementation (each cow received 600 g of 20.8% crude protein of whole cottonseed supplement per day) and exposure to bull (FSBE); (4) grazing plus feed supplementation and no exposure to bull (FSNE). Post-partum cows were observed visually for the standard signs of oestrus (standing to be mounted) twice daily between 07:30 and 08:30h and 17:30 and 18:30h by experienced herdsmen and technicians. Cows in oestrus in the non-exposed group were taken to the bull for 24h for mating. Once weekly, whole milk samples were taken from cows 7-180 d post-partum for determination of progesterone concentrations using RIA kits. Cows with an increase in milk progesterone (P_4) concentration of <1 ng/mL from the weekly milk samples were used to analyse the number of days from calving to the time of resumption of ovarian activity.

The interval from calving to resumption of post-partum ovarian activity for the feed supplemented (FS) cows was 107 d; earlier than the 136 d for the non-supplemented (NS) cows ($P < 0.05$; Table I). Similarly, cows exposed to bulls (BE) resumed post-partum ovarian activity earlier than the non-exposed (NE) cows with a difference of 16 d. Average daily gains for the FS cows were significantly higher than the NS cows. Resumption of ovarian activity for FSBE cows was 95 d, earlier than the 119 d for the FSNE cows ($P < 0.05$; Table I). The intervals to onset of post-partum ovarian activity were 24, 33 and 39 d significantly earlier in the FSBE cows than the FSNE, NSBE and NSNE cows ($P < 0.05$; Table I). The intervals to resumption of cyclic ovarian activity were 9 and 15 d earlier in the FSNE cows than the NSBE and NSNE cows ($P< 0.05$; Table I).

At 120 d post-partum, the proportion of cows that had resumed ovarian activity was 75% for the FSBE cows, which was higher than the values of 42%, 23% and 33% for the NSBE, NSNE and FSNE cows, respectively ($P < 0.05$; Table II). In addition, by 120 d, the proportion of cows with ovarian cyclicity for NSBE cows was 42%; higher than the value of 23% for the NSNE cows ($P < 0.05$). By 150 d post-partum, 100% and 92% of the cows in the supplemented groups (FSBE and FSNE) had resumed cyclic ovarian activity compared with 75% and 69% for the unsupplemented cows (NSBE and NSNE; $P < 0.05$; Table II).

This study indicates that supplementation and exposure to bulls can shorten the length of post-partum anoestrus. This agrees with previous studies which have reported that (Butler and Smith, 1989) and exposure of post-partum cows to bulls (Bolanos et al., 1997) hastened the onset of ovarian cyclicity after calving. The economic benefits of using exposure to bulls and

supplementation to enhance early resumption of post-partum ovarian activity of cattle may serve as a management tool in livestock production.

TABLE I: INTERVALS FROM CALVING TO ONSET OF OVARIAN CYCLICITY AND AVERAGE DAILY GAINS OF POST-PARTUM COWS

Treatment groups	Number of cows	Postpartum interval to onset of ovarian cyclicity (d)	ADG (kg)
Exposure and supplementation	12	128 ± 6^{c}	$0.32 \pm .01^{a}$
No supplementation and bull exposure	13	134 ± 7^{c}	0.34 ± 0.04^{a}
No supplementation and no exposure	14	95 ± 4^{a}	0.45 ± 0.02^{b}
Feed supplementation and bull exposure	12	119 ± 8^{b}	0.42 ± 0.03^{b}
Exposure			
Bull exposure	26	111 ± 5^{a}	0.39 ± 0.02^{a}
No exposure	25	127 ± 4^{b}	0.38 ± 0.02^{a}
Supplementation			
Feed supplementation	26	107 ± 5^{a}	0.54 ± 0.05^{b}
No supplementation	25	136 ± 8^{b}	0.31 ± 0.01^{a}

[a.b.c] Data in columns within treatments with different letter superscripts are significantly different ($P < 0.05$).

TABLE II. PROPORTION OF COWS EXHIBITING OVARIAN ACTIVITY BY 120, 150 OR 180 DAYS POST-PARTUM

Treatment groups	Number of cows	Days Post-partum		
		90-20	121-150	151-180
No supplementation and bull exposure	12	$42(5)^{b}$	$75(9)^{a}$	100(12)
No supplementation and no exposure	13	$23(3)^{a}$	$69(9)^{a}$	100(13)
Feed supplementation and bull exposure	14	$75(11)^{c}$	$100(14)^{b}$	-
Feed supplementation and no exposure	12	$33(4)^{ab}$	$92(11)^{b}$	100(12)

[a.b.c] Data (%) in columns within treatments with different letter superscripts are significantly different ($P < 0.05$). Figures in parentheses are number of animals

AFLATOXINS AS A CAUSE OF HIGH MORTALITY RATE IN FARMED TROUT IN A PERUVIAN RURAL TOWN

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Between 2002 and 2003, an outbreak of a trout's mass death occurred at the intensive fish culture at a Peruvian rural town (Marcara, Huaraz, Peru) where 15,000 from 20,000 fish died. Our objective in the present study was to investigate the high mortality of the trout biomass occurred in period of two months.

This study was conducted after the peak of the outbreak has occurred. We collected samples of fishes, water and fish foodstuff which were examined for aflatoxin, metals, toxics and bacteria. We interviewed people who administered the feed pellet.

Feed sample preparation, transport and storage. The processing of fish feed was at room temperature which was below 16 °C. Once prepared the diet it was keep under an appropriate room for a few days before sending to Marcara town.

Fishes. 20,000 immature trout larval of rainbow trout *(Oncorhynchus mykiss)* was acquired from an official Peruvian fish culture. The fishes were fed twice a day. Adjusted of feed ration was based from the monthly sample weight.

Pellet sample analysis. The samples were analyzed for aflatoxin Bl (AFB1) according to the method previously published (I). The sensitivity is 0.1 ug per 1 kg of sample.

During the fish development until the peak of the outbreak, the foodstuff to fishes was maintained in plastic bags. At this time the storage room temperature was 18-20 ° C between 1.00-2.00 P.M. and the humidity rose close to 90 % at the Marcara facilities.

Mortality development and Effect on survival. The fishes maintained in 4 pods had a normal surviving until end of November, less than 10 specimen dead by month. The fish outbreaks started the first week of December and continuing until the fourth week of January totalizing 15,000 dead fishes from 20,000. See Figure I. The survival of the fish at the first month was less than 50 %. The mortality continues throughout January totalizing 15,000 dead fishes and leaving only 25 % survival.
Laboratory data. The collected samples for analysis were frozen and transported in dry ice to the analysis laboratory. We took the samples on January 23 and it was analyzed on January 25. Aflatoxin Bl was detected in three samples of fish muscle and in the 3 samples of fish feed but it was negative in the 3 water samples. See Table I. The AFB1 concentration was 10 times in the fish feed than in the fish muscle.

In spite of heavy metal residues (lead, mercury and arsenic) were found in the fish samples, those concentrations were below the permissible levels. Volatile toxic residues were negative in water, fish and feed. Only the fish feed samples were contaminated by bacteria (Staphylococcus aureaus).

Under favourable conditions of temperature and humidity, the Aspergillus flavus grows on certain foods and feeds, resulting in the production of aflatoxin Bl (2). For the trout, the highest admissible amount of AFBI in feed is 0.1 ug per kg (3). The data showed suggest that an improper handling of fish foodstuff (18-20 ° C and 90 % humidity) was the cause growing of mould and/or spores and consequently it produced an increased concentration of AFBI in fish feed.

Liver is strategically located between intestinal tract and general circulation. As AFBI concentration ranged in liver between 10 and 100 ppb, this level is capable to produce an acute hepatotoxicity in the fish stocks.

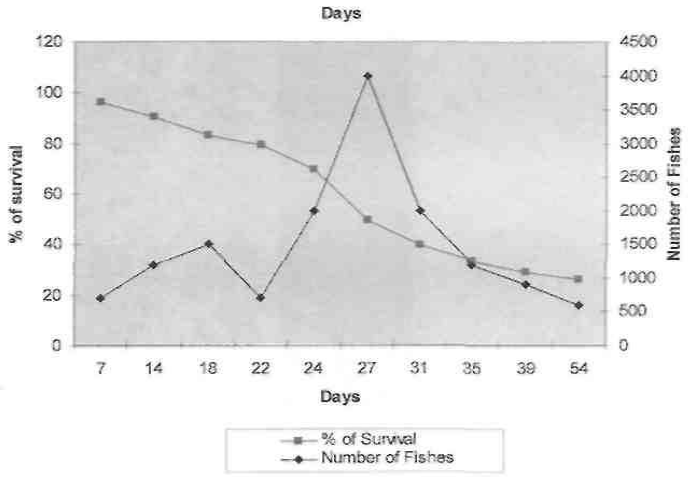

FIG 1. Mortality rate and survival of fish feed by AFBl-contaminated foodstuff

TABLE I. AVERAGES OF AFLATOXIN BI LEVELS IN POD WATER, FISH AND PREPARED DIETS

Samples	Aflatoxin BI (ug/ Kg)
Pod water	Negative
Fish muscle	10
Fish feedstuff	100

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METHANE EMISSIONS FROM ENTERIC FERMENTATION OF REPRESENTATIVE DAIRIES IN PERU: NUTRITIONAL STRATEGIES TO REDUCE THE EMISSIONS

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Estimates of methane emissions from enteric fermentation of cattle in five typical locations of Peru and nutritional strategies to reduce those emissions are presented. The system in the coast considers animals in free-stall and in the highland cattle graze on pastures. Methodology applied was the Tier-2, IPCC [1]. Cows on both grazing systems produce more methane per unit of milk and less methane per animal than in the coast. Strategies than improve quality of forage in the highlands can allow more milk with less methane emission.

The methodology used considered five farm types, which were selected to estimate methane emissions from enteric fermentation. Farm PE-180 located in the coast (50 masl); farms PE-15 and PE-6 located in Cajamarca (north highland; > 2800 masl) and farms PE-1 and PE-4 located in Junin (central highland; 3600 – 4400 masl). The production system in the coast considers animals in free-stall with high use of concentrate and corn forage. Highland units consider grazing on cultivated (PE-15) and natural pasture (PE-6, PE-1 and PE-4) with limited use of concentrate. The methodology applied was the Tier-2, IPCC. Information was collected using specific surveys applied during one year that included rainy and dry season for the highland regions. Previous estimates using Tier-1, IPCC have been presented [2].

The results of methane emissions (Table I) from cattle show that animals on grazing system produce less methane than free-stall. However, when milk production is considered dairy cows on free-stall system produce 0.015 – 0.02 kg methane / kg of milk which is lower than emission from animals on grazing system producing 0.03 – 0.13 kg methane / kg milk corresponding the higher values to animals on natural pasture. Considering total methane emission (lactating + growing animals) in relation to milk production per farm, grazing systems compare even less favourably per kg milk than stall systems. In addition, there is opportunity with the actual cattle genotypes to increase productivity as demonstrated recently [3]. It can be concluded that the high methane emissions in the highland are mainly due to poor quality of forage (high fibre and low N). In contrast, coastal diets have lower fibre content and higher rates of digestion producing less methane emissions per unit of milk produced. Nuclear techniques in conjunction with conventional methods can contribute to develop strategies than improve forage production or utilization allowing more milk with less methane emission. These strategies include improving the quality of pasture (i.e. irradiation); evaluation using tracer techniques of supplementary feeding approaches (grains, molasses, urea), monitoring reproductive performance (progesterone assays) and developing modifiers of the rumen.

TABLE I. METHANE EMISSIONS FROM ENTERIC FERMENTATION

Farm	Category	Average population (head/year)	Milk yield (kg/d)	Estimated Feed Digestibility (%)	Estimate DMI	Methane Emission (kg/ head/ year)	kg CH4 emitted/ kg of milk/ per cow
PE-180	Free-Stall						
Dairy Cows	High producing	120	24	68	21.9	133	0.017
	Middle producing	60	16	65	20.1	117	
Other cattle	Calves pre weaning	10		75	2.1	5	
	Replacement heifers (3-12 m)	50		62	4.8	32	
	Replacement heifers (>13m)	60		60	8.2	59	
PE-15	Grazing						
Dairy Cows	Producing cows	15	13.7	62	15.6	131	0.03
Other cattle	Calves pre weaning	1		70	1.7	6	
	Replacement heifers 3-12 m)	3		58	4.3	32	
	Replacement heifers (>13m)	4		58	6.7	50	
PE-6	Grazing						
Dairy Cows	Producing cows	6	6.4	58	12.9	120	0.05
Other cattle	Calves pre weaning	1		68	3.6	15	
	Replacement heifers (3-12 m)	1		52	3.7	42	
	Replacement heifers (>13m)	3		52	5.5	61	
PE-1	Grazing						
Dairy Cows	Producing cows	1	3.2	52.6	7.5	103	0.09
Other cattle	Calves pre weaning	1		68	3.2	14	
	Replacement heifers (3-12 m)	0.3		52	2.8	64	
	Replacement heifers (>13m)	1		52	4.1	94	
PE-4	Grazing						
Dairy Cows	Producing cows	4	2.6	45.8	6.6	121	0.13
Other cattle	Calves pre weaning	2		68	3.6	16	
	Replacement heifers (3-12 m)	2		45	2.8	64	
	Replacement heifers (>13m)	2		45	4.1	94	

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EFFECTS OF BREED, SEX AND GEO-ECOLOGICAL ZONE ON THE HAEMATOLOGICAL PARAMETERS OF NIGERIAN GOATS

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Haematological indices together with the sex, sex × breed interaction and breed source effects on three major breeds of Nigerian goats were studied using 81 goats (comprising 9 males and 18 females per breed), objective being to characterize and outline the differences and similarities between the breeds in blood parameters. The goats were derived from different geo-ecological zones in the country based on the areas of preponderance of each breed. The breeds studied were: the Sahel goat (SG), Red Sokoto goat (RSG), and West African Dwarf goat (WADG) and haematological values obtained per breed were: 22.52 ± 1.48, 23.04 ± 3.56, and 29.22 ± 4.76 (% PCV); 7.52 ± 0.50, 7.82 ± 1.25 and 9.48 ± 1.60 (g/dl Hb); 2.71 ± 0.23, 3.09 ± 0.64, and 4.10 ± 0.42 (x 10^{12}/l RBC); 11.94 ± 1.10, 11.32 ± 2.03 and 9.23 ± 0.63 (× 10^{9}cells/l WBC), and 83.22 ± 1.67, 76.72 ± 2.30 and 73.34 ± 3.40 (× 10^{6}/mm^3 MCV), respectively. Significant differences ($P < 0.05$) were observed between the breeds, but the platelets, MCH and leucocytes differential counts were similar ($P > 0.05$) for all the breeds. The WADGs were superior to the RSG and SG in PCV, Hb, and RBC counts, but lower in WBC counts and MCV. The SG was similar in most of the haematological profiles examined, irrespective of geo-ecological distance, indicating homogeneity of the breeds. The Sahelian goat breed also outscored other breeds in MCV, showing that the breed has greater propensity to transport oxygen and in situation occasioning oxygen starvation, the breed survives better. This explains the reason for the survival of the breed in arid and semi-arid zone. Gender has no effect on the MCV and the values of 83.22 ± 1.67 × 10^{6}/mm^3, 76.72 ± 2.30 × 10^{6}/mm^{3}and 73.34 ± 3.40 × 10^{6}/mm^3 were observed for the SG, RSG, and WADG, respectively.

IMPROVEMENT OF BEEF CATTLE GENETICS PROVIDED INCREASING SUSTAINABILITY OF BEEF CATTLE PRODUCTION AND PROTEIN CONSUMPTION IN THAILAND

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The rural innovation research and development (R&D) in beef cattle genetics, biotechnology, climate science and production systems, supported profitable and sustainable beef cattle production in Thailand. Department of Livestock Development (DLD) undertakes R&D to achieve continuous improvement in genetics, production technologies to improve productivity, profitability and sustainability of beef cattle production and quality of products. Efficiencies were achieved through improvements in genetics, nutrition and grazing management, use of information, meat science, and reduction in ruminant methane production. This function was essential to maintain long-term production competitiveness and achieve sustained economic growth in rural Thailand, where the beef cattle production was the important livestock production, accounting for 36.99% of the value of livestock production in Thailand.

Molecular, quantitative genetics, and biotechnology tool were being combined in the development of genetic improvement. In 2006, beef meat was imported 1,842.53 thousand tons (0.41% of all consumption, 120.84 baht/kg) [3]. For the big size cattle, such as Tak cattle, Kabinburi cattle (Thai synthetic breeds by DLD, Tak = 62.5 Charoles-Brahman, Kabinburi = 50 Simental-Brahman), and cross breed cattle, they were in fattening period for 6-12 month. Fattening group, they were raised for restaurant, hotel, super market, and steak house.

Data were collected from 2 parts: 1) 354 cattle of experimental trial in DLD part, and 2) 492 fattening cattle of small holders in Tak province and Nakorn Pathom province during October 2004-September 2007. Data collecting was separated into 2 parts (performance data and reference). Data were adjusted by group location month and year to analyze for growth, carcass performance and economic performances).

There were 5 breeds of fattening beef cattle: 1) Thai Native, 2) Thai Brahman, 3) Kabinburi, 4) Tak, and 5) Tajima-Native. The first group was around 41.00 %. They were the smallest size. Farmers raised them by main of grass-fed and some added with concentrate. Meat quality from this group was the lowest (Table I). The meat texture of this group is the most firm. It was suitable for Thai food cooking. Meat from this group was used to make meatball and Thai food. The second group was around 38.50%. They were fattening 3-6 month [1]. Meat from this group was medium quality. Farmers fed them with grass, agricultural by product, and concentrate. Meat from this group was used for general Thai food and steak cooking. The third and forth group were around 20.00%. In this group, it was added with other beef cattle: *Kampangsaen and Ponyangkhum beef cattle.* They were fed higher concentrate, up to 90% of concentrate in the last month of fattening. They were fattening 6-12 month. Farmers fed them following feeding plan of cooperative, so they had the greatest ADG and %carcass. Quality of meat from this group was the best, most tender. Meat from this group was used for steak and shabushabu. Meat from this group had quality the same as import meat. Some of them were better than import meat. It was tenderer. The fifth group was less than 0.5%. They were on experiment to establish new breed by DLD. This group was optimized for Thai farmers, small size and low mature age. Meat from this group had the most tenderness and marbling. Meat from this group had more quality than import meat. It was used for steak and shabushabu.

TABLE I. PRODUCTION PERFORMANCE AND ECONOMIC POTENTIAL OF THAI BEEF CATTLE

	Thai Native	Thai Brahman	Kabinburi	Tak	Tajima-Native
No. of cattle (head)	154	228	120	332	12
Initial weight (kg)	126 ± 41.4	232.8 ± 24.62	276.7 ± 21.79	290.5 ± 21.79	213.9 ± 8.66
Final weight (kg)	305.4 ± 42.39	405.8 ± 36.93	550.6 ± 80.32	584.5 ± 28.90	456.4 ± 66.16
Final age (month)	21.4 ±12.61	21.3 ± 3.47	24.3 ± 2.18	23.3 ± 2.35	21.3 ± 2.18
ADG (kg/d)	0.636 ± 0.457	0.721 ± 0.291	0.913 ± 0.28	0.941 ± 0.111	0.674 ± 0.185
Carcass (%)	55.2 ± 9.56	57.6 ± 2.72	59.6 ± 4.35	59.6 ± 2.88	58.1 ± 2.72
Loin eye area (cm^2)	54.76 ± 6.04	76.2 ± 16.58	106.7 ±17.38	115.9 ± 18.43	83.4 ± 16.58
Shear force (kg) (21 day)	5.6 ± 0.51	5.3 ± 1.67	4.1 ± 1.66	4.8 ± 2.89	2.3 ± 0.88
Net income/head (baht/head)	3276 ± 2906	3217 ± 1449	6012 ± 3324	7336 ± 1583	5626 ± 2824

Farmers could earn money from beef cattle raising occupation. Beef cattle genetic improvement provided sustainable productivity, profitability, quality of products, and sustained economic growth in rural Thailand. Beef meat production would be the mainly red meat production and provided protein sources for consumer in Thailand.

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THAI INDIGENOUS CATTLE PRODUCTION PROVIDED A SUSTAINABLE ALTERNATIVE FOR THE BENEFIT OF SMALL-SCALE FARMERS, HEALTHY FOOD AND THE ENVIRONMENT

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In Thailand, there were 5.66 million indigenous cattle and 1.76 million of their crosses [1]. Farmers raised these cattle integrated with crop and fish in livestock-crop-fish integrated farming systems. These farming systems are in small scales for efficient utilization of available resources and for maximisation of production of diversified products per unit area to increase the income of the farmers and enhance food production. Thai indigenous cattle meat have more specific nutrient that are beneficial for consumers, such as omega 3, omega 6, and CLA. Furthermore, farmers use cattle manures as fertilizer for crop production, production of plankton for the fish and biogas/electric power used in the household. Additionally, Thai indigenous cattle are used for draught power. Consequently, Thai indigenous cattle increased food production and there was minimal cattle waste on farms thus, we could keep the environment clean and green.

Performance data, meat quality, compost production, biogas production, and draught animal and reference were collected from 103 smallholder farmers in the northern part of Thailand, north-eastern, central and southern parts of Thailand during October 2005 to September 2007.

Growth and reproductive performance

Thai indigenous cattle had various skin and hair colour such as red, light brown, black, piebald, and only Kow-Lamphun cattle in northern part of Thailand, orange-pink skin and white hair colour. Their navels were not slackened but attached to the belly. Their dewlaps were also not slacken. The average birth weight was 19.6 kg and the weaning weight at 200 d of age was 137.96 kg (Table I). They had good characteristics of heat tolerance, disease resistance, and high fertility traits. They were the main red-meat source for consumers.

Thai indigenous cattle were main source of red meat for consumption in Thailand. They produced high Omega 3 and Omega 6 in red meat, so their meat was the main source of protein and healthy food (Table II).

TABLE I. GROWTH AND REPRODUCTIVE PERFORMANCES OF THAI INDIGENOUS CATTLE

Trait		Means ± SD
BW	(kg)	19.61 ± 2.86
WW	(kg)	137.96 ±5 6.89
ADG 200 D	(kg)	0.380 ± 0.231
WT 400 D	(kg)	162.56 ± 77.15
WT 600 D	(kg)	201.06 ± 77.90
Age at first calving	(month)	30.54 ± 3.23
Calving interval	(day)	426.65 ± 117.24

TABLE II. CARCASS AND MEAT QUALITY OF THAI INDIGENOUS CATTLE

Trait		Mean ± SD
WT at slaughter house	(kg)	316.23 ± 40.21
Carcass percentage	(%)	55.57 ± 1.71
Lean percentage	(%)	36.33 ± 7.81
Saturated fatty acids	(%)	49.45 ± 3.27
Monounsaturated fatty acids	(%)	37.85 ± 5.89
Polyunsaturated fatty acids	(%)	12.70 ± 5.45
Omega 3	(%)	2.37 ± 0.69
Omega 6	(%)	10.14 ± 4.74

Other utility of Thai indigenous cattle integrated farming system

Farmer raised on average 31.95 heads of Thai indigenous cattle on each farm integrated with crops (rice, corn, pineapple, sugar cane) and fish (striped catfish, catfish, tilapia, crucian carp). Cattle were fed on natural grass, rice straw as the main feed and other by-product from crops. Farmers used cattle manure to replaced chemical fertilizer and produced compost, which was used as fertilizer for crop production. Farmers also used manure to produce plankton for fish. They harvested 85.19 ton of crop production by using compost from manure (Table III) and produced 0.25 ton per rai, which was less than the average country production (0.40 ton/rai) [2]. Average fish production was 100.00 kg per farm per year (147.06 kg/rai), which was similar to fish feeding with manure and concentrate (142.67 kg/rai) [3]. By integrating production in livestock-crop-fish, farming systems on small-scale, farmers produced safety food and gain their income. In addition, they could prevent air pollution, and global warming, leading to clean environment.

TABLE III. PRODUCTION PERFORMANCE OF THAI INDIGENOUS CATTLE INTEGRATED FARMING SYSTEM

Trait		Mean ± SD
Cow No.	(head per farm)	31.95 ± 18.90
Land Owner	(rai per farm)	34.42 ± 28.63
Calf production	(head per farm)	18.15 ± 14.86
Compost production	(ton per farm)	55.15 ± 31.27
Fish production	(ton per farm)	100.00 ± 39.12
Crop production	(ton per farm)	85.19 ± 62.06
Cattle for draught power	(head per farm)	3.00 ± 1.41
Biogas production	(m^3/farm)	12.00

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RUMINAL FUNGI FOR INCREASING FORAGE INTAKE AND ANIMAL PRODUCTIVITY

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In grazing ruminants and in ruminants fed low-quality fodder productivity is usually limited by the amount of feed an animal is able to ingest (intake). Forage is the primary source of energy, so the greater the intake the greater the production. Unfortunately, during digestion in the rumen large and recalcitrant fibrous particles are digested only slowly. These prevent further intake until they are reduced to a small size and released from the rumen [1].

Fibre-breakdown and fibre-clearance are the outcome of two processes: physical disruption of plant tissues by chewing and rumination, and fibre degradation during digestion. The fibre-degrading microbes in the rumen consist of fibrolytic bacteria such as *Ruminococcus albus*, *R. flavefaciens* and *Fibrobacter succinogenes*, fungi and some protozoa. The ruminal fungi, promising targets for improving fibre degradation [2], are associated with increased intake in sheep [3] and with improved growth rates in buffalo calves [4].

This paper describes results from studies on two ruminal fungi; one, which digests recalcitrant lignocellulosic materials, and one which has an unusual capacity to physically disrupt fibrous plant tissues. The aim was to carry out a detailed examination of fungus/plant cell-wall interactions and obtain new information on the modes of action of the two species.

P. tremuloides tissues, resistant to ruminal fibrolytic bacteria but readily degraded by the fungus *Neocallimastix frontalis* [5], are an ideal substrate for studying the fibre-degrading properties of this fungus. Large fragments of *P. tremuloides* were incubated with *N. frontalis* and examined using transmission and scanning electron microscopy. The results showed that cell-wall degradation in fragments progressed in a sequential manner from outer cells to inner cells. Mature fungal fruiting bodies (sporangia), part of the fungal life cycle, and inter-cell fungal transfer via pit apertures were observed.

The initial step in fungal attack involved direct contact between fungal rhizoid (rhizomycelia) and plant cell wall. As invading rhizoids extended into new areas of plant tissue, extra-cellular enzyme complexes were observed. As digestion continued, enzyme complexes migrated from rhizoids and attached to receding plant cell walls. At sites of degradation, plant cell-wall surfaces appeared to be saturated with enzyme complex, and digestion continued until the entire secondary cell wall was removed leaving only between-cell middle lamella tissue. This was resistant to digestion.

In contrast to the thin mycelial rhizoids of *N. frontalis* and other species of ruminal fungi, the rhizoids of *Caecomyces* species are large, bulbous and non-filamentous. These unusual rhizoids have been found to physically disrupt plant tissues during digestion [6]. In a separate experiment, sisal (*Agave sisalona*) segments were inoculated with a *Caecomyces* sp. and the fungus/plant cell-wall interactions during digestion examined. After fungal establishment, the bulbous rhizoids expanded along the axis of the fibre. The segments were split into fibrils at sites of attack. As was observed for *N. frontalis*, cell-wall degradation involved initial contact followed by migration of extra-cellular enzyme complexes to plant cell-wall surfaces. Extra-cellular membrane vesicles were observed and may play a role in degradation. There was no degradation of middle lamellae.

At completion of incubation, the segments were macerated and had a shredded appearance. The expanded bulbous rhizoids filled regions previously occupied by plant cell walls. In an effort to find *Caecomyces*-like activity in the rumen, plant fragments in digesta from a cow were examined.

This revealed the presence of fibrillated plant fragments containing *Caecomyces*-like bulbous rhizoids consistent with the *in rumeno* involvement of *Caecomyces* species in the physical disruption of forage. When a *Caecomyces* sp. was incubated with alfalfa-hay stems, the waxy cuticles, which protect the fragments from bacterial attack, were removed intact. It appears that *Caecomyces* species have a range of unusual fibre-degrading properties including 'cuticle peeling'.

Our observations indicate that whereas all species of ruminal fungi contribute to fibre weakening and breakdown, unique capabilities of *Caecomyces* species make them promising candidates for increasing the rate of particle-size reduction within the rumen, and thus increasing intake and productivity.

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MERINO BREEDING PROGRAM IMPROVES WOOL PRODUCTION IN THE WESTERN USA RANGE SHEEP FLOCKS

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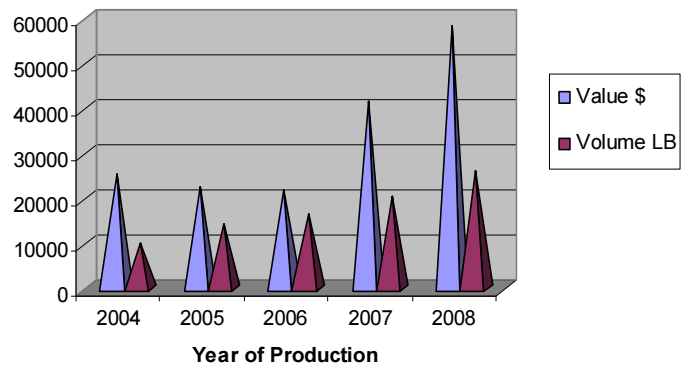
A Merino breeding resource flock was established at Rafter 7 Ranch, Yerington, Nevada through cooperation of the College of Agriculture, Biotechnology and Natural Resources, University of Nevada-Reno (UNR) and The Edwin L Wiegand Trust in 1990. Initially, 500 Rambouillet ewes were purchased from two established breeders in 1990. These ewes were bred naturally or by AI to imported rams from Australia and to rams selected within the flock. Over the 16 years, 16 rams and semen from 41 rams have been imported from Australia. Selection was based on objective wool measurements, subjective assessment, growth rate and reproductive performance traits. The flocks were expanded to 1300 ewes and were bred in 30 single sire-mating groups as of the 2005/2006 breeding seasons.

Flock management is in two breeding lines, one as a registered Rafter 7 Pure Merino flock (n = 650) and the other (Merino x Rambouillet) as Rafter 7 Merino Line (n = 650), which are selected for high fleece weight and quality, twinning and growth traits. The spring lambing flock winters on desert rangelands, is grazed on irrigated pasture from shearing through lambing and early weaning. Merino crossbred ewes showed that wool fibre density, clean wool yield, staple length, and grease fleece weight were increased by 41% per unit area of skin, 15%, 2.5 cm and 1.14 kg per head shorn, respectively. The wool clip is classed on pre-shearing mid-side wool sample tests (OFDA 2000 system). Fleece weight and fibre diameter and staple length measurements are significantly improved over the years. Wool sales from the Rafter 7 Ranch have increased for volume, value and quality over years (Figure 1). Rafter 7 Ranch wool clip has topped seventh consecutive annual shearing for the highest price of US grown wool. Sheep producers from 18 states, and Mexico and Canada have purchased breeding rams and ewes annually from Rafter 7 ranch over the past 12 years. Objective and subjective measurement qualities are significantly improved in sale rams. Over 1000 breeding rams and 500 replacement ewes were distributed to range flocks in the western states in the last decade, which made a notable improvement for fleece weight, fibre diameter and yield in client's flocks.

The dissemination of introduced Merino genetics in the western range sheep flocks will improve wool quality and clip profits, which strengthen a long-term competitive advantage for the US wool and sheep production sectors.

Figure 1. The Rafter 7 Ranch Wool Clip Volume & Value



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IMPROVING MILK PRODUCTION, COLLECTION AND PROCESSING SYSTEMS TO INCREASE SMALLHOLDER ACCESS TO COMMERCIAL MARKET OPPORTUNITIES IN EGYPT

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In Egypt, the contribution of livestock to the agriculture economy is about 31%. Total milk production is estimated at about 2.63 million tones per annum and is growing at a rate of about 1.6% per year. On the other hand, the human population, estimated at about 73 million, is growing at a rate of over 3% per annum. This shows the per capita consumption of milk in Egypt is about 40.4 kg/year, which is much, lower than the worlds per capita that average at about 234 kg/year. Hence, about 14 million tones of additional milk are required to feed the population as per the world's standard. This indicates the existence of wide gap between the potential demand and supply of milk in Egypt. The cows' milk represents 57% of the total milk produced. Buffaloes produced on average about 3600 tons of milk/d. The average daily production being 5.52 kg/d. Buffalo milk represents 43% of total milk produced daily. The price of one kilogram of buffalos' milk nowadays is in the range of 1.75-2.0 LE, while the price of one-kilogram cow, milk nowadays is ranged of 1.3-1.4 LE. Generally, almost all the Baladi cattle are in the hands of the farmers, together with perhaps 99% of the buffalo population (Government farms produce less than 1% of the total milk production, private farms less than 3 %, buffalo private farms about 17 %, the rest owned by Fellaheen. Improvement the milk production in smallholder is a must.

BREED DIVERSIFICATION IN SOUTH WESTERN UGANDA: CHARACTERIZATION OF A NEW CATTLE FARMING SYSTEM

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A new production system in which pastoral communities are becoming more sedentary and are keeping different genotypes (Holstein-Friesians or their crosses and pure Ankole) is emerging in South Western Uganda. In this system the Ankole, cattle are being crossed with Holstein-Friesian and the two genotypes are being kept in separate herds on the same farm. This is in response to the rapidly growing population, new land policies that favour individual land ownership [1, 2] high demand of livestock products in the urban centres and improved rural infrastructure.

As part of a larger research program that aims at evaluating the ecological and economic sustainability of the new pastoral systems, a survey was undertaken of sixteen farmers selected from 3 sub-counties in Kiruhura District in South West Uganda. Two sets of detailed structured questionnaires were used to collect information from the farms. The 1st set was administered at the beginning of the study in April 2007, and the 2nd one was administered on a monthly basis for a period of the last 12 months. On each farm visit performance traits such as milk yield (MY), heart girth measurement (HG) and Body Condition Score (BCS) of the animals were recorded. Comparative MY, BCS and body weight performance for mature cows for the different genotypes are considered here.

Majority (53.3%) of the farmers interviewed stated that they kept the two cattle types (genotypes) because the crossbreds gave them more marketable milk, while the Ankole provides security in case diseases or prolonged drought affected the crossbreds. Another group (19.9%) stated that they still prefer to keep Ankole cattle besides the crosses because they are hardy, while others (13.3%) stated that they kept Ankole for beef production because they were easier to sell off for this purpose and the crosses for milk production. Another 13.3% stated that the crossbreds were kept for income through milk sales and Ankole were kept for cultural reasons.

Different reasons were given for rearing the animals in separate herds: (i) to control breeding (ii) Ankole cattle scare the Holstein Friesian crosses with their large horns (iii) The two genotypes have different grazing behaviour (iv) The two genotypes require different management. One farmer stated that he kept the two genotypes separate because he had enough land that allows for this. According to the farmers' statements the selected farms had, a combined 4,886 animals of this (56%) were Holstein Friesian- Ankole crosses and the rest Ankole. The combined herd sizes were between 91 and 725 animals. While herd, sizes of the different cattle types (genotypes) were between 32 to 453 animals (Table I).

Larger herd sizes of up to 1284 animals have been observed in other studies in which the two genotypes are kept [3]. All interviewed farmers owned the land on which they were grazing the animals, land size ranged between 100 to 750 Hectares. Two of the farmers stated that they had rented additional land to ensure that their animals had enough pastures. The calculated mean stocking density on the farms was 1.36 Hectares per Tropical Livestock Unit.

The crossbreds gave significantly higher daily milk yields and had higher body weights than the Ankole (Table II).

TABLE1. MINIMUM AND MAXIMUM AND MEAN HERD SIZE AND HERD STRUCTURE OF THE ANKOLE AND HOLSTEIN FRIESIAN – ANKOLE CROSSES (CROSSBREDS) IN THE SELECTED FARMS

Ankole (n = 16)					Crossbreds (n = 16)				
Type	Mean	Min.	Max.	S.D.	Type	Mean	Min.	Max.	S.D.
Herd size	134.5	59	453	93.02	Herd size	171	32	325	110.5
Bulls	1.5	1	3	0.63	Bulls	1.7	1	4	0.85
Cows	59.2	20	200	41.9	Cows	73.4	15	150	48.4
Heifers	32.8	2	80	21.93	Heifers	37.3	3	100	25.49
Steers	17.9	2	42	17.50	Steers	17	2	60	23.04
Calves	32.06	6	130	11.56	Calves	44	2	148	40.86

Min. = Minimum, Max = maximum S.D. = Standard deviation

TABLE II. LEAST SQUARE MEANS OF DAILY MILK YIELD AND BODY CONDITION SCORES OF MATURE COWS OF THE ANKOLE AND HOLSTEIN FRIESIAN –ANKOLE CROSSES

Daily Milk Yield			BCS			Body weight (cows)		
Genotype	Litres	S.E.	Genotype	Score	SE	Genotype	Wt*	SE
Ankole (n = 191)	2.2[a]	0.5471	Ankole (n = 1576)	3.36	0.0283	Ankole (n = 1622)	334.2[a]	2.0167
HF 50% (n = 158)	10.6[b]	0.5132	HF 50% (n = 345)	3.28	0.0915	HF 50% (n = 361)	398.2[b]	6.5442
HF >50% (n = 625)	10.1[b]	0.1758	HF >50% (n = 1602)	3.24	0.0297	HF >50% (n = 1641)	395.6[b]	2.1036

HF 50% = F1 Ankole Holstein, Friesian HF > 50% = Crossbreds of greater than 50% Holstein Frisian Kilogram* converted from Heart girth measurement, SE = Standard Error

Other areas that were covered in this study include nutrition of the animals, herd health management (disease occurrence, disease control measures and costs involved), labour (hired and costs involved), challenges of the new production system (fluctuations in amounts of pasture and water available for production and unstable milk prices). The paper reports findings on all the above, highlights existing knowledge gaps and finally appropriate technical interventions are recommended.

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EFFECT OF EVAPORATIVE COOLING SYSTEM ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCES OF CROSSBRED HOLSTEIN FRIESIAN CATTLE IN TROPICAL CONDITIONS

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Effects of evaporative cooling system on productive performances and reproductive performances of crossbred Holstein Friesian cattle in tropical condition were studied. Twenty primiparous 87.5% crossbred Friesian cattle were used in the experiment. They were divided into two groups of ten animals each. One group was kept in evaporative cooling system (EVAP) that temperature reduced by using air force movement through cooling pad. Another group was kept in the open conventional housing system (NEVAP). Temperature-humidity index (THI) was daily calculated according to NOAA [1] and used to differentiate the severity of heat stress in each housing system. Productive performances and reproductive performances were measured starting from parturition until 10 weeks of lactation

Productive performances of experimental animals were shown in Table I. Maximum temperature in EVAP and NEVAP during the study was 29.1, 35.8^{o}C and minimum temperature was 22.2 and 23.6^{o} C respectively. Average THI in EVAP and NEVAP was 77 and 81 respectively. DMI and DMI % BW of cows in EVAP were significant higher ($P < 0.05$) than cows in NEVAP. Animals in EVAP consumed 19.8% more feed than animals in NEVAP. Cows in EVAP produced significantly more milk than cows in NEVAP ($P < 0.01$). Milk yield of cows in EVAP and NEVAP were 16.9 kg/d and 12.6 kg/d respectively. 4% FCM production of cows in EVAP was significantly higher ($P < 0.01$). Cows in EVAP could produce 3.5 kg of 4% FCM more than cows in NEVAP. DMI/4%FCM of cows in EVAP and NEVAP were 0.70 and 0.77 respectively in, which no difference was found. It was found that animals in EVAP consumed 54.4 L of water per day compare to 93.6 L in NEVAP, which was highly significant difference ($P < 0.01$). When water intake per DMI was compared, less water intake was found in EVAP ($P < 0.01$). Cows in NEVAP consume 6.1 L of water/kg DMI more than cows in EVAP. Data of milk compositions were shown in Table I. No significant differences in milk composition between EVAP and NEVAP were found. No difference was found in eating time and ruminating time. However, cows in EVAP spent more time ($P < 0.05$) for total chewing time than cows in NEVAP. The concentrations of individual volatile fatty acids (acetate, propionate and butyrate) from both groups of animals were similar. The total concentration of volatile fatty acids and the ratio of acetate to propionate in cows were slightly higher in EVAP but no significant difference was found. Reproductive performances of cows in EVAP and NEVAP were shown in Table II. No difference was found in all parameters measured, though better performances were shown in animals kept in NEVAP.

TABLE I. DRY MATTER INTAKE (DMI), MILK PRODUCTION AND PRODUCTIVE PERFORMANCE OF CROSSBRED FRIESIAN HEIFERS IN EVAPORATIVE AND NON-EVAPORATIVE COOLING SYSTEM (MEAN ± SE)

Parameters	EVAP	NEVAP
BW (kg)	393.1 ± 2.81	367.6 ± 3.1
DMI (kg)	13.3 ± 0.4[a]	11.1 ± 0.54[b]
DMI/%BW	3.47 ± 0.07[c]	3.03 ±0.06[d]
Milk yield (kg/d)	16.9 ± 0.6[a]	12.6 ±0.6[b]
4%FCM (kg/d)	14.6 ± 0.8[c]	11.1 ±0.5[d]
DMI/4%FCM	0.70 ± 0.04	0.77±0.04
Water intake, (l/d)	54.4 ± 3.5[c]	93.6 ±8.0[d]
Water intake/DMI (l/kg)	4.5 ± 0.26[c]	10.6±0.77[d]
Milk compositions (%)		
Fat	3.49 ± 0.16	3.26±0.16
Protein	3.26 ± 0.04	3.08±0.04
Lactose	5.0 ± 0.02	4.97±0.02
Total eating time (min/d)	227.1 ± 6.9	192.4±6.2
Total ruminating time (min/d)	349.5 ± 5.2	255.9±13.7
Total eating time (min/d)	576.7 ± 4.3[a]	448.3±12.8[b]
VFA (mmol/ml)		
Acetate	119.2 ± 6.4	107.7±8.9
Propionate	42.7 ± 3.4	43.8±3.1
Butyrate	19.0 ± 1.0	19.8±1.4
Max temp ºC	29.1 ± 0.30	35.8±0.33
Min temp ºC	22.2 ± 0.67	23.6±0.55
Average temp ºC	25.4 ± 0.40	28.5±0.34
THI	77 ± 0.5	81±1.39

Different superscript in the same row is significantly different, [ab] ($P < 0.05$), [cd] ($P < 0.01$)

TABLE II. REPRODUCTIVE PERFORMANCES OF COWS IN EVAP AND NEVAP (MEAN ± SE)

Parameters	EVAP	NEVAP
Synchronization rate (%)	82.4	52.9
Maximium size of the largest ovulatory follicle (mm)	14.6 ± 0.5	14.2 ± 0.4
Days to first ovulation (d)	31.4 ± 4.3	26.1 ± 3.6
Conception rate (%) within 60 d after parturition	25.	17.7

Reference:

[1] NOAA, Livestock hot weather stress. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Weather Service Central Region, Regional Operations Manual Letters, 31-76 (1976).

EFFECT OF DIETARY ENERGY INTAKE AND SOMATOTROPIN ADMINISTRATION AFTER WEANING ON GROWTH RATE AND SEMEN CHARACTERISTICS OF GRANADINA GOAT BUCKS

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Twenty-eight growing Granadina goat bucks initially averaging 14 kg BW were divided into four groups of seven animals in a 2 × 2 factorial arranged design. These animals were used to study the effect of post-weaning levels of dietary energy and administration of recombinant bovine somatotropin (rbST) on growth and semen characteristics.

Bucks were offered either 1.0 (2.36 Mcal EM kg^{-1}; 15% CP) or 1.25 (2.95 Mcal EM kg^{-1}; 18% CP) times growth energy and protein requirements during 99 d, in combination with or without subcutaneous administration of 125 mg rbST every 14 d. Goats were weighed biweekly without withdrawal of feed or water. Latex condoms for men inserted into the vagina of three adult does in oestrus were used for semen collection. On the last day of the feeding trial (d 99), blood samples were obtained before feeding for serum metabolites determination using spectrophotometric methods. The experiment was conducted from May to August (breeding season for this breed of goats in northern Mexico).

Because there was no interaction between diet and rbST ($P > 0.15$), only the main effects of diet and rbST administration on growth and semen characteristics are presented. Average daily gains (ADG) were greater ($P < 0.01$) in bucks fed the high energy diet (133 ± 25 vs. 111 ± 23 g day^{-1}). ADG was greater ($P < 0.05$) in bucks treated with rbST than untreated bucks (130 ± 28 vs. 114 ± 23 g day^{-1}). In accordance with the increased ADG with rbST, an increase in ADG in rbST-treated Angora goats has been observed [1].

Percentage of live sperm cells was similar for bucks fed 1.25 of NRC recommendations than bucks fed 1.0. Similarly, bucks on the 1.25 diet had similar sperm outputs than bucks on the 1.0 diet (2282 ± 1137 vs. 1946 ± 529 × 10^6/mL). The percentage of morphologically normal sperm cells, assessed at 26 kg of BW, did not differ between groups. Semen volume (0.51 ± 0.29 vs. 0.55 ± 0.28 mL), sperm concentration (2210 ± 1139 vs. 2055 ± 656 × 10^6/mL), total sperm cells (1233 ± 962 vs. 1014 ± 572 × 10^6), percentage of motile sperm cells (67.1 ± 14.5 vs. 60.9 ± 19.3), and abnormal sperm cells (7.8 ± 3.3 vs. 6.3 ± 4.2) were not affected by rbST. No significant differences due to dietary (24.6 ± 3.2 vs. 23.8 ± 1.2 cm) and hormonal (23.6 ± 2.9 vs. 25.0 ± 1.9 cm) treatments were observed on scrotal circumference. Results of the present study are in agreement with studies in young bulls [2] where chronic applications of somatotropin did not alter semen quality variables.

Dietary and hormonal treatments had no effect on either serum concentration of particular metabolites and minerals, except cholesterol (16% higher for rbST_treated goats). Results from this study indicate that high energy diets markedly increased weight gains in growing Granadina goat bucks, with and without somatotropin treatment, which implies that rapid growth rates were not incompatible with semen production and quality. On the other hand, these results do not depict a potential for enhancing semen production and quality through chronic rbST treatment of growing Granadina goat bucks.

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METHANE REDUCTION PROPERTIES OF TANNIN CONTAINING PLANTS, SIMPLE PHENOLS AND PURIFIED TANNINS IN *IN VITRO* RUMEN FERMENTATION SYSTEM

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This study was conducted in a series of 3 experiments, and aimed at evaluating (1) polyphenol containing plants, (2) simple phenols in the form of phenolic acids, and (3) purified tannins for their potential to reduce methane production *in vitro*.

In experiment 1, polyphenol containing plants (n = 17) were analysed for chemical composition (dry matter, ash, crude protein, ether extract, neutral detergent fibre (NDF) and acid detergent fibre (ADF)), polyphenols content and activity (total phenols (TP), total tannins (TT), condensed tannins (CT) and tannin bioassay) [1], and methane production *in vitro*. Methane was expressed as mL net CH_4 in 100 mL net gas production, decrease of CH_4 compared to methane from hay, and increase of CH_4 after polyethylene glycol (PEG) addition. Regression and correlation analyses were performed between each tannin assay and other chemical constituents for their effect on methane production. Each plant was analysed in duplicate. In experiment 2, six sources of simple phenols (benzoic, cinnamic, phenylacetic, caffeic, p-coumaric and ferulic acids) were evaluated for their potential to reduce methane.

Two levels of each phenol (2 and 5 mM) were added to hay diet before *in vitro* incubation. The simple phenols were prepared by solubilizing the phenols in sodium phosphate buffer pH 6.7 to avoid pH > 7.5, and adding 130 µl of NaOH (10 M) to completely dissolve the phenols. An appropriate aliquot of solubilized phenolics (≤1 mL) was injected into the syringe from the syringe nozzle before dispensing rumen liquor. The measured variables were gas production, methane, organic matter digestibility (OMD), and short chain fatty acids (SCFA: C_2, C_3, C_4, iso-C_4, C_5, iso-C_5, total SCFA) and ratio of C_2/C_3. In experiment 3, effects of four purified tannins [2] from chestnut, mimosa, quebracho and sumach in hay:concentrate diet (70:30 w/w) were evaluated at three different concentrations (0.5, 0.75 and 1.0 mg/mL). The measured parameters were gas production, methane, OMD and individual SCFA. Chesnut and sumach are hydrolysable tannins, whereas mimosa and quebracho are condensed tannins.

The results from experiment 1 showed that there were negative relationships between TP, TT or tannin activity and methane production. The correlation (r) values ranged from –0.59 to –0.75 with $P < 0.05$ for the relationships. Very weak relationship was found between CT and methane production. There were positive and significant relationships between TP, TT or tannin activity and the methane decrease, as well as with methane increase by PEG addition. The highest correlation of 0.79 ($P < 0.001$) was obtained between tannin activity and the methane decrease. Amongst the tannin assays, tannin bioassay (a reflection of tannin activity) was the best predictor of the methane reduction potential of a plant.

All simple phenols studied in experiment 2 were not effective in decreasing methane production at lower concentration (2 mM). At higher concentration (5 mM), benzoic and phenylacetic acids were not effective. Cinnamic, caffeic, p-coumaric and ferulic acids decreased methane production significantly ($P < 0.05$) when added at 5 mM. Caffeic acid at 5 mM was the most effective out of the simple phenols tested and it decreased methane by 6.3% from the control. The magnitude was higher when expressed as decrease of methane per unit organic matter digested and the decrease

was 9.4% from control. After caffeic acid, the order of simple phenols to decrease methane was: p-coumaric > ferulic > cinnamic.

The observation from experiment 3 showed that addition of purified chestnut and sumach tannins at 1 mg/mL to hay:concentrate (70:30) diet significantly decreased ($P < 0.05$) methane production by 6.5 and 7.2% from control, respectively. Lower concentrations (0.5 and 0.75 mg/mL) of these hydrolysable tannins did not significantly ($P > 0.05$) decrease methane production. The addition of mimosa and quebracho tannins (condensed tannins) did not significantly decrease methane production, even at the highest concentration. For all tannins, increase in concentration led to increase in methane reduction (Figure 1). Condensed tannins decreased gas production and OMD more than hydrolysable tannins. The results suggested that hydrolysable tannins are more effective in decreasing methane emissions than condensed tannins, while at the same time hydrolysable tannins did not significantly decrease OMD. The condensed tannins appear to decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins act more through inhibition of the growth and/or activity of methanogens and/or hydrogen producing microbes (direct effect). Work on the study of the microbial ecology using quantitative PCR is in progress and the results from this study will be presented.

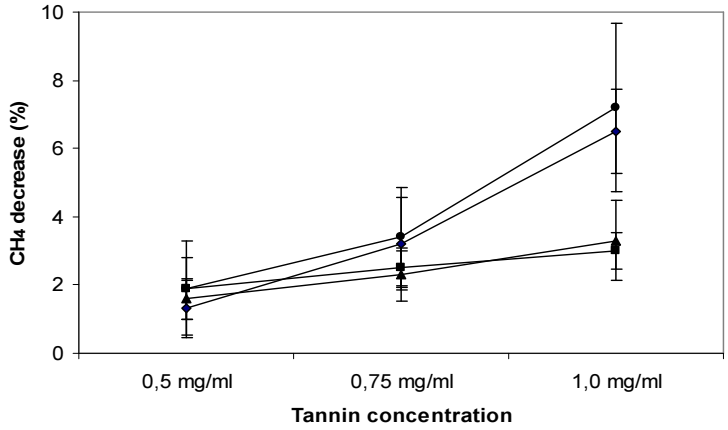

FIG. 1. Effect of purified tannins from chestnut (-♦-), mimosa (-■-), quebracho (-▲-) and sumach (-●-) on CH_4 decrease when added to hay:concentrate diet (70:30 w/w) at 0.5, 0.75 and 1.0 mg/mL concentrations.

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SEASONALITY OF REPRODUCTION AND MT1 RECEPTOR GENE POLYMORPHISM IN AWASSI SHEEP

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In three consecutive experiments seasonal pattern of ovarian activity was investigated in non-suckling awassi dairy sheep.

In Experiment 1. (Exp. 1.) resumption of postpartum (pp) ovarian cyclicity was studied in autumn lambing (AL;n = 27) and spring lambing (SL; n = 38) ewes. Cyclicity was monitored by means of individual progesterone (P4) profiles (milk P4 was assayed trice weekly from day (d) 5 to d100 pp). 89% of AL dams ovulated before d 35 and became cyclic thereafter. Incidence of persistent corpus luteum (CLP n = 5) and short luteal phases (sCL n = 8; CLP and sCL together n = 4) was frequent among non-conceiving dams. In contrast only 39% of SL ewes ovulated before d70. P4 levels during luteal phase in cyclic animals were lower, and length of cycle was longer in SL compared to AL. No CLP or sCL was detected in SL. 61% of SL remained acyclic till the end of trial.

In Expt. 2 influence of additional lighting on the time of first ovulation was investigated in 48 AL ewes. Long-day photoperiod (LD) group (n = 23) was exposed to artificial light from sunset till midnight (approx 16 hours light/8 hours dark). Control group (n = 25) received no treatment (natural photoperiod). Sampling protocol was similar to Exp. 1. Time of first pp ovulation tended to delay in LD animals compared to Control (average 25.87 ± 1.63 vs. 21.5 ± 1.72 d pp; surv analysis P = 0.093; Figure 1.).

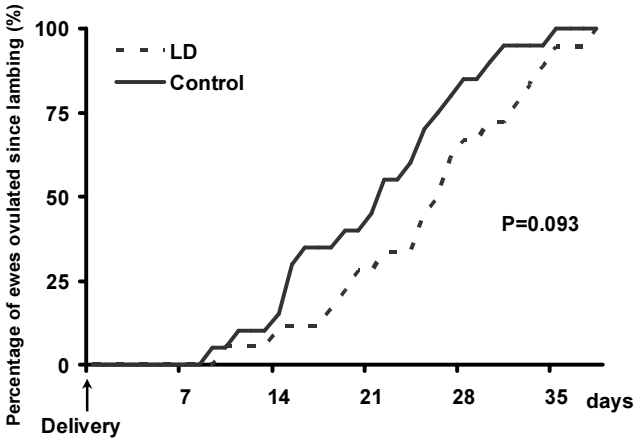

FIG 1. Day of first postpartum ovulation in Long-day photoperiod (LD) and Control groups

In Expt. 3a interaction between melatonin receptor 1a (MT1) gene polymorphism and out-of-season cyclicity was evaluated in 395 dams. Milk P4 level was determined 3 times 7 d apart between 10-12 weeks pp. If P4 level was >4 nmol/L in at least one of the samples, animals were judged cyclic. 10 weeks pp plasma leptin and insulin-like growth factor I was measured. Two RFLP sites (RsaI and MnII) of MT1 gene were determined in all dams. Proportion of out-of-season cycling animals was depending on age (P = 0.003) and leptin level (P < 0.001). Distribution of various MnII and RsaI alleles for the two RFLP sites (n = 395) in the Hungarian Awassi population (Table I) differed from the distribution of the same allels in Israel Local Awassi reported earlier by Notter and Cockett [1].

TABLE I. DISTRIBUTION OF ALLELS FOR THE TWO RFLP SITES. IN CASE OF BOTH RFLP SITES ALLELE „-" MEANS THE ABSENCE OF THE CLEAVAGE SITE

		MnlI		RsaI	
	n	Allele +	Allele -	Allele +	Allele -
Hungarian Awassi	395	0.55	0.45	0.83	0.17

Association between genotype and seasonality was evaluated in animals older than 3 years of age. Regarding RsaI genotypes higher number of −− ewes showed cyclic ovarian activity (P = 0.04), and higher proportion of ++ ewes for MnlI genotype were cycling (P = 0.07). As Expt. 3b milk sample collection was repeated in 43 dams 2 years later with the same protocol, and interaction between genotype and ability for out-of season cyclicity was re-evaluated in those dams (Table II).

TABLE II. ASSOCIATION BETWEEN OUT-OF-SEASON CYCLICITY AND MT1 POLYMORPHISM FOR THE TWO RFLP SITES IN CONSECUTIVE YEARS (N = 43)

Out-of-season cyclicity in		Chi-square test
2005	RsaI	0.092
	MnlI	0.416
2007	RsaI	0.701
	MnlI	0.740
2005 and/or 2007	RsaI	0.012
	MnlI	0.049

Conclusions:

- ovarian function of awassi population became seasonal under temperate continental weather.

- First pp ovulation of non-suckling, autumn-lambing dams may happen very early, even before the completion of uterine involution.

- Additional artificial lightening may retard the time of first pp ovulation.

- in the investigated population MT1 gene polymorphism seem to be a promising candidate for detection of ability for out-of-season cyclicity.

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PRODUCTION SYSTEMS AND CHARACTERISTICS OF INDIGENOUS SMALL RUMINANTS IN SRI LANKA

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Farming operations of small ruminants is one of the most common features in the small-scale livestock farmers, which represents 99% of the total farming population in Sri Lanka. However, the distribution of native small ruminants; goats and sheep, are scattered. The goat population is distributed mostly (72%) in drier areas [1] whereas sheep are concentrated mainly in northern area of the country [2]. Therefore the management systems of these farm animal genetic resources are largely influenced by the socio cultural conditions of the respective areas.

The data collection on farming systems and production characteristics were carried out during the years 2007 and 2008 from the areas in the north central, north-western and northern parts of the island. The farms were randomly chosen based on their representativeness of indigenous small ruminant populations, having confirmed that there was no introduction of exotic breeds within documented past. A pre-tested structured questionnaire was used for data collection from 40 farm families for each small ruminant species. The production status of each species was analyzed separately as they belong to separate regional, social and ethnic categories.

Native goats are kept mainly for meat and manure and rarely for milk under extensive management conditions. The input levels were low, ranging from sub-standard levels to zero level. According to farmers' perspective, native goats are hardy and resistant to common diseases. This was further revealed by absence of disease incidence recorded during the survey. The herd size varies according to the area ranging from 1-2 goat on average in northern area and 6-7 on average in the north-central and north-western areas. The animals were recorded as small compact animals with varying coat colours either polled or horned. Females are prolific, however kids show low growth rates and high mortality before weaning. Breeding is done based on community arrangement using a hired buck. Low milk yield and low growth performance after weaning hinder their chances of being attracted as a genetic asset among rural community. This is mostly highlighted since the goat production is a part of mixed crop-livestock production system. The role-play of goat as an income generator was minimum (0% - 20% of the total income) even in the areas, where the goat production is popular.

Native sheep, know as Jaffna Local sheep are reared in very specific farming system prevailing in northern area of the island. The flock size of native sheep varies from 12 – 254 animals. There were only two farms having more than 200 animals. Animals are usually white with patches of various colours (brown and black), and have extremely short tails. Females usually have no horns but half of males do. Indigenous sheep are small animals with no production potential of wool. Breeding occurs naturally in a close system and no attention has paid for performance improvement but for the number. Single birth is most common though there were very few twinning (2.5%) recorded in the survey area. High lamb mortality rate could be seen due to harsh environmental conditions and lack of attention paid by the farmer

s during lambing season. However, Jaffna Local is the hardy native sheep breed and it is the only local sheep breed in Sri Lanka. Majority of farmers kept sheep as a tradition and as an inherent property while few others (35%) recorded a family income born by selling manure and animals.

Hence sheep production system is essentially a low-input, low-risk and low-return system specific to the area.

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APPLICATION OF NEAR INFRARED SPECTROSCOPY TO IMPROVE ANIMAL PRODUCTION IN DEVELOPING COUNTRIES

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THE GENETICS OF ADAPTATION OF CATTLE TO HIGH ALTITUDE ENVIRONMENT: THE CASE OF BRISKET DISEASE IN NORTH WESTERN ETHIOPIA

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High altitude or brisket disease of cattle is common at high altitude areas. It is characterized by right ventricular hypertrophy and edema of the chest and brisket, because of reduced blood oxygen saturation at high elevation. It is similar to altitude sickness in humans and frequently ends with the death of the affected animal unless transferred to lower altitude. The incidence and severity of the disease increase with altitude [1, 2]. Pulmonary artery pressure (PAP) is an indicator of proneness to the disease. High values (>50 mm HG) indicate high risk and low values (<35 mmHG) indicate resistance to the disease [1]. Analyses of heritability of PAP, mostly performed on Angus cattle in the Rocky Mountains at altitudes of 2000-3800 m indicate a relatively high heritability, in the range of 0.30-0.50 [2, 3, 4]. This provides options for selection, which is performed routinely in the Rocky Mountains.

The Semien Mountains are the highest mountain range of Ethiopia, peaking at the Ras Dashen (4620 m). Cattle are kept at altitudes of up to almost 4000 m. Along the western side of the mountain range, there is a rather continuous drop in altitude down to about 600 m, over a rather short distance (250 km). The cattle in the region are partly of different type (Zebu, Sanga) and partly a mixture (Zenga = Zebu x Sanga). The phenotypic differentiation in terms of body size along levels of altitude is strong [5].

A study was conducted to assess the prevalence rate of high altitude disease and as well as to compare adaptive characteristics of indigenous cattle populations and their crosses with European types towards altitude, in particular, to high altitude disease. In January 2007, 218 animals situated within an altitude range of 1730 - 3500 m were tested for PAP by an experienced veterinarian from Colorado State University. Local breeds and crosses with Holstein Friesian and Jersey were measured.

The results in Table I indicate that no sign of brisket disease is observed among the studied populations. All PAP scores (21- 47 mm Hg) fall within the range of low to moderate risks. Differences in means were not significant for any pair of populations. Some of the readings (values <28 mm HG) for the Semien cattle group measured at 3500 m are out of the range of readings of approx. 100,000 cattle that the veterinarian has taken in the Rocky mountains in the course of 20 years. Crosses of the local cattle with Holstein Friesian and Jersey were not more prone to brisket disease than local cattle measured at the same altitudes. In a study comparing PAP readings in yak, cattle and their crosses [6], the crosses had equally low PAP readings as the yaks. Yaks are known to be resistant to high altitude disease due to an adaptation of vascular system, indicated by thin-walled small pulmonary arteries. The authors speculated about an autosomal dominant gene transmitting genetic attenuation of the hypoxic vasoconstrictor response.

TABLE I. POPULATIONS, THEIR RESPECTIVE LOCATION AND CORRESPONDING PAP SCORES MEASURED IN MILLIMETRES OF MERCURY (MM HG)

Breed	*Altitude /m.a.s.l/*	*Animals*	*PAP scores Mean ± s.d*	*PAP scores Range*
Overall	1730 - 3500	218	33.40 ± 3.94	21 – 47
Overall indigenous	1730 - 3500	126	33.08 ± 3.91	21 – 46
Indigenous				
Fogera	1730	55	32.51 ± 2.95	27 – 42
Wegera	2700	39	34.41 ± 3.44	28 – 42
Semien	3500	32	32.47 ± 5.36	21 – 46
Crosses				
Overall crosses	1730 - 2700	92	33.84 ± 3.96	28 – 47
Fogera x Friesian	1730	8	34.50 ± 2.66	31 – 39
Wegera x Friesian	2700	64	33.42 ± 4.15	28 – 47
Wegera x Jersey	2700	20	35.00 ± 3.49	30 – 41

We conclude that cattle breeds of North Western Ethiopia are genetically adapted to high altitude. To get an insight on the mechanism of adaptation an in depth histological study on the internal anatomy of cardiovascular and respiratory systems of these genotypes is currently being undertaken.

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FERMENTED MIXTURE OF CASSAVA PEEL AND CAGED LAYER MANURE AS ENERGY SOURCE IN BROILER STARTER DIET

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Five parts of sundried cassava peel a (fibrous and low protein) by-product of cassava tuber processing industry was ground and mixed thoroughly with one part of ground sundried caged layers' manure in a vertical feed mill mixer. Rumen filtrate (100ml) from slaughtered bovine, containing rumen microbes was used to spray and innoculate the mixture of the cassava peel and caged layers' manure in a 50 L black plastic vat. The content of the vat was again thoroughly mixed using plastic scoop and was immediately covered airtight with black polythene sheet to ensure fermentation for a period of 14 d. The fermented cassava peel and caged layers' manure (FCPCLM) was analyzed for proximate composition [1] and amino acids [2]. It contained 8.71% crude protein (CP), 11.58% crude fibre (CF), 73.52% Nitrogen free extract (NFE), 2.75% Ether extract (EE), 3.97% Ash. The analyzed essential amino acids in FCPCLM are Lysine 2.16%, Methionine 0.78%, Valine 3.64%, Histidine 1.64%, Leucine 5.13%, Threonine 2.13%, Phenyalanine 3.17%, Arginine 4.00%, Isoleucine 3.01%. FCPCLM was then mixed with other ingredients in broiler starter diets (control) to replace maize at 25% and 50% while other ingredients in the diet remain constant. The objective is to ascertain the performance, serum indices and cost benefit of FCPCLM to partially replace maize as source of energy, which has become expensive because of recent use as raw material for biofuels and to focus on farm residue resource readily available to small-scale farmers for sustainability of poultry products without sophistication in technological approach. The control diet had the following ingredients viz; Maize 40%, Wheat offal 19%, Soybean meal 23%, Fish meal 1.20%, Groundnut cake 12%, Bone meal 2%, Oyster shell 2%, Broiler starter Premix 0.25%, Salt 0.25%, Methionine 0.10%, Lysine 0.1% and Feed antibiotic 0.1%. The prediction equation: metabolisable energy (ME) of FCPCLM = 37 × %CP + 81.8 × %EE + 35.5 x % NFE which is 3157.18 kcal/kg was used to calculate ME. Ninety broiler starter day old chicks of Anark breed, weighing averagely 38.89 g were used in this feeding trial for 28 d. The birds were divided into three groups of three replicates each containing 10 chicks in a completely randomized design experiment.

Results showed a significant ($P < 0.05$) linear increase in the feed intake, body weight gain, feed conversion ratio and protein efficiency ratio. The serum chemistry indices viz; total protein, albumin, globulin and alanine aminotransaminase (EC 2.6.1.2) and aspartate aminotransaminase (EC 2.6.1.1) all indicated good quality protein that is also confirmed by the essential amino acid content stated above. Some cut parts of the carcass of the starter chicks is presented in Table II. Eviscerated weight, wing, head and other cut-parts (all as percent live weight) significantly increased ($P < 0.05$) as the replacement of maize by FCPCLM increased in the broiler chicks' diets. The values obtained for birds fed 25% and 50% replacement for maize were better for all the cut-parts than for those fed the control diet. Conclusively, FCPCLM can be used in broiler starter diet at optimum inclusion level of 50%. This becomes useful and relevant as the price of maize the major component as energy source is on the increase due to it use for biofuels which brought a lot of competition on the cereal and environmental management of poultry waste. This study shows that an alternate to maize can be found in FCPCLM.

TABLE I. PERFORMANCE AND SERUM CHEMISTRY OF BROILER STARTER CHICKS FED FCPCLM

Parameters	Replacement of maize with FCPCLM (%) 0	25	50	SEM
Feed intake (g)	78.75±0.61	88.53 ± 0.92	91.47 ±1.41	0.88
Final Live weight (g)	618±24.7^{c}	741.67 ± 17.4^{b}	805.67 ± 5.1^{a}	26.29
BW gain/bird/d (g)	20.67±0.8^{c}	25.09 ± 0.5^{b}	27.38 ± 0.5^{a}	0.48
FCR	3.90±0.2^{c}	3.63 ± 0.3^{b}	3.39 ± 0.2^{a}	0.16
PER	0.90±0.01	0.83 ± 0.05	0.79 ± 0.0	0.13
Cost of Feed /kg (₦)*	92.91	87.91	82.91	
Cost/kg gain (₦)*	353.97 ± 6.0^{c}	310.19 ± 4.2^{b}	276.98 ± 8.6^{a}	20.67
Total protein (g/dl)	27.11±1.0	25.75 ± 3.2	25.88 ± 3.6	1.38
Albumin (g/dl)	24.05±1.2	22.15 ± 1.0	22.50 ± 2.5	1.95
Globulin (g/dl)	3.06±0.5	3.60 ± 0.01	3.38 ± 0.2	0.56
ALT (g/dl)	15.33±0.7	21.0 ± 0.0	17.01 ± 0.09	1.05
AST (g/dl)	136.0±10.1	124.0 ± 10.6	120.67 ± 9.0	3.49

Means with different superscripts on the same row differ significantly ($P < 0.05$)
*1 USD = ₦118.00

TABLE II. CARCASS AND CUT-PARTS OF BROILER STARTER CHICKS FED FCPCLM

Parameters	Replacement of maize with FCPCLM (%) 0	25	50	SEM
Eviscerated wt (%)	72.72±0.6^{b}	81.30±1.03^{a}	80.68±0.5^{a}	3.96
Wing	2.64 ± 0.2^{b}	5.20 ± 0.4^{a}	5.15 ± 0.1^{a}	3.06
Head	4.74 ± 0.3	4.83 ± 0.2	4.88 ± 0.5	0.34
Neck	4.30 ± 0.1	4.35 ± 0.8	4.34 ± 0.3	0.45
Breast	17.30 ± 2.9	17.63 ± 1.0	17.65 ± 0.2	2.22
Back	12.82 ± 0.2	12.56 ± 0.5	12.53 ± 0.01	2.45
Drum stick	11.67 ± 0.04	11.71 ± 0.01	11.69 ± 0.06	1.15
Thigh	11.45 ± 1.9	11.13 ± 0.6	11.96 ± 1.2	1.16
Whole intestine (cm)	195.01 ± 1.9	202.33 ± 2.5	203.11 ± 1.0	2.22
Small intestine (cm)	172.10 ± 1.1	174.31 ± 1.3	174.0 ± 2.1	1.89
Large intestine (cm)	2.00 ± 0.2^{b}	6.36 ± 2.1^{a}	6.40 ± 1.8^{a}	0.44

Means with different superscripts on the same row differ significantly ($P < 0.05$)

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EVALUATION OF SPINELESS CACTUS (*OPUNTIA FICUS-INDICUS*) AS AN ALTERNATIVE ANIMAL FEED AND WATER RESOURCE DURING DRY SEASON IN ERITREA

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Animal feed and water shortage is one of the main constraints for the livestock sector in arid and semi arid region of Eritrea. The major feed resource comes from the rangeland pasture and crop residue. The quality and availability of these feed resources decreases rapidly following the rainy season. This fluctuating pattern of animal feed supply results in a pattern of gain and loss in animal growth and performance. In a country like Eritrea where feed shortage is such a serious problem, utilization of multipurpose trees and shrubs such as cactus that can cope with low and erratic rain fall, high temperature poor soils, and required low energy inputs can serve as an alternative strategy to reduce the chronic animal feed and water shortage (Barbera et al., 1995). Therefore the aim of this research was to assess the potential of spineless cactus (Opuntia ficus-indica) as an alternative source feed and water for ruminant animals fed poor quality crop residues during the dry season in Eritrea.

A randomized complete block design was used to allocate 24 fat tailed Highland male sheep with initial mean live weight of 21.1kg in two replications and one of four feed treatment groups. Animal in T1 received ad libitum amount of urea treated barley straw alone, while those in T2, T3 and T4 received ad libitum urea treated barley straw supplemented with 175g, 350g and 525g of spineless cactus (DM basis), respectively. At the end of the feeding trial, four sheep were transferred to metabolic crates for the digestibility trial. Data were analyzed using standard analysis of variance (ANOVA) with help of GENSTAT statistical producer software.

Spineless cactus cladodes were high in water and ash content but low in crude protein and low in crude fibre. The energy content of cactus was 65% more than the urea treated straw. The effect of increasing level of spineless cactus on feed and water intake and weight gain is presented in Table I. With increasing level of cactus, there were significant increases in DMI (P <0.001) and body weight performance (P <0.05) while deceased in water consumption (P <0.001). The highest DMI was found in the last two treatments (101.81 and 96.48 BW0.75/d, respectively), compared with the first two treatments (94.35 and 87.57 for g/kg BW0.75/d, respectively). The trend of water intake of sheep with increasing level of spineless cactus pear is presented in Figure 1. Sheep in T1 consume more water (2 litres/d) than the other treatments (0.85, 0.51, 0.15 litres per day for T2, T3 and T4, respectively). In East African countries, during the drought season animal daily travelled for more than 14 km to reach to watering point (Ndikumana, 2002). This justify cactus's extremely important role in saving drinking water for livestock during the dry season. The highest body weight gain (51.9g/d) was found when sheep received 350g DM of cactus (T3), while the lowest was in the control diet (26.8g/d). About a 22% body weight improvement was achieved in this study, which is quite interesting as animals loss body weight normally during the dry season, although cactus pear is abundant and succulent in this season. In this study it was evident that cactus pear supplementation improves diet digestibility. The metabolism trial demonstrated that available energy intake (DOMI or TDNI) was directly related to animal performance in the feeding trial. In conclusion, feeding cactus in combination with urea treated barley straw can significantly increased animal performance and feed intake, and significantly reduced water intake. Therefore, utilization of cactus pear as an animal feed could play a significant role in promoting sustainable livestock production by providing with an alternative feed as well as water source.

TABLE I.THE EFFECT OF INCREASING LEVEL OF SPINELESS CACTUS ON FEED AND WATER INTAKE AND WEIGHT GAIN

Parameters	Treatments				LSD
	T1	T2	T3	T4	
Gain (g/d)	26.8[b]	33.3[b]	51.9[a]	47.2[a]	12.26
Cactus DMI (g/d)	0	175	350	525	
Total DMI (g/kg BW0.75/d)	94.35[b]	87.57[c]	101.81[a]	96.48[b]	3.418
Water (l/d)	1.98[a]	0.78[b]	0.57[c]	0.18[d]	0.03

Means with different superscripts (a–d) in the same row differ significantly (P <0.001) LSD=Least significance difference

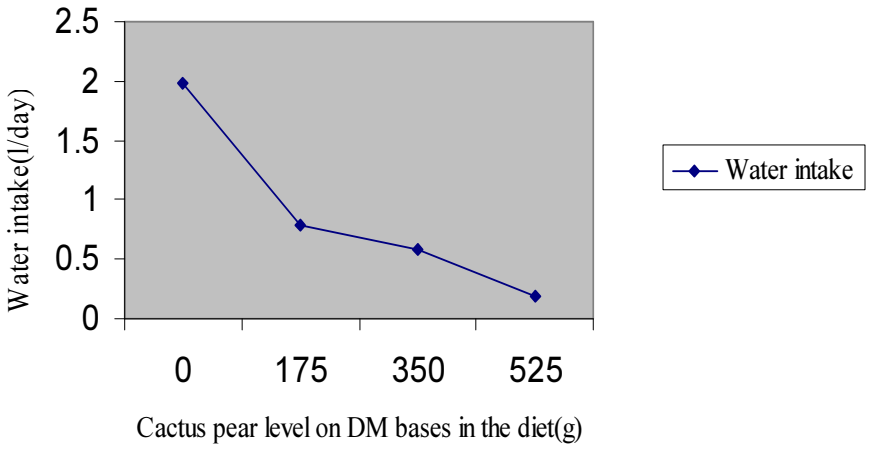

FIG 1. Trend of water intake of sheep with increasing level of spineless cactus pear

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STRATEGIES AND EFFORTS TO RESTORE SMALL RUMINANTS POPULATIONS IN SIERRA LEONE: A CASE STUDY OF SOUTH EASTERN SIERRA LEONE

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Two hundred (200) small ruminants, goats (180 doe and 20 buck), were restocked in forty-eight villages within three years. A twenty minute Rapid Rural Appraisal was conducted in each operational village for two weeks to know the communities and animals reared before and after the war. Participatory Rural Appraisal followed immediately. Beneficiaries used this opportunity to decide how they wanted the restocking to be done. During PRA, animal rearing communities comprising 12 men and 8 women were selected by the entire communities in each village, with no interference from thieves. Each group was headed by a Chairman, who organized the entire village to formulate animal rearing by-laws. Criteria were developed for selection of villages, group members and construction sites. During the first year, 20 villages were selected.

Each village was restocked with 10 goats (9 doe and 1 buck).At the end of first year, each restocked village repay 5 doe and 1 buck. 120 goats were collected at the end of first year and redistributed to 12 villages. At the end of second year, 72 goats were recollected from 12 villages and the project contributed 8, which were later redistributed to 8 villages. At the end of the third year, the 8 villages repay the same number of goats and this same quantity was redistributed to the remaining 8 villages (5 doe and 1 buck). Remaining goats in each village was shared to either family household or compound. Town thieves receive two female goats on behalf of the village and animal rearing groups are compensated at the end of redistribution. Small ruminants are the most expensive items in rural Sierra Leone when compared with other items weight by weight. Small ruminant restocking helped built damaged and burnt down houses, restart life, pay school fees and above all bring unity among scattered families or community. Adopting the above methods will increase small ruminant population within a short period in war torn countries. Constant attention, vigilance, cooperation and unity are means to successful restocking.

BASELINE ASSESSMENT MARCH 2004

Location	Number of goats	Total
Largo	14	14
Gondama	3	3
Bandajuma	12	12
Jimmi bargbo	6	6
Total	35	35

ANIMALS RESTOCKED JUNE2004.

Location	Number of villages	Number of goats
Largo	6	60 (54 doe and 6 buck)
Gondama	3	30 (27 does and 3 buck)
Bandajuma	5	50 (45 doe and 5 buck)
Jimmi bargbo	6	60 (54 doe and 6 buck)
Total	20	200

GOAT EVALUATION 2007

Location	# of villages	Animals Restocked	Birth	Death	Sold	Loan	Others	Current #	Total #
Largo	6	60	247	64	14	30	13	126	307
Gondama	3	30	110	19	12	14	5	60	140
Bandaguma	5	50	188	38	12	30	5	103	238
Jimmi Bargbo	6	60	206	49	11	36	3	107	266
Total	20	200	751	170	49	110	26	396	951

REMOTE SENSING AND GIS APPLICATIONS TO DETERMINE THE POTENTIAL GRASSLAND AREAS TO BE UTILISED BY BEEF CATTLE IN THE HIGHLANDS OF EASTERN TURKEY

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In this study, it was aimed to determine the potential grassland areas in the highlands of Eastern part of Turkey with special reference to Kars Province located in the East, using Remote Sensing (RS) techniques and Geographic Information Systems (GIS) applications. Kars province has a unique place in the region in terms of both the number of cattle and sheep (large and small animals) and the larger area of grassland available [1]. In general, beef cattle production is carried out under extensive conditions and based on grasslands in Eastern part of Turkey where Kars Province is located and 41.4% of the whole grassland area of the country is present [2].

Study area covered provincial boundaries of Kars with an altitude of above 2000 m. The area of Kars province is 918.117 ha. It lies between 260 000-390 000 km East, 4 420 000 - 4 530 000 km North according to UTM Geographic Coordinate System. Ardahan province is in the North; Agri in the South; Erzurum in the West and Armenia in the East. Figure 1 shows geographical location of the study area.

LANDSAT 5 TM satellite images taken in 2005 were used and land use and land cover classification maps were produced using GIS. In order to determine the status of grasslands, red (0,45-0,52 μm), near infra-red (0,52-0,60 μm) and infra-red (0,63-0,69 μm) bands of Landsat images were used and unsupervised classification was applied and the distribution map of grasslands showing the present status was produced (Figure 2).

It was found that 2/3 rd of the total area of Kars province is grassland. However, in terms of plant cover density this accounts for only 1/3 rd of the total area. In other words, only 181 275.7 ha of the 638 393.5 ha of whole grassland area is in a better status in terms of plant cover to be utilised by cattle. It was also found that the best quality grasslands for beef cattle production lies in the North-west part of the province starting from the West of Sarikamis forests to the North-western range of Allahuekber mountains and to the foothills of Erdagi mountains (Figure 3).

It was concluded that in this region where the economy is based on animal production, determination of grassland areas, stocking rates, estimation of biomass available for grazing, the length of vegetation period and monitoring the change in grassland must be included in Regional Development Plans and the results obtained from this study can be beneficial for the improved beef cattle production in the Region.

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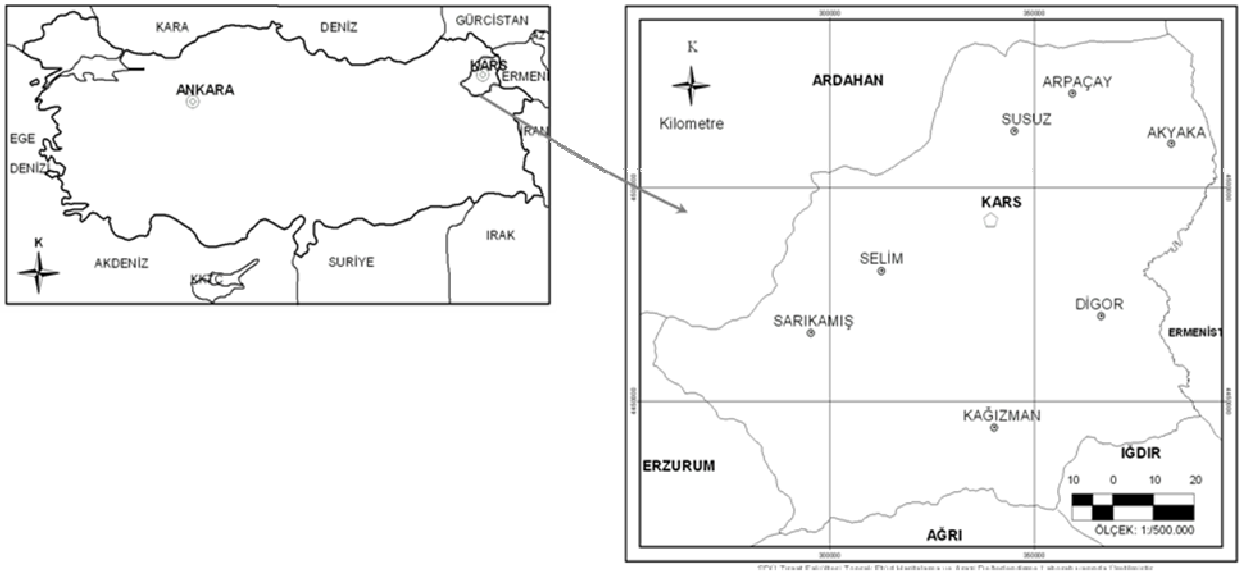

FIG 1. Geographical location of the study area

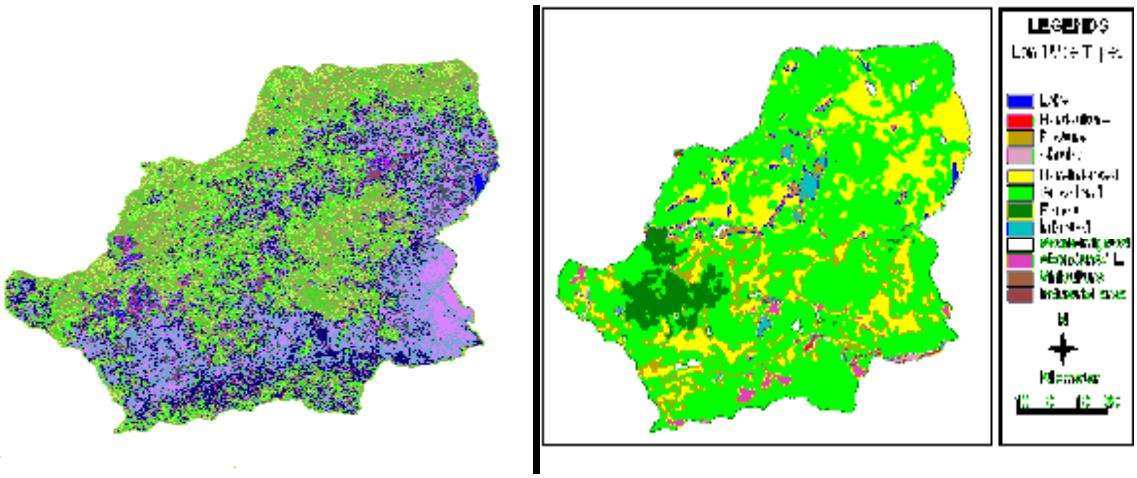

FIG 2. Classified satellite data (A) and the map produced from these data (B)

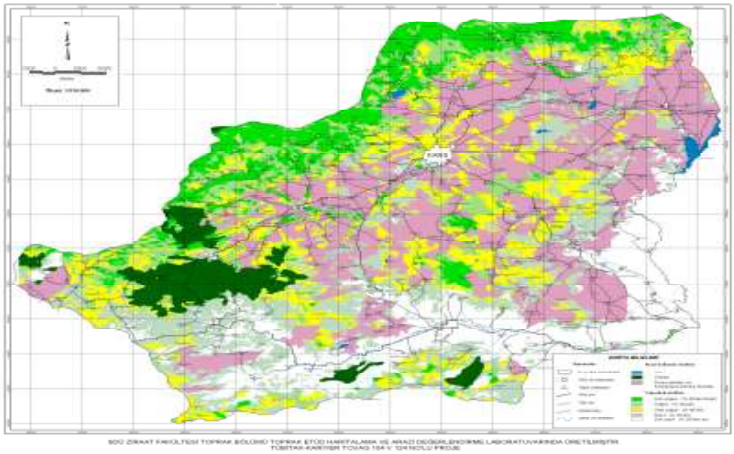

FIG 3. Land use map of Kars and grassland classification distribution.

RUMEN ECOLOGY OF SWAMP BUFFALO AS INFLUENCED BY DIETARY SOURCES

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Four, 3-year old rumen fistulated swamp buffalo bulls were randomly assigned to receive dietary treatments according to a 2 × 2 factorial arrangement in a 4 × 4 Latin square design. Factor A = 2 sources of energy (cassava chip and corn cob) and factor B =2 levels of urea in concentrate mixture (15 and 30 g/kg urea). The treatments were as follows, T_1 = cassava chip with 15 g/kg urea, T_2 = cassava chip with 30 g/kg urea, T_3 = corncob with 15 g/kg urea, T_4 = corncob with 30 g/kg urea. All buffaloes were supplemented with respective concentrate at 5 g/kg BW and 50 g/kg urea-treated rice straw was given *ad libitum*. Rumen fluid was collected and determined for direct count of protozoa and fungal zoospores, and group of bacteria were measured using roll-tube technique. Methanogenic and predominant cellulolytic bacteria populations were determined by using molecular techniques including real-time PCR and PCR-DGGE techniques. Briefly, Community DNA was extracted by the RBB + C method [1]. Primers for cellulolytic bacteria for PCR and real-time PCR were chosen from previously published sequences that demonstrated species-specific amplification [2]. For Methanogens primers were employed as described by Denman et al., [3]. For methanogen diversity, the V3 region of eubacterial *rrs* genes was amplified and PCR products were resolved on 80 g/L polyacrylanide gel with a 300-600 g/L denaturing gradient for 16 hr at 85 voltage. The gel images were captured using Photo documentation. All data were analyzed as a 2 × 2 factorial arrangement in a 4 × 4 Latin square design using PROC GLM of Statistic Analysis System.

It was found that types of energy source had an effect on protozoal population being lowest in buffaloes fed on corncob while fungal zoospores and groups of bacteria including total viable, cellulolytic, amylolytic and proteolytic bacteria were not changed by different energy sources and urea levels (Table I). Real-time PCR analysis results are presented in Figure 1. When compared among treatments, methanogenic bacteria were the lowest in cassava chip + 30 g/kg urea treatment while higher level of urea (30 g/kg) also resulted in lower methanogens. Predominant cellulolytic bacteria (*R. flavefaciens, F. succinogenes* and *R. alus*) analyzed were variable among treatments. *F. succinogenes* were found the highest across dietary treatments especially in cassava chip + 15 g/kg urea treatment as compared to *R. flavefaciens*, and *R. albus.* However, *R. flaveflaciens* were found highest in corncob + 15 g/kg urea treatment, while *R. albus* were highest in cassava chip + 15 g/kg urea and corncob + 30 g/kg urea treatments, respectively. The result analyzed by PCR–DGGE is presented in Figure 2. As shown, the methanogenic diversity were similar among treatments, which presented 7 bans of DNA. However, development of better conditions in the future could provide better bands in identifying the diversity and will offer useful information with regards to rumen microbial diversity in swamp buffaloes.

It could be concluded that corncob and urea at 15 g/kg could be efficiently utilized in the rumen and thus, could provide good fermentation end products for the host swamp buffaloes. Further research on effect of various levels of corncob replacement on meat and milk productivity in ruminants using molecular techniques should be investigated.

TABLE I. EFFECT OF ENERGY SOURCES AND UREA LEVELS ON MICROBIAL POPULATION IN SWAMP BUFFALOES

Items	Cassava chip		Corn cob		SEM	Significance[1]		
Urea, g/kg	15	30	15	30		C	U	C*U
Direct count, x10^5 cell/ml								
Protozoa	17.2	14.8	8.4	7.8	1.54	**	ns	ns
Fungal zoospores	2.2	2.4	2.4	2.2	5.27	ns	ns	ns
Roll-tube technique, CFU/ml[3]								
Total viable bacteria, × 10^{10}	4.9	5.5	5.5	4.4	4.95	ns	ns	ns
Cellulolytic bacteria, × 10^9	3.2	3.3	3.8	3.0	2.56	ns	ns	ns
Amylolytic bacteria, × 10^8	1.1	1.2	1.3	0.9	1.18	ns	ns	ns
Proteolytic bacteria, × 10^8	1.0	1.1	1.1	0.9	0.98	ns	ns	ns

[1] C = cassava chip vs. corn cob, U =15 vs. 30 g/kg urea, C*U = energy sources and urea levels interaction

SEM = standard error of the mean, ** P < 0.01, ns = non-significant (P > 0.05)

[2] CH_4 = (0.45 × acetic acid) – (0.275 × propionic acid) + (0.4 × butyric acid) according to Moss et al., (2000).

[3] CFU = colony forming unit

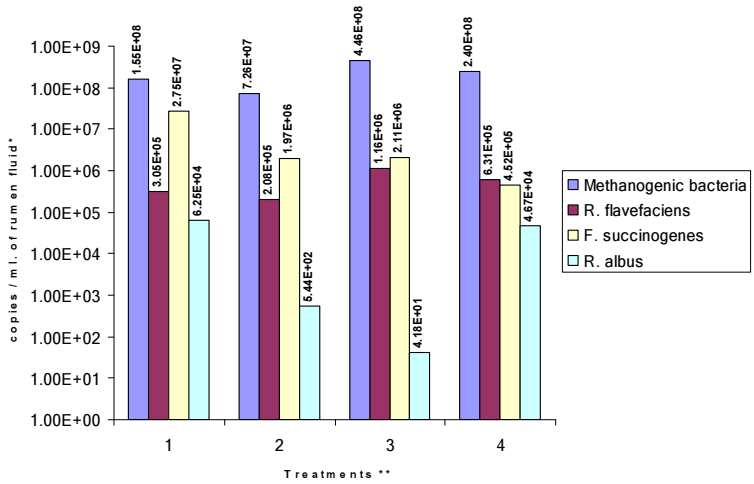

*FIG 1. Effect of energy source and urea levels on quantification of rumen bacteria by using real time PCR. * The values were calculated with total DNA purified from 1 ml. of rumen fluid. ** T1 = Cassava chip + 15 g/kg urea, T2 = Cassava chip + 30 g/kg urea, T3 = Corn cobs + 15 g/kg urea and T4 = Corn cob + 30 g/kg urea*

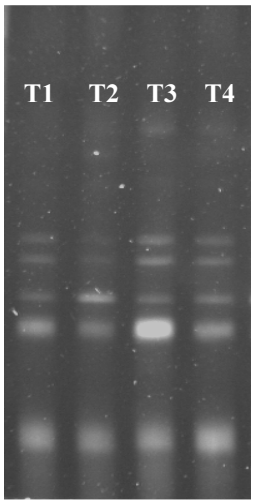

FIG 2. Photographed gel after DGGE eletrophoresis of 16s DNA fragments from 4 treatments of rumen fluid. Lane: T1 = Cassava chip + 15 g/kg urea, T2 = Cassava chip + 30 g/kg urea, T3= Corn cobs + 15 g/kg urea and T4 = Corn cobs + 30 g/kg urea.

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LIFETIME PERFORMANCE OF IMPORTED AND FARM BRED JERSEY COWS IN HOT AND HUMID CLIMATE OF TAMIL NADU, INDIA

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Milk production and reproduction records of imported and farmbred Australian and Danish Jersey cows were collected over a period of 29 years (1978 to 2006) from Exotic Cattle Breeding Farm, Eachenkottai, Tamil Nadu, India. This farm was established as a bull mother farm with the import of purebred Jersey heifers (n = 150) from Australia in 1978 and 1979. Subsequently, 120 purebred Jersey heifers were imported from Denmark in 1995. Investigations on lifetime performance of Jersey cattle (i.e., both imported and Jersey cattle born and bred on the farm) have been made. The data were grouped according to different age at first calving and were analysed [1] to study its influence on various lifetime performance traits.

The overall means (± se.) for longevity, productive herd life, lifetime milk production and lifetime calf crop of imported Australian Jersey cows (n = 119) were 2797.9 ± 96.3 d, 2032.9 ± 95.5 d, 7605.2 ± 426.1 kg and 3.24 ± 0.17 respectively and the corresponding estimates for farm bred Australian Jersey cows (n = 391) were 2494.7 ± 42.0 d, 1471.8 ± 44.8 d, 4759.3 ± 72.7 kg and 3.07 ± 0.09 respectively.

The overall means (± s.e.) pooled over all age at first calving group for longevity, productive herd life, lifetime milk production and lifetime calf crop of imported Danish Jersey cows (n = 97) were 2500.9 ± 71.4 d, 1744.3 ± 71.3 d, 4568.2 ± 212.0 kg and 2.75 ± 0.11 respectively and the respective values for farm bred Danish Jersey cows (n = 50) were 1823.4 ± 62.7 d, 695.3 ± 61.2 d, 1425.7 ± 143.6 kg and 1.45 ± 0.11 respectively.

The study revealed that the age at first calving had significant ($P < 0.05$) to highly significant ($P < 0.01$) effect on different lifetime performance traits. The imported and farm bred Jersey cows calving at younger ages had better performance with regard to all the lifetime performance traits studied. The longevities observed for the imported and farm bred Jersey cows were lower than the values reported for Jersey cows maintained at Livestock Farm, Palampur, India [2]. The lifetime milk production of imported Jersey cattle estimated in the present study was much lower than the value of 13,026 ± 382 kg reported for imported Jersey cows maintained at Jersey Cattle Farm, Banavasi, Andhra Pradesh, India [3].

Based on the performance study, it may be concluded that Jersey cattle did not perform satisfactorily at this hot and humid climatic conditions and it appeared that the imported Australian Jersey cows had better performance over other categories. The distinctly lower performance of farm bred Danish Jersey cows might be due to genotype x environment interaction and also due to the decline in general management during the period of maintenance of imported Danish Jersey cattle.

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EFFECTS OF THE LEVEL OF FEEDING ON CHANGE IN BODY RESERVES, CALVES' GROWTH AND POST-PARTUM OVARIAN ACTIVITY IN THE ZEBU GOBRA

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Forty-eight pregnant zebu cows were equally divided in 2 groups (GO and GC). They were fed on natural pasture and only GC received a concentrate, 1.76 FU and 489.9 DNM/kg DM. From the 7th day *post-partum* to the 4th of lactation, cows in each group were reallocated in 2 lots, a control (GOO and GCO) and a complemented lot (GOC and GCC). Body weight (BW) and score were monthly recorded in cows and calves. Plasma progesterone was determined from weekly sampled blood. Cows BW at calving didn't differ, 264.2 kg in GO and 269.7 kg in GC. Concentrate supply increased BCS (1.5 *vs.* 0.5, $P < 0.05$) at calving and complemented cows lost more BCS (-0.9 in GCO and -0.5 in GCC, $P < 0.05$). At week 21 of lactation, calves from cows in GCC gained more BW when compared to those in GOO (43.4 *vs.* 34.6 kg, $P < 0.05$). The intervals between calving and reactivation of the ovarian cycle were 31 d in GCC *vs.* 42 d in GCO, and 56 d in GOC *vs.* 78 d in GOO. The percentage of reactivation of the ovarian cycle was 50, 42.8, 27 and 25% in these respective groups.

EFFECTS OF A SHORT ENERGETIC SUPPLEMENTATION DURING THE TRANSITION PERIOD OVER THE OVARIAN POSTPARTUM REACTIVATION IN COWS

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As it is known the nutritional status of how the cow enters to a new production cycle is determinant for the negative energetic balance (NEB) behaviour during the postpartum period. The follicular development and the ovulation rate are directly related with the NEB[1]. There is also a positive correlation between the number of presented heats and the fertility at the first artificial insemination (AI)[2]. That's why it is important to understand the relation of the nutritional balance during the transition period on the reproductive axis in the cow.

The effect of a short energetic supplementation during the transition period over the return of the ovarian activity postpartum in dairy and beef crossbred cows was evaluated in the north-eastern of Honduras. During the period of November of 2007- September 2008 a total of 32 cows were used for this experiment, 16 treated (T1) and 16 as control (T2). The T1 group was treated beginning 21 d before the expected calving date and until 21 d postpartum. The diet consisted in the prepartum period in a palm nut by-product and wheat by-product (slow degradation energy sources) and in the postpartum: corn meal, molasses (fast degradation energy sources), and palm nut and wheat by-product. In both periods the supplement was offered in two pound per day divided in two portions. The T2 group was not supplemented at all and in was managed as usual in the farm. The diet was complemented for all the cows with grazing in *Brachiaria decumbens* pastures. In both groups the postpartum ovarian activity was measured by Progesterone detection in blood serum using the Radioimmunology analysis (RIA) technique. Samples were taken twice a week, starting at the day 18th postpartum and until the presence of an onset heat in the cow.

Both treatments showed a marked behaviour on the presence of first onset heat postpartum; been the mean of 87 d for the T1 cows compare with 133 d for the T2 cows, with a difference of 46 d between both groups ($P < 0.01$). This reflects that the nutritional balance has a direct impact in the reproductive axis in the cow during the postpartum period. As a side evaluation the T1 group showed a more marked body weight loss 11.44% compare with 5% weight loss in the T2, ($P < 0.01$), probably because when a cows enters the postpartum period with a higher nutritional status also have more energy (as fat) stored and available to move and use during the NEB postpartum.

```
Source        DF     SS    MS      F      P
 Tratamiento   1   8556  8556  36.20  0.000
 Error        14   3309   236
 Total        15  11865

 S = 15.37   R-Sq = 72.11%   R-Sq(adj) = 70.12%


 Individual 95% CIs For Mean Based on
 Pooled StDev
 Level  N    Mean  StDev  --+---------+---------+---------+-------
 1      16  133.38  11.13                        (-----*-----)
 2      16   87.13  18.67 (-----*----)
            --+---------+---------+---------+-------
80        100       120       140
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FIG 1. Anoestrus length vs. treatment

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Source        DF     SS     MS     F      P
Tratamiento    1  13110  13110  9.31  0.009
Error         14  19710   1408
Total         15  32820

S = 37.52   R-Sq = 39.95%   R-Sq(adj) = 35.66%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    ----+---------+---------+---------+-----
1      16  -103.13    (---------*--------)
2      26   -45.88                      (---------*--------)
                     ----+---------+---------+---------+-----
                       -120       -90       -60       -30
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FIG 2. Body weight loss vs. treatment

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ULTRA STRUCTURAL CHANGES INDUCED DURING TRANSPORTATION OF BOVINE OOCYTES

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The aim of this study was to examine the ultra structural changes induced in the bovine cumulus-oocytes-complexes (COCs) during holding in a medium commonly used for transporting ovaries or COCs from the slaughterhouse to the *in vitro* fertilization laboratory. Good quality COCs retrieved at the slaughterhouse were immediately transferred to Dulbecco's phosphate buffered saline (D-PBS) maintained at two different temperatures i.e. 2-4^{0}C and 35-37^{0}C and were held in this medium for 1, 3, 6 and 12 hours, respectively before processing for transmission electron microscopy.

Changes were seen in COCs held at 2-4^{0}C for 1h, which were more marked at 3h and later of the holding periods. Prominent changes in the corona cumulus cells included dilatations of the rough endoplasmic reticulum, the Golgi complexes, the mitochondria, ballooning of cristae and increased incidence of lipid droplets. Changes in the ooplasm included swellings in the mitochondria and the Golgi complexes, ballooning of cristae, reduced incidence of cortical granules and increased incidence of lipid droplets. In COCs held at 35-37^{0}C for 1h, changes were not marked, however, obvious changes were observed with increase in the holding period. These included changes in the corona cumulus cells such as marked intercellular spaces between corona cells, their elongation, vacuolation of the cytoplasm. Changes in the ooplasm, included enlargement of the perivitelline space, reduced incidence of the cortical granules, and presence of numerous large-sized lipid droplets. The incidence of these changes increased with increase in the holding period. The results of the experiment demonstrated that holding of COCs in D-PBS at two temperatures and for various lengths of time induced ultra structural changes in them, which were more marked at low temperature and longer holding periods.

These ultra structural changes in COCs could contribute towards low viability and subsequent development following *in vitro* procedures. In view of these observations it is preferable to culture COCs within one hour of their retrieval and a holding temperature of 35-37^{0}C is relatively better than the low temperature of 2-4^{0}C.

DIVERSITY FOR GDF9 AND FECB IN THREE-WAY CROSSBRED SHEEP AND CORRELATION WITH LITTER SIZE

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In this experiment, genetic structure and correlation between genetic diversity and reproductive traits in three-way crossbred sheep, which was Poll Dorset ×（Tan sheep × Small Tailed Han sheep）, were analyzed with PCR-SSCP on the base of candidate gene as GDG9 and FecB. The results indicated, as for GDF9, there were two types of genotype in three-way crossbred sheep, which was AA and BB, their frequency was 0.19 and 0.81 respectively. The average lambing numbers for individuals with AA genotype was 2.50, which was obviously higher than that which was 1.68 for individuals with AB genotype（$P < 0.05$). As for FecB, there were two types of genotype in three-way crossbred sheep, which was CC and CD, their frequency was 0.58 and 0.42 respectively. The average lambing numbers for individuals with CD genotype was 2.33, which was obviously higher than that which was 1.50 for individuals with CC genotype（$P < 0.05$). The heterozygote genotype of GDF9 and lambing numbers for three-way crossbred sheep had a negative correlation. The heterozygote genotype of FecB and lambing numbers for three-way crossbred sheep had a positive correlation.

TANDEM INHIBIN GENE IMMUNIZATION TO INDUCE SHEEP TWINNING

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Inhibin (INH) is a type of glycoprotein hormone secreted by testicular sertoli cell and ovarian granulose cell. The protein is structurally a heterodimer composed of two sub-units α and β. The development of animal follicle and the fertility could be improved by inhibin immunization. However, the utilization of active and passive immunization of inhibin immunization in animal production was restricted due to its difficulty in preparation and the higher cost. The innovation and development of gene vaccine made it practical and effective to improve the reproductive performance of sheep through inhibin immunization. To improve the follicular development, ovarian ovulation and number animals farrowed, different types of inhibin gene vaccines were constructed and used to immunize mice, rats, sheep and cattle, but the immunization was not so effective as expected. In this sense, it is necessary to explore the novel methods in inhibin production and the new strategies in immune reproductive technology. To examine the feasibility of developing the inhibin gene as vaccine for sheep, we constructed the recombinant plasmid of tandem inhibin gene and investigated its effect in sheep twinning after the gene immunization.

The synthetic DNA sequence of α-subunit (1 to 32) of inhibin in swine was used as the template to amplify the forward inhibin (*FINH*) gene sequence with the first double of primer. Then, the *FINH* was used as the template to amplify the reverse inhibin (*RINH*) gene sequence with the second double of primer. After the two sequences were combined, they connected with T carrier. They were transfected into the *E.coli.* by cultivating and multiplying, then the plasmid was extracted. After enzyme digestion, tandem inhibin gene and eukaryotic vector pcDNA3.1 were connected, the recombinant plasmid (pcDNA-*DINH*, pcDNA-double inhibin) expressed in the eukaryon would be obtained. The adjuvant, sheep complement 3d (sC3d), was cloned from the tissue in the liver of sheep by RT-PCR. The gene was inserted into pMD18-T-simple, pMD18-T and pGEM-T Easy vector. After pMD18-sC3, pMD18-sC3d and pGEM-sC3d vectors were constructed, it was sub cloned in pSG-DPPISS and pcDNA-*DPPISS-DINH-mC3d3* vectors expressed in the eukaryon to construct pcDNA-*DPPISS-DINH-sC3d3* (pcDNA dog preproinsulin signal double inhibin sheep complement 3d). Using the recombinant plasmid, pcDNA-*DPPISS-DINH* and pcDNA-*DPPISS-DINH-sC3d3* were transfected into BHK-21 cell with liposome. Then, the western blot was conducted to identify *DINH* and *DINH-sC3d3* fusion protein.

Sixty adult ewes of Gansu Alpin Merino were randomly divided into 5 groups, each with 12 individuals. The individuals treated with pcDNA-*DPPISS-DINH* were divided into two groups according to the immunization dosage of 0.2mg and 0.4mg. Similarly, the sheep transfected with pcDNA-*DPPISS-DINH-sC3d3* were divided according to the dosage of 0.3mg and 0.6mg. The comparative group was treated with 2ml of saline water. The successive three gene immunizations were conducted every 20 d and the first immunization was done 60 d before mating. The method of immunization was intramuscular injection. Artificial insemination was conducted after oestrus.

In the experiment, the inhibin fusion plasmid constructed included 32 amino acid sequence at N-terminal in α-subunit, in addition, through tandem two antigenic determinants; the molecular weight of the immunogen was increased. The immunity efficiency was strengthened. The gene immunity can increase animal reproductivity with fusion plasmid. The coding region of the complement C3d gene in sheep had 909 nuclesidases that coded 303 amino acid residues (GenBank Accession: EF681138) [1]. That the complement and antigen were injected in animals can increase the immune reaction of the antigen. After BHK-21 cells were transfected with the recombinant plasmid pcDNA-*DPPISS-DINH* and pcDNA-*DPPISS-DINH-sC3d3*, expression level was increased. The results of sheep twinning after tandem inhibin gene immunization were shown in Table I.

TABLE I. THE REPRODUCTION RATE OF SHEEP IN DIFFERENT GROUPS AFTER THREE IMMUNIZATION

Group	Twins	Monotocous	No lambing	Total	Number of lambs born	Twinning rate
0.2mg *DINH*	1	8	3	9	10	11.1^{b}
0.4mg *DINH*	1	7	4	8	9	12.5^{b}
0.3mg DINH-sC3d3	2	6	4	8	10	25.0^{c}
0.6mg DINH-sC3d3	1	6	5	7	8	14.3^{b}
Control	0	8	4	8	8	0^{a}

Note: Value in the same column with different small superscripts mean significant difference values ($P < 0.05$); same superscripts mean no statistical difference ($P > 0.05$).

In this experiment, α-subunit (1 to 32) gene in tandem inhibin was cloned. The new type of molecular adjuvant, C3d, was cloned from the tissue in the liver of sheep. The recombinant plasmid, pcDNA-*DPPISS-DINH* and pcDNA-*DPPISS-DINH-sC3d3* were constructed successfully and expressed in the eukaryon. After immunization, the twinning rate of sheep was 12.5% and 25.0%, respectively, which was significantly higher ($P < 0.05$) than the control group. The construction of recombinant plasmid of tandem inhibin gene made the theoretical and technical basis for developing the inhibin gene as vaccine for sheep.

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GENETIC DIVERSITY IN ALPACA (*VICUGNA PACOS*) POPULATIONS USING 10 MICROSATELLITE MARKERS

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Alpacas from Puno, Junín and Huancavelica were analysed by a panel of 10 microsatellite markers [1] in order to determine the level of genetic diversity among these populations. A sample of two hundred and sixty five non-related alpacas showed allelic polymorphism identifying a total of 144 microsatellite alleles. Alleles number ranged from 6 (YWLL40) to 28 (YWLL08), with a mean number of alleles per locus (MNA) of 14.40. The polymorphic information content ranged from 0.635 (YWLL40) to 0.942 (YWLL08) with a mean of 0.7975, while expected heterozygosity (H_E) ranged from 0.692 (YWLL40) to 0946 (YWLL08) with a mean of 0.8207. All loci except LCA37 (heterozygote deficit, $P < 0.01$) were in HWE and all populations except one from Muñani - Puno (heterozygote deficit $P < 0.01$) were in HWE. All population pairs were genic and genotypic differentiation (exact G test) except for two Junin populations ($P < 0.01$).

The relative magnitude of gene differentiation among populations was evaluated with F-statistics, frequency of private alleles [2] and number of migrants [3]. F_{IS} value for all populations was 0.014 (Rho_{IS} = 0.015) with the highest value of 0.046 for Sector Carniceria B – Huancavelica. No significant ($P > 0.01$) inbreeding effect (F_{IS}) was detected in alpaca populations. F_{IT} value was 0.037 (Rho_{IT} = 0.0318) for all population and F_{ST} value for all populations was 0.024 (Rho_{ST} = 0.0171). A F_{ST} value of 0.024 ($P < 0.01$), implied that 97.6% of the total genetic variation was from genetic differentiation within each population and only 2.4 % of the genetic variation existed among populations. Two populations from Huancavelica (Sector Pallccapampa and Sector Carniceria A) have highest F_{ST} value (0.753) and Rho_{ST} (0.736) with a frequency of private alleles of 0.0207 and number of migrants of 7.8359 in all alpaca populations. A Neighbor-joining tree was constructed based in Nei's genetic distance D_A [4] and standard genetic distance D_S [5] using DISPAN software. Four cluster were identified (Fig. 1 and Fig. 2): Junin (Cochas and Pachacayo), Puno (Muñani and Lacchoc), Huancavelica 1 (Sector Carniceria A) and Huancavelica 2 (Sector Pallccapampa).

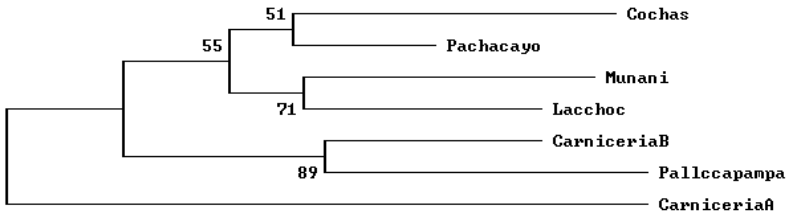

FIG. 1. Neighbor-joining dendogram of seven alpaca populations based on D_A Nei's distances using DISPAN software. The digits at nodes are the per cent occurrence in 10000 bootstrap replicates.

Individual assignment test and Bayesian clustering analysis [6] were used to evaluate population structure using Structure v2.2 software. Structure analysis showed three clusters (Fig. 3): (i) Sector Carniceria A - Huancavelica, (ii) Sector Pallccapampa – Huancavelica (include Sector Carniceria B) and (iii) Junin - Puno (include Cochas, Pachacayo from Junin, Muñani from Puno and Lacchoc from Huancavelica). Substancial genetic differentiation between Huancavelica with Junin and Puno were found. The preliminar results showed that alpaca populations in Peru maintained high genetic diversity within populations and a low, although significant, genetic differentiation between

populations. This work was supported by grants from IAEA (TC Project PER 05/027), INCAGRO Project, Ministry of Agriculture of Peru (Research Contract No. 05-0010) and FINCyT Project, Ministry of Agriculture of Peru (Contract No 007 FINCyT-PIBAT 2007).

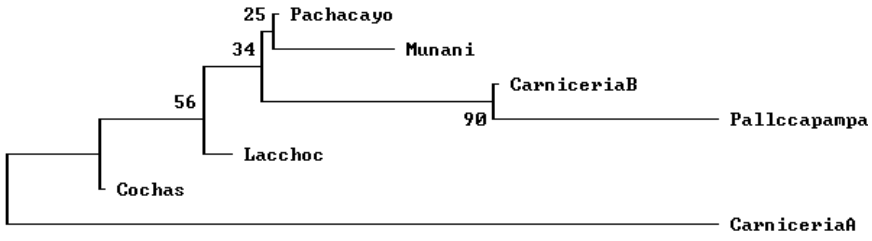

FIG. 2. Neighbor-joining dendogram of seven alpaca populations based on D_S standard genetic distances using DISPAN software. The digits at nodes are the per cent occurrence in 10000 bootstrap replicates.

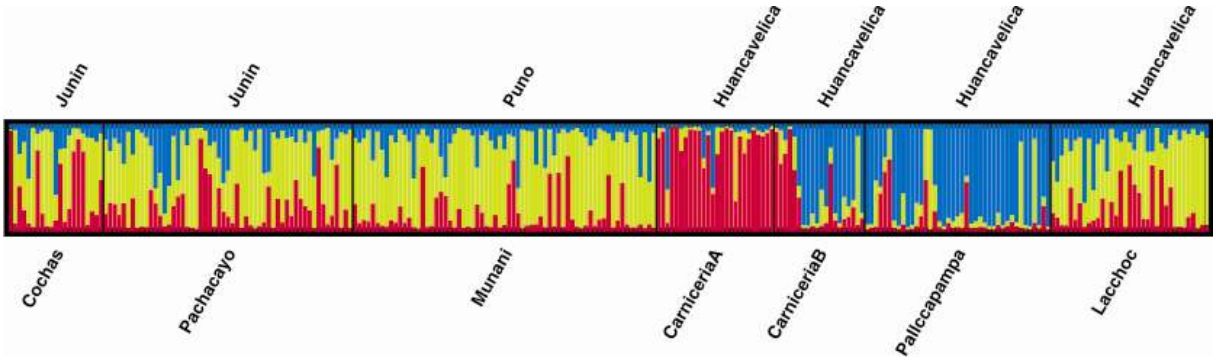

FIG. 3. Structure analysis for seven alpaca population from Junin, Puno and Huancavelica based in Bayesian clustering analysis (K=3) using Structure v2.2 and Distruct v1.1 software. The length of the segment in red, yellow or blue shows the individual's estimated proportion of membership in that cluster. Black lines separate the individuals of different populations. Populations are labelled below and regions are labelled above.

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IN VITRO PROLIFERATION OF SPERMATOGONIA AND PREPARATION OF RAMS FOR TRANSPLANTATION

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In order to enhance the proliferation of spermatogonia, and to screen a most effective technique for preparation of recipient rams, we have for the first time developed an *in vitro* system for proliferation of ram spermatogonia and then investigated the effects of three methods on depletion of endogenous spermatogonia.

Spermatogonia were enzymatically isolated from 3-month-old ram or caprine testes, purified by discontinuous Percoll gradient centrifugation, and cultured on the monolayer of testis somatic cells in DMEM/F-12 supplemented with 10 ng/ml of EGF and 0.01% 2-mercaptoethanol, with or without 2.5%, 5% or 10 % FBS in a humidified atmosphere of 95% air-5% CO2 at 37 ºC. Medium was changed every 2-3 d and the monolayer changed monthly. Cells and their morphological changes in culture were studied and photographed with a camera installed on a Nikon inverted microscope (TS-100). The cells were also stained with PGP 9.5, BrdU and C-kit to help identify the types, mitosis and meiosis of the germ cells. Spermatogonia were collected after culture and cryopreserved in liquid nitrogen. In the depletion experiments, twelve 5-month-old Han rams were randomly divided into four experimental groups. The animals in each group were treated with LH (5 mg), LHRH (200ug), buslfan (2.5mg/kg body weight) or placebo respectively every three weeks for three times. Rams were weighted and the sizes of their testes were measured every two weeks. After three weeks from the last treatment, the samples of testes were obtained. The samples of testes were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and viewed under a microscope.

A number of clones/colonies of spermatogonia were formed in a couple of weeks of culture in DMEM/F-12 containing 2.5% FBS, 10 ng/ml of EGF and 0.01% 2-mercaptoethanol, and the spermatogonia in the culture maintained over an experimental period of 3 months, while increasing FBS to 5% or 10% shortened it to 4-5 weeks as spermatogonia differentiated into spermatids rapidly as the concentration of FBS increasing. Complete removal of FBS resulted in poor proliferation and differentiation of spermatogonia. The following cells and structures were observed and recorded: Type A and type B spermatogonia, paired and aligned spermatogonia, bridges, clones/colonies of spermatogonia, spermatocytes, Sertoli cells and their junctions, spermatids, and premature spermatozoa.

Treatment with buslfan resulted in significant reduction in the size of testes and complete depletion of germ cells in rams, while LHRH and LH showed lesser and the least suppressive effect.

A SOLUTION ON IDENTIFICATION AND REARING FILES IN SMALLHOLDER PIG FARMING

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In order to meet government supervision of pork production safety as well as consumer's right to know what they buy, this study adopts animal identification, mobile PDA reader, GPRS and other information technologies, and put forward a data collection method to set up rearing files of pig in smallholder pig farming, and designs related metadata structures and its mobile database, and develops a mobile PDA embedded system to collect individual information of pig and uploading into the remote central database, and finally realizes mobile links to the a specific website. The embedded PDA can identify both a special pig bar ear tag appointed by the Ministry of Agricultural and a general data matrix bar ear tag designed by this study by mobile reader, and can record all kinds of inputs data including bacterins, feed additives, animal drugs and even some forbidden medicines and submitted them to the centre database through GPRS. At the same time, the remote centre database can be maintained by mobile PDA and GPRS, and finally reached pork tracking from its origin to consumption and its tracing through turn-over direction. This study has suggested a feasible technology solution how to set up network pig electronic rearing files involved smallholder pig farming based on farmer and the solution is proved practical through its application in the Tianjin's pork quality traceability system construction. Although some individual techniques have some adverse effects on the system running such as GPRS transmitting speed now, these will be resolved with the development of communication technology. The full implementation of the solution around China will supply technical supports in guaranteeing the quality and safety of pork production supervision and meet consumer demand.

ANALYSIS AND CONSTRAINTS OF GOATS' PRODUCTION IN NORTHERN NAMIBIA – A CASE STUDY IN ONESI AND RUACANA CONSTITUENCY

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The paper describes small-scale goat production in Omusati region, Namibia by considering the objectives, husbandry practices, indicators of productivity and barriers to small-scale goat development. The study is based on a questionnaire survey of sixty small-scale farmers keeping goats in Onesi and Ruacana constituencies. The results show that small-scale farmers (83%) mainly keep goats for prestige and as a store of wealth. The goats are kept under the communal grazing system, with limited supplementation (27%). On average 20 to 40 goats comprising mainly of local breeds and a few mixed breeds (local and Boer goats) are reared. The average kidding percentage is 42% with a kid mortality rate of 60% and average adult mortality rate of 27%. Limited marketing of goats occurs mainly in times of financial need and goat off-take rate is about 17.6%.

The goats are consumed during important social events. Milk output is low and milking is done by boys not adults and goat milk collected is used by the household to feed children. Few farmers de-worm their goats or control diseases in their stock. The major barriers to small-scale goat production in the study area include: (i) feeding, (ii) disease control; (iii) marketing, (iv) breeding and v) access to water and grazing land. The general strategy should focus on increasing productivity through improved extension services focusing on husbandry practices such as feeding, disease control (to reduce mortalities), and marketing services (to increase off-take).

Deeper analysis of the of production parameters regarding kidding rate, prolificacy, mortality rate and off-take rates shows that productivity levels on small-scale goat production farms are low compared to other semi-arid regions in Sub-Saharan Africa. Therefore, in order to improve the performance of small-scale goat production in the study area, there is a need to increase kidding, prolificacy and off-take rates and to reduce mortality rates especially by utilizing veterinary inputs (disease control) and improving feeding through livestock extension services. This should be accompanied by improvements in the breeding stock and marketing arrangements for goats, and by access to rangeland with adequate water and grazing. Through livestock extension services, small-scale farmers should be encouraged to adopt a number of existing animal husbandry technologies such as: (i) preservation and utilization of crop residues for supplementary feeding; (ii) de-worming of livestock; (iii) take farming as a business and learn to market their goats and livestock regularly utilize market incentive schemes; and (iv) rotational grazing on communal rangelands.

The lack of record keeping among small-scale farmers needs attention in particular in view of the globalization of agricultural markets, which may require farm records for traceability of products. The need for reliable and globally acceptable recording systems will therefore become inevitable for globally traded products. The government through extension service should promote record keeping among farming communities and organizations including small-scale goat farmers. It should also link recording keeping to institutions (universities e.g. UNAM) with technical capacity to process the data in timely manner and interpret the results for farmers' immediate use in management decisions.

Finally, there is great potential for goats to contribute more to the livelihood of the people in the NCAs. Early and major productivity gains can come through improved husbandry practices (feeding), veterinary services (to reduce losses to diseases) and marketing (to increase off-take). Breed improvement is a possibility but takes time and sustainability is in low-input smallholder conditions is uncertain

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ENVIRONMENTAL DESCRIPTORS INFLUENCING PERFORMANCE OF THE NGUNI ECOTYPES

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Nguni is an indigenous breed of cattle in the Southern Africa, specifically found in Swaziland, South Africa, Zimbabwe and Namibia. Due to the ten years of civil war, cattle numbers in Mozambique was reduced from 1.6 million head in 1976 to approximately 200,000 in 1992. After 1996, large numbers of Nguni cattle were imported from South Africa into Mozambique as part of the livestock restocking program. A nucleus herd of Nguni cattle was established at the breeding station, Posto de Fomento do Impaputo (PFI), near Maputo and next to the Swaziland border. PFI has also a separate breeding nucleus of the Landim cattle. Both these ecotypes are registered at the Nguni Breed's Society of South Africa. This study whose results are reported here aims to determine the environmental descriptors that influence the performance of the Nguni and the Landim cattle ecotypes in Mozambique. Results from the analysed data will help to provide information for sustainable country level utilization and conservation programs in the region. Reproductive and productive data, between 1997 and 2008, were analysed for the two ecotypes using PROC GLM from SAS (1999). Variation sources such as type of breed, place of origin of foundation herd, parity, season and year of calving were taken into consideration in preliminary runs.

Results of the preliminary runs on PFI herds indicate that the age at first calving (AFC) was 1089.2 ± 193 d and the calving interval (CI) was 437.6 ± 98.8 d on average for both ecotypes. For AFC there were interactions between the year and season of calving (Nguni or Landim; $P < 0.05$) and place of birth (Chobela, RSA or Impaputo; $P < 0.01$) for both ecotypes. CI decreased as the number of parities increased. A significant difference ($P < 0.0001$) was found on CI for the place of herd's birth, parity and interaction between the year and season of calving (dry and rainy seasons). It is concluded that there is sufficient data to demonstrate within and between population variations in the different ecotypes in terms of reproductive performance. A more complete analysis, which includes information from both ecotypes and data from South Africa as well as other similar environments, should be done.

These results can thus be used for the design and implementation of breeding and sustainable conservation programs for the Nguni and the Landim ecotypes under Mozambique and South Africa as well as similar environments.

MOLECULAR CHARACTERIZATION OF BULGARIAN LIVESTOCK GENETIC RESOURCES AND THEIR OPTIMIZED UTILIZATION FOR ANIMAL PRODUCTION

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This study was undertaken to determine the genetic structure and the diversity between 2 local cattle breeds from Bulgaria, the Rhodope Shorthorn and Grey cattle. A panel of 11 microsatellites was used for the evaluation. For these loci, allele frequencies, heterozygosity, HWE, genetic disequilibrium were determined. Both populations displayed a relatively high level of genetic variation as estimated by allelic diversity and heterozygosity. Heterozygosities ranged from 0.5424 /SPS 115/ to 0.8983 /TGLA 227/ for the Rhodope population and 0.6333 /TGLA 53/ to 0.9333 /TGLA227/ for Grey cattle, with similar average values for the two groups – 0.7858 and 0.7757.

This study contributes to the knowledge of the genetic diversity, genetic structure and to the molecular characterization of small populations on the brink of extinction. Since the actual implementation of a sustainable program for the conservation of animal genetic resources requires a wide variety of technologies and approaches it is now possible to characterize them at DNA level. Both Grey and Rhodope Shorthorn cattle breeds were genetically characterized by using DNA markers. The characterization of Bulgarian cattle local breeds with microsatellite loci is useful to identify high informative markers for each breed while simultaneously would facilitate the genotypic identification.

All loci were polymorphic and this indicates that the microsatellite markers used are suitable for genetic diversity study. The comparison between the two local breeds shows that they display a remarkable high variability. This clearly suggests that these breeds have potential value to be preserved as genetic resources. The highest value of Bulgarian local breeds is determined of the genes they possess as a source of their excellent adaptive capabilities, high resistance to diseases and ability of good meat and milk quality.

More work and analysis will be required in the future to increase the efficiency of studying a larger number of microsatellites. Additional information on productive, morphological, and fitness-related traits of these breeds is needed, however, as these factors should also be taken into account when ranking breeds for preservation purposes.

GENE EXPRESSION PROFILING OF DIFFERENTIALLY EXPRESSED GENES IN BULL TESTICLE BETWEEN DIFFERENT SCROTAL CIRCUMFERENCE USING DDRT

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To identify tissue-specific expression gene in testicle of differential scrotal circumference bulls and analyze the function of the specific gene on the development of the bull's scrotum in this study. The DDRT-PCR and Reverse Northern Blot Analysis were used to identify tissue-specific expression genes in bulls with differential scrotal circumference.

The experiment was designed sixty 6-month-old crossbreeds (Charolais with indigenous Fuzhou female). These were raised under the same age, cross generation, raising condition and management. When the feeding was over after 6 months, the scrotal circumferences of bulls were measured. Four bulls were selected and classified into two groups, and the difference of scrotal circumference is significant between the two groups ($P < 0.01$). A group was consisted of two bulls with larger scrotal circumference 26±2.5cm. The control group was two crossbreed bulls with smaller scrotal circumference 17±2.2 cm. When the scrotal circumferences were measured, the bulls were castrated by surgical operations. A piece of tissue (2 by 2 by 2 cm) was removed from the deeper area of the testis and stored in liquid nitrogen. A small section (0.5 by 0.5 by 0.5 cm) was used for total RNA extraction by using the TRIZOL reagent kit (GIBCO/BRL, Bethesda, MA, USA). The RNA was prepared for DDRT-PCR experiments and quantitative real-time PCR.

The results were shown that six genes corresponded to genes of known or inferred function; either the bovine gene or the likely human orthologue and three genes or ESTs were unknown. Bos taurus similar to galactosidase, beta 1-like; Bos taurus similar to Kinesin heavy chain isoform 5C; Bos taurus similar to ankyrin repeat domain protein 15 isoform and Bos taurus ebd-P2 pseudogene were founded both highly expressed in bulls which had bigger scrotal circumference by qRT-PCR. Their functions may be involved with sperm maturation in the epididymis, sperm protection and preventing the ascent of microorganisms into the adjacent testes and responsible for converting immature sperm into competent functional cells, and movement of spermatozoa.

TABLE I. RESULT OF SEQUENCE BLAST OF DIFFERENTIAL GENE EXPRESSION IN BOVINE TESTIS

No.	Primer	Gene bank	Similarity	Gene name
A54	$H2T_{11}A$, H-AP5	gi55415674	142/145 (97%)	Bos taurus ebd-P2 pseudogene
A55	$H2T_{11}A$, H-AP5			Unkown
A56	$H2T_{11}A$, H-AP5			Unkown
A57	$H2T_{11}A$, H-AP5	gi55415674	142/145 (97%)	Bos taurus ebd-P2 pseudogene
A58	$H2T_{11}A$, H-AP5			Unkown
A59	$H2T_{11}A$, H-AP5	gi76639092	223/224 (99%)	Bos taurus similar to F-box only protein 21 isoform2
A62	$H2T_{11}A$, H-AP6	gi54013468	238/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
A63	$H2T_{11}A$, H-AP6	gi54013468	239/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
A71	$H2T_{11}A$,H-AP7	gi76681130	292/293 (99%)	Bos taurus similar to septin 10 isoform 1
G31	$H2T_{11}G$, H-AP3	gi54013468	239/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
G53	$H2T_{11}G$, H-AP5	gi54013468	215/216 (99%)	Bos taurus mRNA for proline-rich protein P-B
G63	$H2T_{11}G$, H-AP6	gi54013468	239/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
C13	$H2T_{11}C$, H-AP1	gi54013468	234/240 (97%)	Bos taurus mRNA for proline-rich protein P-B
C16	$H2T_{11}C$, H-AP1	gi76609893	584/585 (99%)	Bos taurus similar to Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2), transcript variant 1
C18	$H2T_{11}C$, H-AP1	gi54013468	239/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
C42	$H2T_{11}C$, H-AP4	gi54013468	239/240 (99%)	Bos taurus mRNA for proline-rich protein P-B
C44	$H2T_{11}C$, H-AP4	gi76671770	480/485 (98%)	Bos taurus similar to ankyrin repeat domain protein 15 isoform a
C62	$H2T_{11}C$, H-AP6	gi76610658	392/395 (99%)	Bos taurus similar to galactosidase, beta 1-like
C72	$H2T_{11}C$, H-AP7	Gi83405409	460/470 (97%)	Bos taurus cDNA clone MGC:134370 IMAGE:8054193

ISOLATION OF DIFFERENTIALLY EXPRESSED GENES BETWEEN STEER AND BULL LONGISSIMUS BY SSH AND Q-PCR STRATEGY

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Numerous research studies have been conducted to assess growth and meat characteristic differences between bulls and steers. In general, results have indicated that bulls grow more rapidly (15 to 17%), utilize feed more efficiently (10 to 13%) to an age or weight end point, and produce higher yielding carcasses with less fat and more muscle than steers. Steers have a slowly growth rate, more intramuscular fat, tenderer meat as compared with bulls.

For further study on muscle gene expression profiles between bulls and steers, we constructed subtracted cDNA libraries between *Longissimus* muscles from three Chinese Simmental steers and three Chinese Simmental bulls with same age and same raising condition using suppression subtractive hybridization, genes that were differentially expressed in steer vs. bull *Longissimus* muscle were identified.

More than 300 clones were randomly selected from each subtracted cDNA library. By PCR analysis, 223 positive clones were isolated from the subtracted cDNA libraries, by steers as Tester, bulls as Driver, respectively.

By *Longissimus* muscles from steers and bulls cDNA as probes, high-throughput screening was carried out. We selected 84 differential expressed clones for further analysis, which showed that they represent 10 ESTs, all of them are known in cattle.

Three functional genes, which are *ACTG2*, *TPM2* and *IGF-1*, were chosen to do qRT-PCR to confirm the expression differentiation between steer LD tissue and bull LD tissue. The genes expressed in the former tissue were 1.96、2.41、2.89 times higher, respectively, than in the later tissue. These results implied that new candidate genes could be selected form the SSH library constructed in this research, and this could be a way to make the base of steer muscle special trait.

TABLE I. DIFFERENTIAL EXPRESSED EST IDENTIFIED FROM SUBTRACTIVE CDNA LIBRARY

Homologue gene	Frequency	NCBI accession No.	Identity %
DUSP26 dual specificity phosphatase 26	2	NW_001494415.1	99%
TPM2 tropomyosin 2	2	NM_001010995.1	97%
MUSTN1 musculoskeletal, embryonic nuclear protein 1	3	NM_001040589.1	97%
ACTG2 actin, gamma 2, smooth muscle	1	NM_001013592.1	92%
LOC521822 FADS2 Similar to fatty acid desaturase 2	3	NM_001083444.1	98%
IGF-1 insulin like growth factor 1	2	NM_001077828.1	97%
HSL lipase, hormone-sensitive	2	NM_001080220.1	97%
LOC505543 Bos taurus similar to KIAA0246 protein	4	NW_001494118.1	99%
SH3KBP1 SH3-domain kinase binding protein 1 soform 2	1	NW_001508842.1	98%
LOC616654 ankyrin repeat and SOCS box-containing 17	3	NW_001494789.1	98%

SESSION 2: EFFECTS OF NUTRITION, REPRODUCTION, GENETICS, AND ENVIRONMENTAL FACTORS ON ANIMAL PRODUCTIVITY

MANAGING LIVESTOCK IN DEGRADING ENVIRONMENTS

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While overgrazing is often blamed for environmental degradation, there is clear evidence that livestock are not inherently damaging to rangelands or farming landscapes, and, in fact, may be required for their sustained health and profitability. Moderate to heavy grazing has, in some cases created highly resilient and ecologically sound systems while under-grazing has resulted in dense woody growth and reduced species diversity [1]. Conversion of rangelands into intensive crop/fodder production has also led to progressive loss of diversity, species connectivity and ability to recover [2]. Well-managed livestock in either a grassland or mixed crop/livestock system offer a highly efficient method of increasing the production of high quality food with minimal environmental impact [3].

Although an ecological case can be established for the continued use of livestock in degrading landscapes, the reality is, livestock will only be grazed responsibly if the owner receives a benefit from the process. Importantly, by providing a potentially profitable option, the revegetation of degraded or partly degraded landscapes may take place through the expenditure of private rather than public funding. Given the vastness of the landscapes in question and the urban priorities in the expenditure of public funds, significant progress is only likely if profitable solutions are available.

This use of livestock may or may not contribute to the return of the landscape to its original state. In some cases stable vegetation that provides some of the functional benefits of the original landscape, combined with the productive benefits of a profitable livestock system may be the best option available. This then provides an opportunity to design a landscape based on a range of predetermined objectives which include both profit and ecosystem services. For example, in Western Australia, the revegetation of 10% of the 1 million ha of saline land with halophytic shrubs and salt tolerant forage has resulted in a system that provides valuable out of season feed for livestock, slows or reverses the elevation of saline groundwater tables and reduces salt and sediment run-off into waterways.

Meeting the nutritional and environmental requirements of livestock grazing degraded environments will always be a challenge. Both over- and under-grazing are likely to result in a loss of species diversity [4]. As livestock have the ability to select plants with higher digestibility, adequate nitrogen (crude protein) and low or manageable anti nutritional compounds, loss of diversity will be accompanied by a decline in nutritive value and palatability and reduced ability to deal with toxins [5]. In the case of over-grazing the decrease in diversity and nutritive value will be accompanied with a decrease in feed availability. Management of livestock, vegetation and the interaction between the two is critical for productive grazing of such fragile environments.

Where loss of diversity is a consequence of previous over-grazing, complementary feeding can improve feed intake, feed conversion efficiency and therefore productive potential. Examples include the provision of energy supplements to improve the utilisation of plants that contain high levels of non-protein nitrogen or to facilitate the breakdown of anti-nutritional compounds in the forage. Similar strategies can be applied to revegetation with selective planting to complement the composition and availability of other feed resources. Where revegetation is an option, there will be additional benefits in assessing the plant options for palatability, nutritive value, anti-nutritional properties, shelter and possible medicinal properties as well as selected ecosystem benefits. These do not need to be long-term and expensive plant breeding projects but may be well designed and localised screening programs based on indigenous plant species with known natural advantages. For reasons described above revegetation should complement rather than replace existing feed resources.

Strategies to improve the intake and utilisation of forages in degraded environments have the potential to induce further degradation if not accompanied by improved grazing management. Technologies are now available that allow remote monitoring of livestock condition and behaviour. This type of direct information provides incentive and opportunity for livestock managers to tactically manipulate feeding and production. For example, monitoring change in both livestock and forage are grazing management options. While monitoring feed supply is the more traditional method advocated in high input grazing, more information and control may be available from direct livestock monitoring in the more extensive, low input systems that characterise degraded landscapes. Livestock will begin to lose weight long before feed supply is exhausted and live weight change may provide information on both available biomass and diversity of feed sources. Similarly, animal behaviours, such as time spent walking, grazing and feeding are related to feed supply and diversity. Grazing and feeding behaviour can be further assessed with the use of stable isotope techniques applied to feed and water intake. These techniques have the potential to optimise the combination of livestock production and ecological stability that will be required for the long-term productive use and revegetation of degraded landscapes.

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FOUNDATIONS, FALLACIES, AND ASSUMPTIONS OF SCIENCE FOR LIVESTOCK DEVELOPMENT

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Animal improvement in the developed world in the last 50 years has been able to assume consistent management systems based upon intensive inputs set within a temperate environment with all the physical infrastructures of Western society including rapid communications, financial systems up- and down-stream companies and agencies waiting to buy and sell – plus a compliant consumer market. Bu contrast, the social, physical and economic environments in developing countries where most livestock are located are very variable, often unreliable and distant from large markets. Further the livestock resources are diverse. Even more important, these animals often represent the total life resources of those who care for them – a dependency often extending to the remote rural community. In 2007 half the world population lived in rural locations and 3 billion of these are dependent upon or associated with livestock.

While there is a move in a few locations to set up intensive livestock units to serve the growing mega-cities in urban parts of the developing world, a high proportion of livestock and their keepers seek a sustainable life for themselves and their extended families in rural areas. Contemplating Western technology transfer to improve indigenous livestock in remote rural communities is an entirely different and risky matter. It is well known that deep consideration must be given to the social structure, customs and values of the livestock keepers about which much has been written elsewhere.

Genetics is always seen as a major tool for bringing about improvements in livestock production and health. In the last five years a major new issue has emerged in the application of genetics to livestock improvement which has yet to have its impact on the thinking and genetic models used in the West.

Molecular genetics opens new horizons for radical change in the life processes of animals with great potential for improvement in health and production. This is uncharted territory. The scientific imagination sees the universe of DNA as an opportunity to reshape biodiversity to yield ever more efficient economic performance. Caution is needed. Emerging genome research reveals that control of gene expression is far more complex than conveyed in our current genetic models. In developing countries use of radical molecular genetics presents us with issues of risk, accountability, authority, power, ownership, morality, the nature of animals and the sustainability of life for rural people in remote areas.

Our current genetics models on which manipulation of DNA has been built derive from the discovery of the molecular architecture of DNA by Watson and Crick in 1953. This was followed in 1958 by Crick's announcement of the "Central Dogma of Molecular Biology" which he reaffirmed in Nature in 1970. This states that information flows only one way: DNA-RNA-Protein described by the terms Replication, Transcription, Translation. Our linear Enlightenment thinking also led us to conclude "one gene – one protein"

This model led to the concept of "Junk DNA" that stated that most DNA is inactive and left over from the evolutionary process. Nevertheless, based on one-gene one-protein we expected the Human Genome Project (HGP) to reveal up to half a million genes in the human genome. It was an incredible surprise to find only 20,000, no more than the nematode Caenorhabditis elegans. With hundreds of thousands of mammalian proteins it is clear that 20,000 genes multitask. This means it is difficulty to anticipate effects of transgenes.

The new situation has been recently summarized by Dr. John Mattick of Institute of Molecular Bioscience in Brisbane that challenges the evolutionary junk theory by saying “Most of this DNA is transcribed into non-coding RNA and consists of a hidden layer of gene regulation that directs the development of complex organisms. Expression depends on which tissue the genome is directing”. In the last two years there was an Editorial in Nature editorial arguing we must go back to the beginning of molecular genetics. The ENCODE Project which examined 1% of the human genome in fine detail concluded the same. Dr. David Schubert in Salk Institute considers that GM foods have been launched too early with unknown risks. Stress proteins are emerging as a highly important factor in modifying gene expression.

Perhaps of greatest importance to the question of using molecular genetics in the developing world for livestock improvement is the growing evidence for Epigenesis. The mammalian genome is a complex of DNA, RNA of many types and proteins, which seem to be engaged routinely in passing information around that modifies gene expression. Since this raises the question of information being fed into the genome and the genes from the environment, the whole issue of adaptation of livestock to differing environments is open to review. There is even a case for rethinking the total rejection of Lamarckism.

We face risks from genetic engineering that are asymmetric: Black Swan risks with Low Probability and High Impact. Conventional risk assessments using mathematical probabilities not good enough. Once is too many.

Clearly we should not be against research. In fact more is needed. The main issue is the need for great caution in applying molecular genetic techniques and projects based upon them until we gain better understanding of the inner world of the mammalian genome.

EARLY STIRRINGS OF LANDSCAPE GENOMICS: AWAITING NEXT-NEXT GENERATION SEQUENCING BEFORE TAKE-OFF

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Development of low impact sustainable agriculture and a growing use of adapted breeds is of priority to most countries in the world, and is in particular of key importance to developing countries/emerging regions. The level of adaptation of livestock breeds to their environment has to be measured in order to reach better understanding of the relationship between environment and the adaptive fitness of livestock populations, as well as to favour production systems based on adapted breeds. According to ILRI [1], landscape genomics is a long-term promising approach when regarding study of adaptation. A wide definition considers this field being an "emerging area at the interface of natural resources management and the genome sciences" (Dr Williams, Nicholas School of the Environment and Earth Sciences, Duke University, USA). Population genomics, geo-environmental and statistical methods can be combined to assess the level of association between specific genomic regions of living organisms and environmental factors [2, 3, 4]. Processing of these many association models is based on data sets made up of hundreds of environmental parameters and thousands of molecular markers of thousands of animals. Applied to livestock genetics, landscape genomics purpose is to uncover what genetic material fits with which region of the world. A widespread use would support decision making and recommendations in order to favour raising of the right breed in the right place, thus leading to sustainable and adequate income for resource-poor farmers, reducing impacts on the environment, and making better goods available to consumers.

Early stirrings of landscape genomics applied to livestock research were heretofore limited to a few studies carried out on sheep [4] and goats [5]. They were based on the use of SAM software (http://www.econogene.eu/software/sam/) as well as of population genomics theoretical approaches for results validation (see [6], and references therein). In comparison with the potential of landscape genomics, existing analyses were restricted to approximately one hundred environmental parameters related to a reduced number of genetic markers (<1'000) for a maximum number of 2'000 animals. Let us see how these figures are expected to evolve within a rather short lap of time.

On the one hand, the number of available environmental databases (NASA SRTM, NASA MODIS, Norwich CRU, etc.) gradually increases and their quality improves (better resolution). Most of these global data sets are freely available throughout the Internet and can be used for a comparable description of production environments worldwide. No revolution will occur in the short-term with this category of information, but the diversity of the sources (new satellites, new environmental monitoring capacities) and the quality of data will go on improving progressively.

On the other hand, the amount of molecular data to be analysed will more than likely very shortly and rapidly expand (P. Taberlet, personal communication). In 2004, after the 13 years and a 3 billion dollars human genome sequencing project, the American National Institutes of Health (NIH) proposed a new challenge: sequencing one human genome for $1'000 [7]. The objective has not yet been reached, but next generation of sequencers will allow researchers to get to genomic data faster and at a lower cost. In particular, theoretical potential of Single-molecule/Nanopore sequencing is undeniable [8]. Based on this technology, with a 100 nanopores in parallel, a mammalian genome could be sequenced in 24 hours with the main cost being the chip itself, probably around $1'000 [9]. Several alternative low cost sequencing technologies are under way, and the $100 complete sequencing of individuals is about to become real (P.Taberlet, personal communication). It means that in the immediate future, any research project in livestock genetics will be given the opportunity to analyse the entire 3.4 billion base pairs per animal.

We therefore have little time to pave the way. Naturally, researchers in molecular biology already started the conception of new approaches to analyse the large data sets produced by the "next next" generation sequencing technologies. On the GIS & statistical side, it is necessary i) to stress the importance of geographic coordinates recording in any new project with animal sampling campaigns, and ii) to reinforce software and to make them more sophisticated. As the association analysis process is rather straightforward, the challenge will mainly consist in improving algorithm's efficiency and robustness. This is important because now the perspective clearly is the opportunity to analyse the complete genome of hundreds of thousands of sampled animals worldwide, and to compare it to hundreds of variables constituting progressively enhanced environmental data sets. In other words: models potentially constituted of billions of base pairs time hundreds of eco-climatic parameters corresponding to hundreds of thousands of individuals. In such a context, let us imagine the richness of the information collected by the GLOBALDIV project (www.globaldiv.eu) with the gathering of data from different European projects on livestock genetics (RESGEN, ECONOGENE, AVIANDIV, EFABIS)! Forthcoming global analyses will really push us towards a new dimension of analysis, and therefore lead to new ways to assess genetic diversity. From then on, all conditions will be right for landscape genomics to take off, and to make it possible to fully profit from its contribution in order i) to better understand animal's adaptation to their local environment, and ii) to support adequate decision making in livestock biodiversity conservation, i.e. raise the right breed in the right environment.

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INTERACTIONS AMONG NUTRITION, HEAT STRESS, AND REPRODUCTION IN TROPICAL CATTLE

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Efficient reproductive performance of lactating dairy cattle in tropical/subtropical environments throughout the world is impacted by a multiplicity of factors such as: the physical environment, social-economic status of producers, available nutrients, adaptability and genetic composition of cattle, intensive or extensive management systems, and available reproductive technology. Seasonal periods of reduced fertility are associated with concurrent increases in temperature and humidity, availability of nutrients, and elevations in body temperature detrimental to ovarian function, oocyte competence and embryo development.

Implementation of heat abatement facilities can enhance both pregnancy rates and milk production. However, pregnancy rates are not restored to levels of the cooler season. Bulls that transmit a high tolerance to heat stress have daughters with higher pregnancy rates, a longer productive life, but lower milk yields [1]. Continued selection for milk yield without consideration of heat tolerance likely will result in greater susceptibility to heat stress. Various genes regulate heat tolerance such as the slick hair gene that contributes to a greater tolerance of lactating dairy cows to heat stress that likely improves fertility [2]. Furthermore, *Bos Taurus* x *Bos indicus* embryos, in response to an *in vitro* heat shock, have a higher rate of blastocyst development acquired through *boss indicus* genes from the oocyte or imprinting of certain embryonic paternal genes [3]. With the known gene sequences of the bovine genome, identification of heat tolerance genes of *boss indicus* breeds offers the potential of introducing these genes into less heat tolerant breeds. Upgrading of heat tolerant *boss indicus* cattle to certain percentage of dairy breeding increases milk production and sustains tolerance to heat stress while maintaining a level of resistance to parasites and diseases.

An array of refined reproductive technologies is available to better manage the reproductive performance of dairy cows [4]. Synchronized timed artificial inseminations (TAI) prior to the heat stress period will improve herd pregnancy rate. Since high temperature causes embryonic death during the first three cleavage divisions, embryo transfer of more advanced healthy embryos at day 7 will bypass the early heat sensitive period to partially restore pregnancy rates. Development of vitrification procedures for storage of *in vitro* produced embryos, that develop normally post-transfer of the embryo, will increase the impact of this reproductive strategy. Dairy heifers can now undergo TAI with high fertility during summer to avoid seasonal breeding and parturitions. Optimized TAI programs coupled with heat abatement systems reduce the impact of poor oestrus expression during summer. With intensive proper management of bulls for natural service during both cool and hot seasons, herd pregnancy rate was comparable to that of a re-occurring TAI program. This resulted from intensive bull management and increased service opportunities for bulls during a defined period. However, the net cost per cow per year was greater for a natural service program than a TAI program.

Feeding lactating dairy cattle to meet their nutrient requirements is a challenge depending upon location, environmental conditions, and management systems. A production comparison was made of lactating cows over 14 weeks when grazed on annual rye- rye grass pasture or housing in a free-stall barn in which a fat supplement, enriched in linoleic and linoleic fatty acids (+/-), was part of a grain supplement (pasture group) or total mixed ration (free-stall group). Cows on pasture produced less milk between 8 and 14 weeks postpartum [5]. Cows on pasture + fat had an earlier return to oestrus, accumulated more progesterone in plasma during the postpartum period, and had higher conception rates. The occurrence of no oestrus expression during re-occurring ovulatory periods emphasized the importance of TAI programs that are not dependent on heat detection.

Inclusion of specific nutraceuticals in the diet to improve reproductive function offers an exciting new dimension to dairy cattle management [6]. Feeding organic Se-yeast pre- and postpartum in summer to Florida dairy cattle improved selenium status, enhanced neutrophil function and humoural immune/antibody responses, reduced incidence of fever, and improved both uterine health and subsequent fertility. Sequential feeding of diets enriched in linoleic fatty acid (i.e., pro-inflammatory) during the pre- and early postpartum period followed by diets enriched in omega-3 fatty acids (i.e., anti-inflammatory) from fish oil (i.e., EPA and DHA) in the breeding period benefited milk production and pregnancy rate per TAI. Feeding a pro-inflammatory fat supplement improved immune status (i.e., neutrophil function, cytokine production and secretion of acute phase proteins) whereas the anti-inflammatory diet enhanced embryo survival. Such nutritional approaches offer the potential to reduce use of hormones and antibiotics.

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ORAL PRESENTATIONS

EFFECT OF VARIOUS LEVELS OF COCONUT OIL ON METHANE EMISSION FROM YAKS GRAZING ON WINTER PASTURE OF THE TIBETAN PLATEAU

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This experiment sought to establish the response to increasing levels of coconut oil supplementation on methane emissions from grazing yaks. Three mature female yaks (weight 178 ± 5 kg) were randomly assigned to different plots of the same winter pasture for grazing**,** and supplemented with three levels of coconut oil in 0 g/d, 60 g/d and 120 g/d whilst each animal was fed 1 kg oat hay per day in an incomplete (three periods) multiple Latin-square design experiment. Each period was extended to18 d to complete which was followed a similar design to Sutton et al., [1], d 1 to 12 were designed to allow the rumen to adjust coconut oil exposure environment, from day 13 to 18 yak were measured methane output and herbage intake. Methane emissions were measured by using a modification of SF_6 tracer gas technique [2]. The herbage intake were determined by using the chromium oxide, each day the cows were dosed with two gelatine capsule containing 10 g powdered chromium oxide, the chromium oxide content in the faeces was determined by atomic absorption spectroscopy according to the method of Williams et al., [3].

A linear reduction in methane output occurred (145, 117 and 88 L/d) as the levels of coconut oil in the diet increased (0, 60 and 120 g/d) ($P < 0.01$) with the greatest reduction at the 120 g/d (Table I). As the level of coconut oil increased dry matter intake (DMI) decreased, however these differences were not statistically significant at the various levels ($P > 0.05$). The proportional reduction in CH_4 output was greater than the proportional reduction in DMI and hence CH_4 L/kg DMI decreased from 25.9 L/kg when no coconut oil was given to 17.1 L/kg when 120 g/d coconut oil was given, and the methane yield (Y_m) were declined from 5.6% to 3.7%. These data demonstrate that the inclusion of coconut oil at levels from 0.0433% to 0.0883% of DMI reduces CH_4 production with no adverse effect on DMI up to the 60 g/d level when the yak grazing on the winter pasture.

TABLE I. EFFECT OF COCONUT OIL SUPPLEMENTATION ON METHANE OUTPUT FROM GRAZING YAK

	Coconut oil (g per head per day)		
	0g	60g	120g
DMI (kg/d)	5.6	5.3	5.2
CH_4 (L/d)	145	117	88
CH_4(L/kg DM)	25.9	22.1	17.1
“Methane yield” (Ym; %)	5.6	4.8	3.7

Most feed contains 18.4MJ gross energy per kg DM, methane energy content 55.65MJ/kg, so that a typical Ym value of 6% corresponds to 19.8 g CH_4/kg DM (27.72 L CH_4/kg DM) intake.

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FEEDS FROM GENETICALLY MODIFIED PLANTS (GMP) – NUTRITIONAL AND SAFETY ASSESSMENT

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The cultivation of GMP increased worldwide from 1.7 (1996) to about 114 million ha [3]. Currently, soybeans (60), corn (24), cotton (11) and canola (5 % of global GM area) are the most important GMP. They are modified mainly for agronomic traits. Such plants are characterized by so-called input traits (GMP of the first generation) without substantial changes in composition or nutritive value. GMP of the second generation (with output traits) should contain more valuable nutrients (e.g. amino acids, fatty acids, vitamins, enzymes etc.) or less anti-nutritive substances (e.g. mycotoxins, inhibitors, allergens etc.). Nutritional and safety assessment of feeds from GMP is a large challenge for animal nutritionists. The paper reviews feeding trials done with various animal species and categories in many countries.

Up to now more than 100 feeding studies with food producing animals were published. Since 1997, 18 studies were performed at the Institute of Animal Nutrition of the FLI to determine the effect of first generation GMP feeds on the nutrition of dairy cows, growing bulls, growing and finishing pigs, laying hens, chickens for finishing, as well as with growing and laying quails. This research was recently summarized by Flachowsky et al., [3]. The composition of feeds was analysed, and animal studies were used to assess nutritional qualities, including parameters such as digestibility, feed intake, health and performance of target animal species, and effects on food quality derived from the animals. Reproduction was also considered in generation studies with quails [5] and laying hens.

Both chemical analyses and the animal studies reveal no significant differences between GMP feeds and their isogenic counterparts and hence strongly support their substantial equivalence. Our results agree with more than 100 studies published in the literature and reviewed recently (Table I).

TABLE I. SUMMARY OF PUBLISHED DATA TO COMPARE FEEDS FROM GM PLANTS OF THE FIRST GENERATION (WITH INPUT TRAITS) WITH THEIR ISOGENIC COUNTERPARTS

Animals (Species/categories)	Number of experiments	Nutritional assessment
Ruminants		No unintended effects in composition (except lower mycotoxins concentration in Bt plants) No significant differences in digestibility and animal health as well as no unintended effects on performances of animals and composition of food of animal origin
Dairy cows	23	
Beef cattle	14	
Others	10	
Pigs	21	
Poultry		
Laying hens	3	
Broilers	28	
Others		
(Fish, rabbits etc.)	8	

Mycotoxin contamination of some GM-crops is lower than of non-GM, which may be one exception to their substantial equivalence. For example, Bt maize is less severely attacked and weakened by the corn borer and might have a greater resistance to field infections, particularly *Fusarium* fungi, which produce mycotoxins. Evidence of reduced mycotoxin contaminated in GMP has been demonstrated in some but not all cases, as summarized by Fachowsky et al., [4]. As present, detailed

standardized test procedures for testing feeds from the second generation of GMP are being developed by EFSA [2] and ILSI [6].

The consumption of feeds from GMP resulted in the intake of DNA and proteins including transgenic DNA and proteins; therefore, studies were conducted on their fate during processing, within the gastrointestinal tract of animals, and the potential to which extent transgenes or their products may be incorporated into animal tissues. Studies in this area were excellent by reviewed recently by Alexanders et al., [1]. It can be summarized that transgenic DNA is characterized by unique behaviour compared to native plant-DNA during feed treatment and in animals. Furthermore, there is no evidence that transgenic (novel) proteins are characterized by unusual chemical/physical properties distinct from native protein.

The following conclusions can be drawn:

- Presently, over 600 million hectares of GMP have been cultivated worldwide.
- Most animal studies have been done using first generation GMP.
- No unintended effects in composition (except lower mycotoxins) or nutritional assessment of feeds from first generation of GMP were registered in any of the more than 100 studies with food producing animals.
- Novel experimental designs are necessary for the nutritional and safety assessment of feeds from second generation of GMP.
- Transgenic DNA and novel proteins show similar properties as native DNA and proteins during feed treatment or in animals.
- Case by case studies is necessary to answer open questions.

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MULTIVARIATE CHARACTERIZATION ON MORPHOLOGICAL TRAITS IN BURKINA FASO SHEEP

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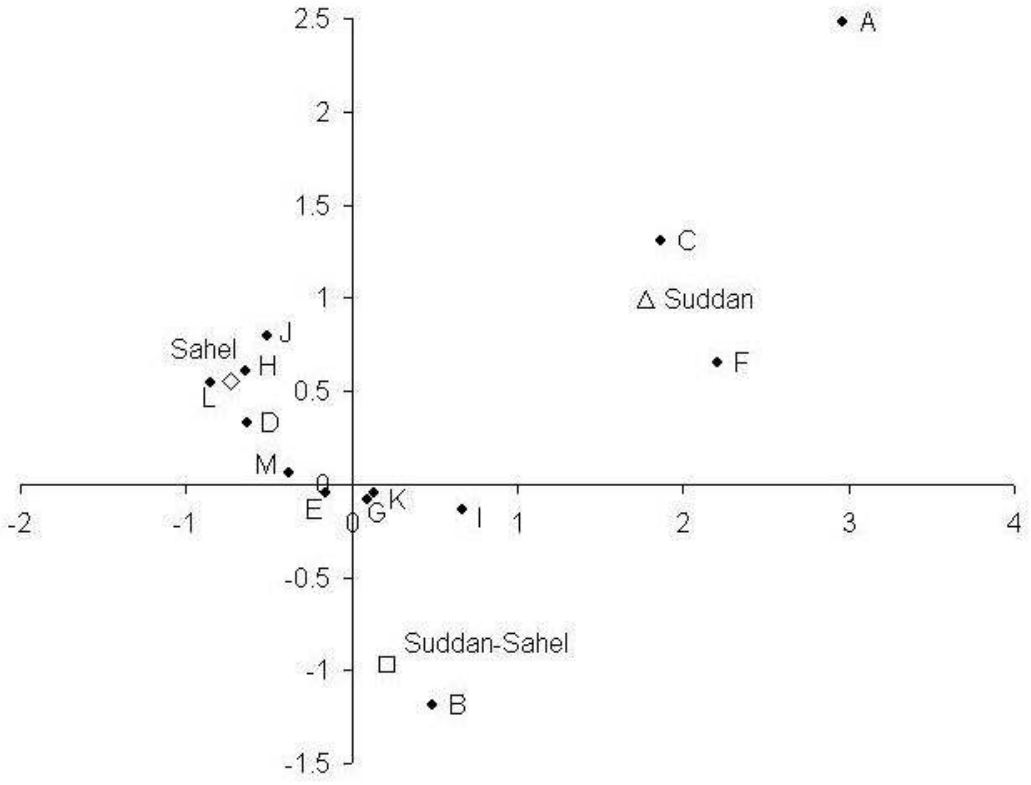
A total of 6440 female sheep from Burkina Faso were scored for seven body measurements and four qualitative morphological traits. Sampling included the three main environmental areas and sheep breeds (Table I) of Burkina Faso: the Sahel area (Burkina-Sahel sheep), the Sudan- Sahel area (Mossi sheep) and the Sudan area (Djallonké sheep).

Canonical analyses showed that differences in body measurements between the Sudan and the Sudan-Sahel sheep were small even though most body traits showed higher average values in the Burkina-Sahel sheep: the shortest Mahalanobis distance was found between the Sudan and the Sudan- Sahel populations (1.54), whilst that between the Sudan and the Sahelian populations was the largest (7.88). Discriminant analysis showed that most Sudan (Djallonké) individuals (60.85%) were classified as Sudan-Sahel (Mossi) individuals whilst most Burkina-Sahel individuals were classified into their environmental area of sampling (89.5%).

Correspondence analyses (Figure 1) indicated that the Burkina-Sahel sheep population clustered together with dropping ears, black and brown colour patterns and presence of wattles, the Sudan sheep were closely associated with long hair and vertical and curled ears and that the Sudan-Sahel sheep did not have clear associations with qualitative phenotypic traits. At the morphological level, the Sudan-Sahel (Mossi) sheep population can be considered a geographical subpopulation belonging to the Djallonké breed, showing some particularities, namely larger body size, due to the particular environmental condition of the area in which it is managed and a continuous gene flow from Sahelian sheep, The information reported in this study will be the basis for the establishment of further characterization, conservation and selection strategies for Burkina Faso sheep.

TABLE I. DESCRIPTION OF SAMPLING

Provinces	Sampling number		Environmental areas		
	Villages	Individuals	Sahel	Suddano-Sahelian	Suddanian
Bazéga	4	245		245	
Boulgou	6	340			340
Boulkiemdé	9	532		532	
Gnagna	10	601	420	181	
Kossi	4	240		240	
Kourweogo	1	121		121	
Mouhoun	7	427		427	
Oudalan	8	490	490		
Sanmatenga	13	785	484	301	
Seno	14	871	871		
Sissili	9	561			561
Tapoa	9	555		555	
Yatenga	11	672	672		
Totals	105	6440	2937	2602	901



A: vertical ears; B: horizontal ears; C: curled ears; D: dropping ears; E: short hair; F: long hair; G: absence of wattles; H: presence of wattles; I: white coated; J: black coated; K: spotted in black; L: brown coated; M: spotted in brown

FIG 1. Among qualitative variables relationship assessed via correspondence analysis.

COMMUNITY BASED PRODUCTIVITY VETERINARY SERVICE FOR SMALLHOLDERS DAIRY FARMERS IN BANGLADESH

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Bangladesh needs to change the dairy industry growth rate from the current rate of 2.0% to at least 6.0% for providing consumers with half the amount of required milk by the year 2025 against a population growth rate of 1.6%. Farmers' income increase would equal to US$ 676.3 - 1730.6 per year if all of them operated their farms as good as the 20% best farmers in the community are doing with regard to increasing milk production per cow per day, increasing lactation length, decreasing age to first calving, and decreasing calving interval [1]. We report here a model of delivering productivity veterinary services to smallholders' dairy farms through farmers' groups and associations, which would substantially increase their income.

In the most dairy populous area of the districts of Satkhira, Sirajgonj and Chittagong, we selected about 250 farms and divided them into groups of 10 farms. One farmer of the group worked as the Group Leader. One veterinarian following a previously set schedule visited 10 farms in a day every month where the Group Leader was kept informed. Thus during 25 working days of a month, the veterinarian visited 250 farms. Twenty-five group leaders made an association.

Data reported here were from four of such associations constituting 1000 farm families during a period from March 2005 to June 2006. To guide delivering the service, follow up its outcome and collect field data, we developed five forms. These forms are named as (1) farm inventory, preventive health and feed management; (2) reproduction and reproductive problem management; (3) mastitis management; (4) general health management; and (5) economics data collection forms. A breeding calendar was developed to keep necessary records. A Microsoft Access based database application was customised matching with the forms to record and analyse the data and to produce a herd summary. At farm visit, the veterinarian checked results of earlier interventions and schedules of deworming and vaccination. The veterinarian then checked the breeding calendar for reproductive events of the cow and especially examined cows bred 35 or more days earlier for pregnancy, cows that gave birth 60 or more days before for ovarian cyclicity and cows that failed to conceive after three consecutive services. A clinical diagnosis was made and treatment and or management changes were prescribed. Heifers that were more than two years old but had not shown oestrus were examined for ovarian cyclicity. The veterinarian also looked the drying off date of the cow, milking hygiene and post milking teat dipping. Additionally, the farmer could call a veterinarian if any emergency or general cattle health care issue arose in the farm.

We examined 1849 animals in 862 farms. In follow-up examinations, 47 to 82% of anoestrous cows and heifers resumed their oestrous cycle, 42 to 73% cows and heifers with history of repeated conception failure and or uterine infections conceived, 78% mastitis cows produced a normal score at California Mastitis Test and 88% recovered from general illness. A database on health problems of cattle was made, which would guide future delivery of veterinary service and education of veterinary students effectively. Examination at scheduled visit identified cyclic animals, which the

farmers would otherwise consider anoestrous. In the Satkhira district, about 80% farm families had an income increase ranging from US $ 1.0 to US 19.4 per cattle in 30 d in a cattle population with average milk yield 7.0 L/d (Figure 1). In the District of Sirajganj, the beneficiary farmers had a significantly higher return rare than the non-member farmers (Figure 2; $P < 0.05$). Institutionalization of the service delivery model described here in collaboration with farmer associations will help increasing farmers' income and maintaining a healthy stock in the smallholder dairy industry.

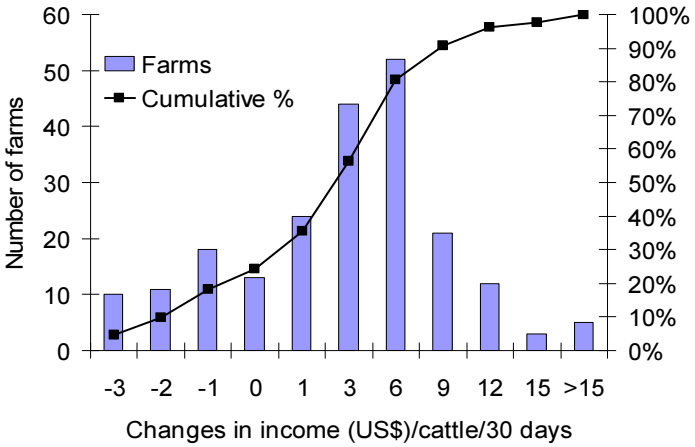

FIG 1. Income increases from productivity veterinary services in the farm families in Satkhira (number of farms = 214)

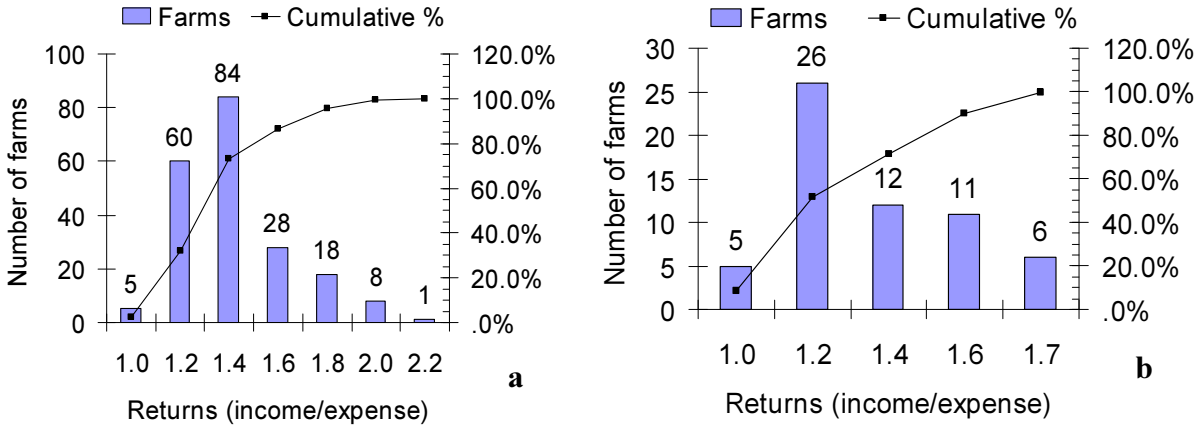

FIG 2. Distribution of returns; (a) 204 farms under community-based dairy veterinary service (CDVS), (b) 60 control farms; the difference was significant ($P < 0.05$).

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PROGESTERONE LEVELS IN THE OVARIAN UTERINE AND SYSTEMATIC VENOUS BLOOD IN ALPACAS WITH EMBRYO MORTALITY

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South American camelids is one the limited options that Peruvian Highland people, has to get economic incomes. It is possible to get fibre, meat, and animals as product from this animal production system; however, reproductive efficiency is not good because birth rate and embryo mortality are around 45-55% and 50%, respectively (Fernández Baca, 1970). There is not so much information about between age, pathogen agents, and genital tract alterations in relation to embryo mortality. This means that improving genetic programmes or breeding systems cannot fulfil the goals of an efficient production system.

New Zealand reports 24% embryo mortality between 21 – 30 days of gestation (Ridland *et al*., 1993). According to Boland (2000), there is no relation between peripheral serum levels and ovary-uterus circulation levels of progesterone; so embryo survival would be relational to progesterone levels in the ovarian and uterine veins. In this study we have considered serum progesterone levels, in uterus-ovarian circulation in luteal phase animals compared with early pregnancy and embryo mortality process.

Twenty open female alpacas with recorded previous parturitions were used. They were breed with a male when they showed sexual receptivity and was found an 8 mm follicle in any of the ovaries (Bravo, 1999). Ovario-hysterectomy was performed in four animals nine days after breeding to use their serum samples as diestrus references, and the rest (16 animals), ovario-hysterectomy was performed after positive pregnancy diagnosis, that was monitored every other day by ultrasound during gestation or until embryo mortality occurred.

There was an ultrasound evaluation of ovaries and uterus from day 15 post breeding until day 90 or when embryo mortality evidences was found. This was considered to have occurred when embryo cardiac beat decreased, embryo motility lost, or suspended particles in foetal fluids apparition.(Adams, 1989; Parraguez ,1997). Blood samples were taken from jugular vein, from breeding day, and twice a week until finish experiment or surgery. In 16 alpacas, females that continuing with gestation left from experiment, only animals with embryo mortality were surgically intervened. The anaesthesia protocol included ketamina 10%, tramadol 0.1%, xilacina 20% and atropina 0.03% (Hinostroza, 2008). Before ovario hysterectomy, blood samples were collected from uterine and ovarian vein. Uterine and ovaries resected were macroscopically evaluated. Serum was harvested and kept frozen until progesterone levels were measured by radioimmunoassay (Coat-A-Count Progesterone In-vitro Diagnostic Test Kit).

Serum progesterone levels were different between local and systemic circulation (jugular vein), in relation with reproductive status of animals (Table I). Three cases of embryo mortality were found.

Case 1: Embryo mortality on day 19 of gestation. Macroscopically, chorionic membrane was observed in left horn, and corpus luteums were found in both ovaries. In addition, systemic progesterone values were similar to those observed during gestation and diestrus period.

Case 2: Embryo mortality on day 40 of gestation. Caseose structures were observed in the left horn, both ovaries with CL, progesterone levels decreasing, and ultrasound images showed gradual process of embryo structures lost.

Case 3: Embryo mortality around 69 days of gestation. Previous ultrasound images showed gestation process and evidence of suspended particles in foetal fluid. Gestation occurred in the left horn and CL was present in the in right ovary.

Embryo mortality (EM) is a multifactorial process that needs to be elucidated in alpaca. In the present study, we found three cases from 16 alpacas; it means 28% of total, a lower value as compared with Fernández Baca's results where they found 50% until day 31 of gestation. We found only one case in this period and the other 2 cases were evidenced in more advanced gestations, Case 2 (40 days) and Case 3 (69 days). We considered late EM very important, because it means that several females that can be diagnosed as pregnant at the middle or final part of breeding season will result open in delivery period, without opportunity to be breed, in order to improve birth rate in the flock.

The process can be explained in ultrasound images, embryo degeneration, which it is suggested an autolysis process, that occur after cardiac beat suspension, gradual decreasing of placental fluids volume, that finish with the presence of remnants, as reported by Ginther (1985). This process would not involucrate the progesterone secretion of CL, because in the 3 cases, it is evident that serum hormone levels are higher than 1 ng/ml in ovarian vein blood, and that is considered (Table I) as functional corpus luteum (Raggi, 1999).

When comparing progesterone levels in Case 1 with gestation and diestrus serum progesterone concentrations, values were similar, but highly different from serum progesterone levels originated from ovaric vein in alpacas 1 and 13 days post breeding, where values for progesterone were basal, and that is coincident with follicles in ovaries (Echevarría, 2007).

Progesterone level in ovarian vein sample is in relation to ovarian progesterone secretion as was showed by Stefanczyk-Krzymowska (1998), because this blood vessel has its origin in the ovary, later it receives affluent from uterus. Progesterone levels in uterine vein sample resulted similar with jugular vein, possibly because a dilution of progesterone concentration with blood from uterus.

Progesterone secretion from ovary is compatible with a functional corpus luteum in these cases of embryo mortality in alpacas, so it would not be involved in the origin of the process.

TABLE I. SERUM PROGESTERONE LEVELS IN ALPACAS OF VARIOUS REPRODUCTIVE STATUSES

Reproductive status	Days post breeding	Progesterone (ng/ml)		
		Jugular vein	Ovarian vein	Uterine vein
Diestrus	9	2.2 0.4	67.5 ± 12.1	5.9 ± 0.5
Normal gestation	73	4	68.3 ± 8.9	4.7
Embryo mortality (Case 1)	19	3.4	65.3	4.2
Embryo mortality (Case 2)	40	0.4	8.7	1.4
Embryo mortality (Case 3)	69	0.5	4.8	1.1

IS EMBRYO TRANSFER A USEFUL TECHNIQUE FOR SMALL COMMUNITY FARMERS?

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Several researchers have provided sufficient evidence to sustain that the best crossbreeding program to produce milk in the tropics is the direct cross between Bos Taurus and Bos indicus (F1). The problem arises when the farmer faces the challenge to breed the crossbred animal. If the choice is to cross with Bos Taurus the resulting product is quite vulnerable to the harsh environmental conditions in the tropics. If, on the other hand, the selection is to sire with Bos indicus then the offspring will be deficient in milk production. Another alternative is to transfer F1 embryos to F1 dams, hence avoiding the hazards of crossbreeding. Although the technique of embryo transfer (ET) has been available for many years, there are several pitfalls at least under tropical conditions, which need to be considered.

Selection of recipients for embryo transfer

The first one is related to the selection of the recipients. These are usually animals displaying spontaneous oestrus or treated with drugs to synchronize their oestrus. The short comings of either of these methods have been experienced by Montiel et al., [4]. In short, the use of spontaneous oestrus is time consuming and inaccurate and the response to oestrus with an ensuing ovulation can be as little as 30% if the animals selected are not in reasonable body condition. Embryo transfer programs in small community farms can be tricky because the selection of recipients is restricted to a few animals in the herd and the distance between farms can pose a serious threat to the success of the program. Thus, just because of this constraint, several government programs have ceased to be functional when the subsidy runs out.

Production of embryos (F1)

The main components of successful embryo production can de divided into three. The quality of the superovulatory response in the donor cow, the ability of the individual to recover as many embryos as possible and the accuracy of the technician to judge the quality of the embryo destined for freezing which has proven to be a difficult task at least under tropical conditions [1, 5]. In relation to the former, figures for embryo production can vary enormously, although some groups demand that the number of good quality embryos cannot be less than eight [3]. However, others have not been so successful [4]. In short, the superovulatory response can be directly related to the follicular dynamics now of treatment.

The recovery of embryos can be difficult especially as it has been reported that almost 30% of the donor cows have curved cervices increasing the difficulties in negotiating the catheter [6]. Embryo production can be affected if the conditions are not favourable, in a detailed study, Marquez et al., [7] showed that the number of healthy embryos evaluated by their resistance to freezing and their degree of apoptosis, was affected if the embryos were produced in the spring or the autumn.

Economical feasibility of ET among small community farmers

Government organizations in developing countries have launched initiatives to popularize the evident benefits of ET, particularly in enterprises not bigger than 50 cows per unit. These programs have experienced a high degree of acceptance, especially those with a substantial subsidy. However, when the program folds UP, it has proven not to be sustainable for the farmers themselves, thus disappointment is the natural course of events [4]. In a recent study [2] found the estimation of the cost involved in the preparation of the donor and embryo recovery was about US 600.00. The

average number of embryos recovered was 3.8. Taking into consideration the cost of gestation, calculated by the percentage of animals pregnant (27%); the cost for preparing the donor, the technique of embryo transfer and the cost of production of the embryo itself, the overall cost per gestation was $1320. Considering a 50-50 ratio of males-females born, the cost for a replacement heifer was 2640 dollars, which surpassed by far the commercial cost of a crossbred heifer (approximately 900 dollars).

Considering the difficulties in distributing F1 embryos among farmers in small enterprises, the cost of production and the low success rate found in terms of fertility, for the time being IT does not seem profitable for farmers themselves to sustain a program of this investiture. Government organizations would need to play a more active and systematic role to ensure the reduction of the costs inherent embryo transfer techniques.

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ESTABLISHMENT OF MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET) TECHNOLOGY FOR GOATS IN SRI LANKA

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Multiple ovulation and embryo transfer (MOET) has been done successfully in goats in some countries (Chen et al., 2008). It can be used to multiply the genetically superior animals and to make elite herds with increased production potential. There have been no previous reports on successful MOET in goats in Sri Lanka. Therefore, this study was carried out to establish techniques for *in vivo* production and transfer of goat embryos in Sri Lanka.

Genetically superior does (n = 7) were subjected to super ovulation for i*n vivo* embryo production using a protocol modified from that of Batt *et al* (1993). Progesterone releasing intravaginal pessaries (45 mg, Cronolone) was inserted on Day 1 of the programme. The does in group 1 (n = 3) were stimulated on Day 8 with injections of pure porcine Follicular Stimulating Hormone (pFSH), while those in group 2 (n = 4) were stimulated with pure ovine Follicular Stimulating Hormone (oFSH). Equine Chorionic Gonadotrophin (eCG) was given to all does in the evening of Day 8. Subsequent injections of pFSH (group 1) or oFSH (group 2) were given in the morning and evening on Day 9 and Day 10. All does were injected with prostaglandin analogue (263 µg/ml cloprostenol sodium) in the morning of Day 9 and vaginal pessaries were removed in the evening of Day 10. On Day 11, pFSH or oFSH was injected in the morning and Gonadotropin Releasing Hormone (GnRH) was injected in the evening. Immediately after the GnRH injection does were exposed to breeding with a genetically superior Jamnapari buck for 48 hours. Embryos were collected surgically 7 d after oestrus, by flushing of the uterus with embryo flushing medium containing lactated Ringer's solution with 1% bovine serum albumin at 37^{o}C through a mid ventral laparotomy. The quality of the embryos was assessed microscopically and those considered to be of good and excellent quality were transferred surgically to oestrus synchronized recipient goats (n = 6) 7 d post-oestrus.

The ovarian parameters measured and mean numbers of embryos recovered after superovalation are given in Table I. The total number and the quality of the embryos recovered from each group of does are shown in Table II. Following embryo transplantation, 4 of the 6 recipient does were diagnosed pregnant by ultrasound at day 35. The first goat kid born (named 'Peradeniya Kumari') was a single healthy female with 3.6 kg birth weight at full term. Two more does kidded, resulting in four healthy kids with birth weights of 3.2 kg (♀), 1.8 kg (♀), 1.6 kg (♂) and 1.2 kg (♂), while an abortion was observed in one doe. During the first six weeks the average weight gains of the first two kids born were 152.3 and 149.2 g/d, respectively.

TABLE I. SIZE OF THE OVARIES, NUMBER OF CORPORA LUTEA AND MEAN NUMBER OF EMBRYOS RECOVERED IN THE TWO GROUPS OF RECIPIENTS

Parameters		Group 1	Group 2
Size of the ovary, Mean ± SEM			
	Length (cm)	2.4 ± 0.1	2.5 ± 0.2
	Width (cm)	1.2 ± 0.1	1.7 ± 0.1
	Thickness (cm)	1.1 ± 0.1	1.5 ± 0.2
Number of corpora lutea (range)		6-10	11-17
Mean number of corpora lutea		7.6 ± 1.2	14.25 ± 1.2
Mean number of embryos per animal		4.3 ± 2	4.25 ± 2

TABLE II. THE NUMBER AND QUALITY OF EMBRYOS RECOVERED IN THE TWO GROUPS OF DONORS

Group	Hormone used	Quality of embryos produced		
		Excellent	Good	Poor
Group 1	pFSH	02	08	03
Group 2	oFSH	04	11	02
Total		06	19	05

The results showed that valuable, genetically superior female goats can be multiplied using embryo transfer. The superovulatory response, quality and quantity of the embryos were better with oFSH than with pFSH. Although the number of embryos recovered was high in both groups, only some of the embryos were transferred due to the lack of sufficient number of recipient goats. The resulting offspring showed high growth rates and good survivability. Further experiments are warranted to optimize the protocols under Sri Lankan conditions and to compare the data statistically.

In conclusion, the birth of healthy goat offspring through MOET technology is reported for the first time in Sri Lanka, indicating the feasibility of multiplying superior goats through this technology.

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INSULIN RESISTANCE IN DIFFERENT FORMS OF HYPERKETONEMIA AND IN COWS AFFECTED BY PUERPERAL METRITIS

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In dairy cows selected for high milk production the phenomenon of insulin resistance (IR) seems to play a pivotal role both in adaptation to the postpartum negative energy balance and in the aetiology of some periparturient metabolic disturbances. Perturbation of pancreatic insulin secretion and insulin sensitivity of peripheral tissues has been documented in the pathogenesis of abomasal displacement [1], cystic ovarian disease [2], excessive lipid accumulation in the liver and ketosis [3]. In human population and in laboratory animal models pro-inflammatory cytokines like tumour necrosis factor-alpha (TNF-α) play an essential role in the development of IR that occurs in association with obesity, acute infections and endotoxaemia. A similar interaction between the intensive release of pro-inflammatory cytokines and IR has been recently explored also in ruminants [4].

This trial was conducted in high-yielding dairy cows challenged with standard intravenous doses of glucose and insulin in different time intervals to parturition. The aim was to determine the grade and time-related changes of (i) glucose-stimulated insulin increase and (ii) insulin-induced glucose decline, furthermore (iii) the interrelation of these challenge tests with plasma levels of metabolites and metabolic hormones in cows showing various ketone pattern with and without puerperal metritis.

28 multiparous Holstein cows (previous 305 FCM day milk: 8331±192.8 L) were subjected to intravenous glucose tolerance test (IVGTT) on day -18, 7 and 70 around calving. Plasma ßOH butyrate (BHB), non-esterified fatty acid (NEFA), glucose, insulin, insulin-like growth factor I (IGF-I) and leptin levels were measured regularly from –18 d before, till d 70 after calving. Cows were milked out twice a day. The course of postpartum uterine involution was checked regularly, and cows showing clinical signs of bacterial complications were treated with antibiotics combined with repeated administration of PGF2α. All cows showing oestrus on day about ≥50 were inseminated and those not returning to oestrus were checked for pregnancy by rectal palpation on day 45-60 after AI. Resumption of ovarian activity was monitored by the means of individual milk progesterone profiles

Based on BHB level and clinical examination cows were categorized in four groups: 1. Normoketonemic (NK) throughout the study (n = 9); 2. Transiently hyperketonemic (HK; n = 7); 3. Continuously HK (n = 7); 4. Continuously HK with severe puerperal metritis (HK+PM; n = 6). Cows with continuous HK showed lower insulin and leptin levels than their normoketonemic mates from day 4-10 before calving, and these tendencies existed throughout the study. The plasma leptin and insulin patterns of cows with transient ketone increase around parturition represented an intermediate situation. Insulin area under the curve (AUC) and maximal insulin response to glucose were significantly lower in the early postpartum period than in late pregnant or mid lactating animals ($P < 0.001$) and in cows affected by continuous HK and PM ($P < 0.001$, Fig. 1). There was no effect of time or health status on glucose turnover rate and glucose half-life ($P > 0.05$). Normoketonemic cows ovulated earlier than cows from group 2, 3 or 4 (day 26.2 ± 3.34; 7 ± 4; 44 ± 4; 50.1 ± 5; $P = 0.002$). Insulin AUC and maximal insulin response in cows affected by HK and PM were still lower on d 70 pp ($P = 0.051$ and $P = 0.23$) than in normo- or hyperketonemic animals.

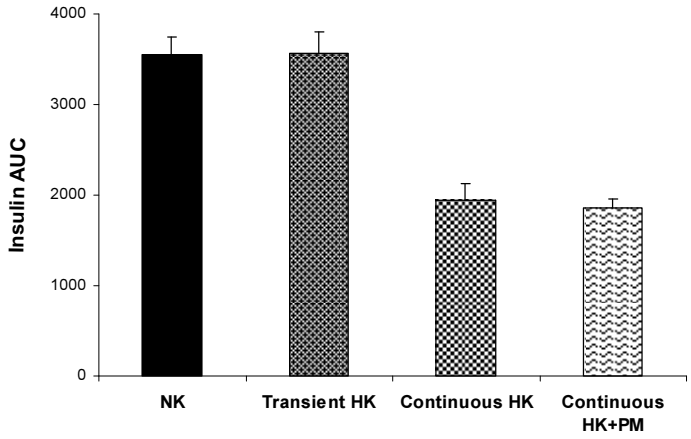

FIG 1. Insulin area under the curve (AUC) in response to glucose infusion (GTT) on day 7 after calving in normoketonemic, transiently hyperketonemic, continuously HK cows and in continuously HK cows affected by puerperal metritis.

In conclusion we showed that pancreatic ß-cell function and the biological potency of insulin is impaired in cows with long-term hyperketonemia. Short term elevation in plasma BHB and free fatty acid may not potentially induce further increase in peripheral tissue IR in the early lactation. Severe puerperal genital diseases like puerperal metritis and mastitis can further accentuate insulin resistance in dairy cows, with long term effects on metabolism and reproduction.

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REPRODUCTIVE PERFORMANCE OF LACTATING DAIRY COWS TREATED WITH INTRAVAGINAL PROGESTERONE IN A TIMED ARTIFICIAL INSEMINATION PROTOCOL

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Lactating cows of five dairy herds that had failed to display oestrus were enrolled in an experiment to investigate the effect of treatment with a progesterone releasing intravaginal device and Estradiol Benzoate (EB) and the injection of D-cloprostenol at device removal (day 7). A second dose of EB was injected at day 8 and artificial insemination (AI) was carried out on day 9 with or without the display of oestrus. No significant improvement on pregnancy rate at first service was observed as a result of this treatment. However, the overall pregnancy rate was improved.

The programme was carried out in five dairy farms in two dairy basins (Central and Cordillera) in Paraguay. The dry cows in each herd with a past history of poor reproductive performance detected through progesterone determination in milk (n = 153) were divided in two groups, of 116 and 37, respectively. Concentrates (3 kg/d) were given to the cows of the larger group to ensure recovery of body condition after calving (if farms normally provided concentrates at the dry period, this had been measured and adjusted to 3 kg if necessary). Body condition scores (BCS) were recorded by palpation of the tip of the transverse processes of the lumbar vertebra (scale 1-5) at calving and at 60–70 d post calving for cows in both groups. Ultrasonographic examination of the ovaries was performed to ensure the selection of cows that did not present any pathology in either the ovary or in the uterus.

Cows that were found with corpus lutea (CL) were treated with D-cloprostenol (PG 0.075 mg/mL) to induce oestrus. Cows that did not show heat after this procedure were subsequently treated for fixed time artificial insemination (FTAI), with the injection of 2 mg of Estradiol Benzoate (EB) and the insertion of an intravaginal device containing 1 g of progesterone at day 0 (60-80 d post calving). Removal of the intra vaginal insert and treatment with 2 ml of D-cloprostenol (PG 0,075 mg) at day 7 and injection of 1 mg of Estradiol Benzoate (second doses) at d 8. Cows were inseminated 30 hours later at a fixed time with or without heat observation.

Among all cows in both groups, 55 (35.95%) showed heat before 60 d and were bred according to the reproductive strategy of each farm. Pregnancy diagnosis was carried out through ultrasonographic examination at 35 to 60 d after services and reconfirmed by rectal palpation at 90 d. Pregnancy rate was defined as the percentage of pregnant cows related to the total bred. Figures 1 and 2 are examples of progesterone level in most cows in the farms where the experiment was carried out. The average interval from calving to first ovulation was 78 d. However, the first AI service occurred about two months later (133 d) on average, indicating some problems in either heat expression by the cattle or heat detection by the farmers.

Small differences in fertility and response to treatments were observed among the groups of cows that underwent the treatment either for prostaglandin treatment or fixed time artificial insemination (FTAI). Overall first service per conception rate was greater for the cows not fed concentrates prior to calving (25% vs. 8%; $P < 0.05$). However, with continued inseminations, the overall pregnancy rate was similar (38.5% and 45.0%; $P > 0.1$) for cows fed concentrates and non-fed group respectively. Important differences between the interval from calving to first service was observed among the two groups of cows (113.3 vs. 91.9 d), as the non fed concentrates group was about 21 d shorter. The interval from calving to conception was shorter in the supplemented group (144.9 d), though this difference was not statistically significant. Cows that underwent treatment via fixed time

artificial insemination (FTAI) showed overall better response than treatment with prostaglandin alone, conception rate at first service was higher (23.1%; $P < 0.05$), total conception rate was also higher (53.8%). Significant difference was also observed in days from calving to first service, this interval was shorten by about 15 d for cows that underwent FTAI treatment, calving to conception was also shorten though this was not statistically important. These results suggest that the problems observed with anoestrus, failure to conceive and long calving intervals problems were not primarily due to nutritional factors, but rather to another set of influences and additional in depth study is required to identify these factors, however these different treatments have shown to be useful tool to improve conception rate and shorten calving to conception intervals.

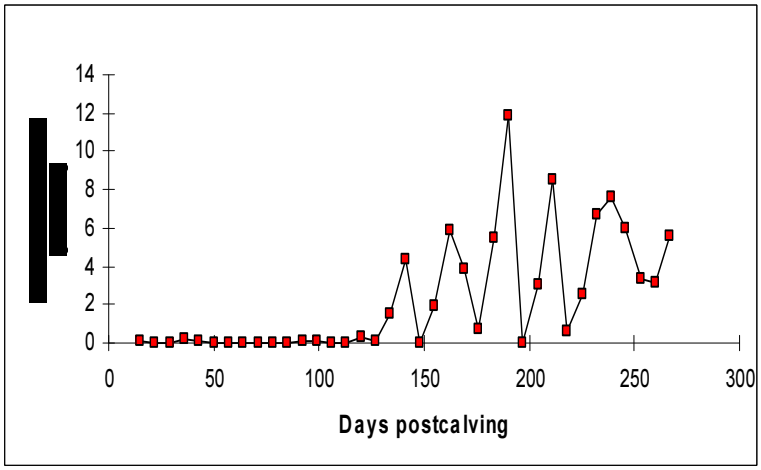

FIG. 1. Shows an anoestrus cow for more than 100 d post calving followed by 3 normal cycles, without report of heat. First service was reported at d 212 post calving. Second service was at d 233 and conception was confirmed.

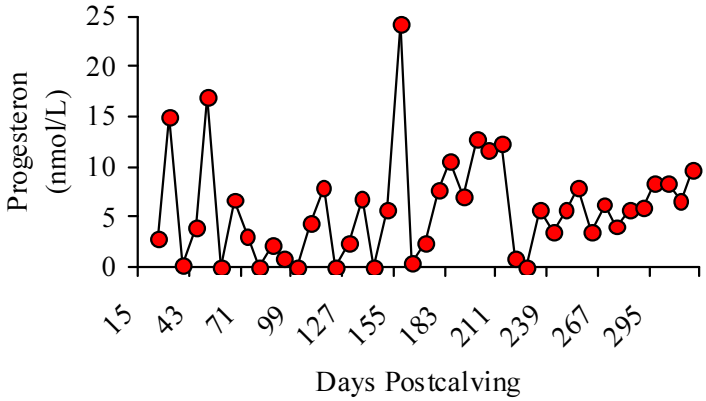

FIG 2. Shows progesterone level of a post-calving cow that had a regular ovarian activity, but inadequate heat observation.

This cow had the first ovulation at d 20 post-calving, followed by 7 regular cycles without the report of heat. Cow probably was served by a bull, there was not heat neither a service reported but cows conceived at d 162 post calving. Pregnancy was interrupted and cow was served at d 218 and conception was confirmed. This is a case of a cow that apparently was in anoestrus by about seven months, but actually was cycling normally.

TABLE I. CONCEPTION RATE AT FIRST SERVICE (CR), TOTAL PREGNANCY RATE (PR), INTERVAL FROM CALVING TO FIRST SERVICE (ICS) AND FROM CALVING TO CONCEPTION (ICC) FOR SUPPLEMENTED AND NON-SUPPLEMENTED COWS IN THE THREE POSTPARTUM REPRODUCTIVE TREATMENTS

Treatment	CR (%)	PR (%)	ICS (d)	ICC (d)
Supplemented	7.7 ± 0.1^{a}	38.5 ± 0.1^{a}	113.3 ± 4.9^{a}	144.9 ± 21.4^{a}
Not supplemented	25.0 ± 0.1^{b}	45.0 ± 0.1^{a}	91.9 ± 7.5^{b}	208.8 ± 47.8^{a}
FTAI	23.1 ± 0.1^{a}	53.8 ± 0.1^{a}	95.9 ± 7.0^{a}	143.8 ± 35.3^{a}
1PG	9.5 ± 0.1^{b}	35.7 ± 0.1^{a}	$110.8 \pm 6.0^{a\,b}$	150.1 ± 30.1^{a}
2PG	0	29.4 ± 0.1^{a}	121 ± 4.9^{b}	186.2 ± 44.6^{a}

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EFFECT OF TEMPERATURE AND HUMIDITY ON HEAT STRESS RESPONSES IN VIETNAMESE YELLOW CATTLE

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Four female cattle (local Yellow breed) 8 months of age were fed a diet of 50% urea-treated and 50% untreated rice straw with free access to water. The levels of temperature /humidity were random combinations of 25, 29, 35 and 39°C and 70, 80 and 90 (%) humidity, achieved in experimental chambers fitted with air conditioners, heaters and ultrasonic humidifiers. The treatments were based on natural condition frequently occurring in an indoor animal house in the area in summer.

Physiology indexes as breathing rate, heart beat rhythms, feed and water intake were measured**.** Total RNA were isolated from leukocytes to identify Hsp70 (heat shock protein, a biochemical stress indicator) by RT-PCR with the primer pairs used designed using the bovine primers for Hsc70 and Hsp70 [1] if they presenting while the cattle in heat levels treated.

The results showed that the heart beat increased in direct proportion to the air temperature but was not affected by humidity levels. The breathing rate increased when temperate exceeded 35°C. The body temperature only increased when chamber temperature reached 39°C and humidity was 90%, which represented a serious stress to the animal ($P < 0.001$).

The feed intake reduces 12% after increasing the temperature from 25°C to 29°C ($P < 0.001$). The water intake was double at 39°C of chamber's temperature than 25°C, even when the humidity level reaches to 90%.

At the level of temperature and humidity 29°C/80% (THI = 81), Hsc70, a importance member of Hsp70 family, were appeared (Figure 1). At 29°C/90% and 35°C/70%, Hsp 70-1 and Hsp 70-2 were also presented (Figure 2).

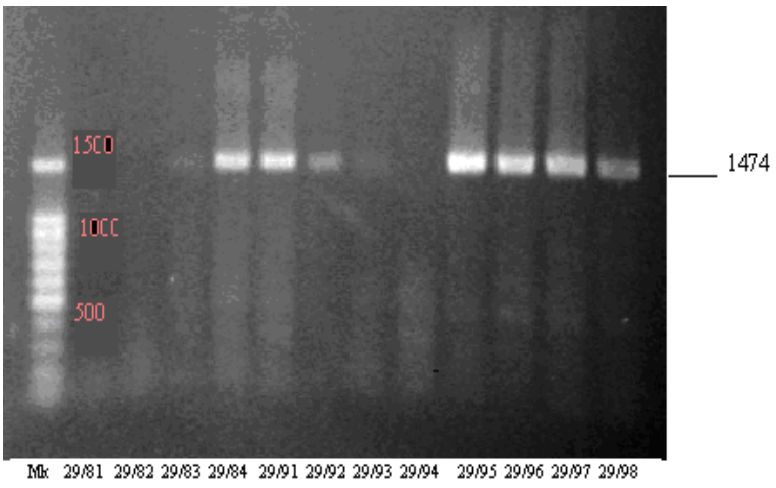

FIG 1. Identification of the Hsp70

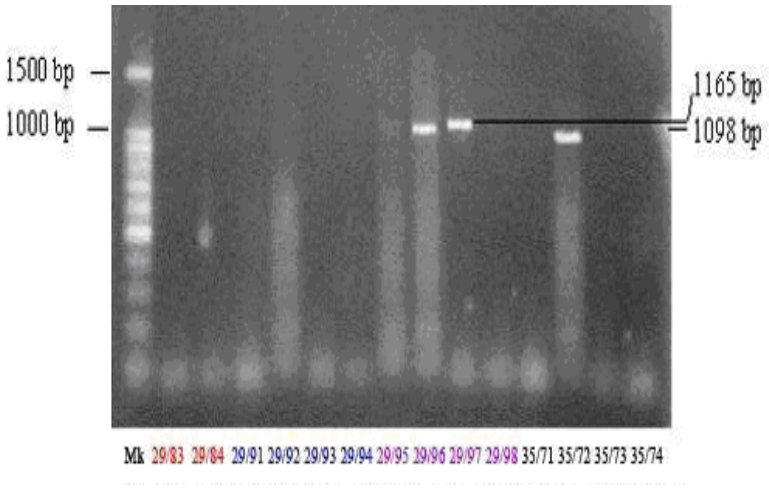

FIG 2. Identification of the Hsp70-1 and Hsp70-2

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METHANE EMISSION BY LIVESTOCK IN INDIA AND MITIGATION STRATEGIES

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India has 226.1 million cattle, 96.9 million buffaloes, 59 million sheep and 124.5 million goats, 18.5 million pigs, 0.9 million each of camels and donkeys, 0.8 million horses/ponies and small a number of yak, mithun, and mules [1] (FAO Production Year Book, Vol. 57: 2003). These animals produce 13.46 Tg methane/year (including emissions from animal wastes). The methane emission factors (kg/head/year) for different categories of animals in India are lower than in Europe and North America. Based on the studies conducted at Indian Veterinary Research Institute, methane emission factors for cattle, buffalo, sheep and goat varied from 25.6 to 57.6, 28.9 to 52.7, 2.6 to 4.1 and 3.3 to 4.3, respectively. These variations are mainly due to differences in body weight, type of feed (high vs. low fibre) and individual behaviour (high or low methane producers) of the animals. Mitigation of methane emission is essential to protect the environment from the greenhouse effect and at the same time improve feed conversion efficiency.

Strategies to mitigate methanogenesis

- Non-productive or low productive animals should be replaced with either high producing indigenous cattle/buffalo or high producing crossbred cattle. If we target to enhance the population of high producing cattle from the present figure of 23 million to 46 million within next 5 years, the excretion of wastes and methane will be increased by only 3.2% and 1.7% respectively, but the production of excreta and methane per unit of livestock productivity will be considerably reduced.
- Any effort made to enhance degradability of poor quality feeds results in an improvement in nutrient availability accompanied with a decrease in methanogenesis. For example, urea ammoniation of straw may serve three purposes, firstly it enhances degradability of straw, secondly the supplemented non-protein nitrogen source stimulates microbial protein synthesis in the rumen and lastly it reduces methanogenesis.
- Plant secondary metabolites (saponins, tannins, lignins, essential oils, etc.) have anti-microbial activities to protect the plants against invasion by microbes. This plant property can be exploited for control of undesirable microbes in the rumen. The initial screening experiments have indicated that the extracts of plants containing secondary metabolites are effective against methanogenesis and ciliate protozoa, and some have an adverse effect on degradability of feed in the rumen [2, 3]. Based on the results of *in vitro* screening experiments, a few plants and some mixtures of plants have been selected for inclusion in the diet of ruminants to study their effect on *in vivo* methane emission as estimated by open circuit respiration calorimeter.
- A mixture of *Allium sativum, Syzygium aromaticum, Foeniculum vulgare* and *Mentha piperita* (oil) in the ratio of 2:1:2:1 respectively, was fed to buffaloes at the rate of 2 g/kg of feed. Although each of the plants individually inhibited *in vitro* methanogenesis, but the level of mixture feeding might not be sufficient to have a similar effect on *in vivo* methane emission.
- *Terminalia chebula* and *Allium sativum* both inhibited *in vivo* methane emission, but *T. chebula* was more effective than the latter. Both these plants both separately and as a mixture improved dry matter digestibility of feed by 10.6 to 11.3% in (Table I). In another experiment, a mixture of 3 plants fed to cattle calves at 2% of DM intake on alternate days inhibited methane production by 9.4% and the body weight gain was 8.7% (448 *vs.* 412 g/d) higher as compared to control, with no adverse effect on digestibility of different nutrients.
- The results of the *in vivo* experiments conducted so far indicate that plants containing

secondary metabolites, showing promising results in *in vitro* experiments, do have a potential to be used as rumen modulators for controlling methane emission in ruminants. The levels used in *in vitro* experiments are usually very high and may not be usable in *in vivo* experiments. Therefore, the level of feeding of plants additives has to be standardized for practical application to get inhibition in methanogenesis without any adverse effect on nutrient utilization.

TABLE I. EFFECT OF PLANT SECONDARY METABOLITES ON *IN VIVO* METHANE EMISSION AND DIGESTIBILITY OF FEED DRY MATTER

Plant	Methane inhibition (%)	Digestibility of dry matter (%)	Animal species
Terminalia chebula (1% of dry matter intake)	24.0	11.3 (+)	Sheep
Allium sativum (1% of dry matter intake)	11.9	11.1 (+)	Sheep
Terminalia chebula and *Allium sativum* (0.5% each of dry matter intake)	23.5	10.6 (+)	Sheep
Mixture of three plants (2% of dry matter intake on alternate days)	12.0	No effect	Cattle calves

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EFFECT OF ETHYL LINOLENATE ON METHANOGENESIS AND RUMEN FERMENTATION IN SHEEP FED DIETS WITH DIFFERENT FORAGE TO CONCENTRATE RATIOS

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Methane produced by enteric fermentation in ruminants means not only a severe loss of feed energy for the animal, but also causes, as an important greenhouse gas, ecological problems [2]. Therefore, developing feeding strategies with methane-suppressing impact is desirable. Long-chain unsaturated fatty acids are known to have a high potential in suppressing ruminal methanogenesis [3]. In the previous study with different type and level of octadeca-carbon fatty acids, it is found that linolenic acid had the most efficient methane-suppressing effect *in vitro* [4]. In this trial, the effect of ethyl linolenate (LNE) was studied on methane emission and rumen fermentation *in vivo* from finishing sheep given diets differing in the forage to concentrate ratio (F:C) using simple box chamber.

The experimental design was a 4 × 4 Latin square including four dietary treatments. Eight male Huzhou sheep were paired with fistulated and not fistulated each at the beginning of the experiment and the pairing of animals was consistent throughout the trial. Four pairs of sheep were fed a forage-based diet without (F; F:C = 70:30, dry matter basis) or with LNE (FL; F:C = 70:25, 5% LNE); a concentrate-based diet without (C; F:C = 30:70) or with LNE (CL; F:C = 25:70, 5% LNE), respectively. The diets were given in equal portions twice a day at 8:00 and 16:00. Methane emission and fermentation parameters including ammonia-N, volatile fatty acids and microbial protein were determined using the methods described previously [1].

Addition of LNE in different F/C ratio diets had the same diurnal pattern of methane emission (Figure 1). Methane emission was rapidly increased to peak two or three hours after the feeding, and then decreased slowly until the next feeding. Addition of LNE decreased methane emission by 17.3 and 33.8% in forage-based and concentrate-based diet, respectively (Table I). Ruminal pH was increased by inclusion of LNE. Diet type had no effect on total volatile fatty acid, but LNE supplementation decreased total volatile fatty acid significantly ($P < 0.05$). Inclusion of LNE decreased molar proportion of acetate and butyrate and increased propionate proportion in concentrate-based diet, while had little effect on the fermentation pattern in forage-based diet. Ammonia-N concentration and microbial protein mass were decreased significantly by addition of LNE ($P < 0.05$). Forage-based diet had lower ammonia-N concentration than concentrate-based diet. However, diet type had no effect on microbial protein mass. It is concluded that addition of LNE can inhibit methane emission in both forage- and concentrate-based diets significantly, which is beneficial for economy and environment.

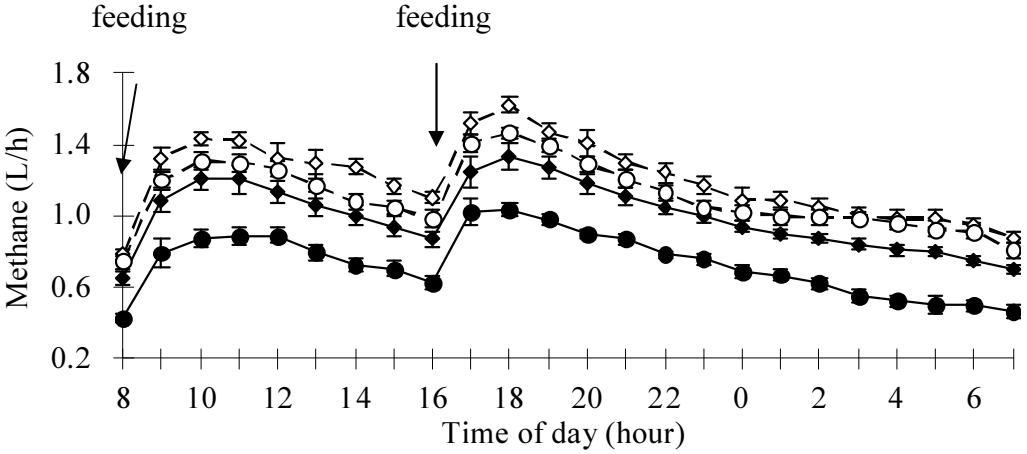

FIG 1. Diurnal pattern of methane emission from sheep fed a forage-based diet without (◇) or with ethyl linolenate (◆; 5g/kg DM); concentrate-based diet without (○) or with ethyl linolenate (●; 5g/kg DM), respectively. The vertical bars indicate SEM.

TABLE I. METHANE EMISSION AND RUMINAL PARAMETERS FOR SHEEP FED DIETS WITH OR WITHOUT ETHYL LINOLENATE AT DIFFERENT RATIOS OF FORAGE TO CONCENTRATE

Diet	Forage-based		Concentrate-based		SEM	P-values [1]		
Ethyl linolenate (g/kg)	0	50	0	50		D	S	D × S
Methane (l/d)	28.9^{a}	23.9^{b}	26.6^{a}	17.6^{c}	0.8	< .01	< .01	0.02
Ruminal pH	7.14^{ab}	7.33^{a}	6.90^{b}	7.13^{ab}	0.09	0.02	0.03	0.84
VFAs (mmol/L)	68.6^{a}	54.6^{ab}	62.2^{ab}	49.7^{b}	4.1	0.26	0.02	0.88
Molar proportions (%)								
Acetate	77.4^{a}	76.9^{a}	71.7^{b}	69.3^{c}	0.7	< .01	0.14	0.30
Propionate	13.7^{b}	14.8^{b}	16.6^{b}	22.6^{a}	1.3	< .02	0.03	0.11
Butyrate	9.0^{ab}	8.3^{b}	11.7^{a}	8.1^{b}	0.8	0.15	0.02	0.10
NH_3-N (mg/dL)	12.3^{c}	10.7^{d}	20.7^{a}	16.8^{b}	0.4	< .01	< .01	0.15
Microbial protein (mg/ml)	1.95^{a}	1.55^{b}	1.95^{a}	1.69^{ab}	0.11	0.25	0.01	0.76

$^{a, b, c, d}$ Means within the same row sharing no common capital letters are different at $P < 0.05$

[1] D = effect of forage/concentrate ratio; S = ethyl linolenate effect; and D × S = interaction effect

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POTENTIAL OF TROPICAL PLANTS TO EXERTING DEFAUNATING EFFECTS ON THE RUMEN AND TO REDUCE THE METHANE PRODUCTION

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The objective of this work is to present a summary of the principal results obtained in Cuba in relation with the potential of different tropical plants used as animal feed, with possibilities of exerting defaunating effects on the rumen and to reduce the methane production. The assays were carried out in areas of the Institute of Animal Science located in 22º 53´ of north latitude and 82º 02´ western, 92 m over level sea. The plants studied were *Sapindo saponaria, Morus alba, Trichanthera gigantea, Tithonia diversifolia, Gliricidia sepium Leucaena leucocephala,* Stysolobium *aterrimun* and *Arachis pintoi.* The leaves with petioles and young stems were collected simulating animal selection. Grasses used as forage in the assays to obtain mixes of grass:foliages were *Pennisetum purpureum* Cuba CT-115 or Star grass (*Cynodon nlemfuensis*).

Phytochemistry screening was carried out. The experiments were conducted in vitro system. Rumen fluid was strained as inoculum from two rumen-fistulated crossbreed Zebu steer fed low quality forage. To determine methane production, the mixture of gases in the fermentative process was collected in each time at interval of 4, 8, 12 and 24 hours and the methane production were determined by flame ionization in gas chromatograph**.**

The phytochemical analysis indicated the presence of tannins, saponins and others secondary compounds with antiprotozoal and antimethanogenic properties. It is believed that saponins, tannins and other secondary compounds present in many plants have effects of suppressing the methane production, reducing rumen protozoa counts, and changing rumen fermentation patterns (1). *Enterolobium* and *Leucaena* show high content of tannins and moderate levels of saponins. *Morus alba* presented moderate presence of saponins and triterpenes, while the content of secondary metabolites in *Tithonia* and *Gliricidia* were not very high (Table I).

Studies in relation with the effect of inclusion of the foliages in the diet of grass forage on microbial populations in the rumen indicated that 15% of *Leucaena* and *Gliricidia; 20% of Sapindus and A. pintoi* as well as *40% of S. aterrimum,* affect negatively the protozoa population en el rumen. *Gliricidia* reduced this population from 45.71 to 2.57 x 10^5 cel/mL. *Enterolobium,* however, increased the number and activity of the cellulolytic fungus and total viable bacteria, although it didn't exert defaunating effect, at least, under the experimental conditions in which the studies were conducted. The inclusion of 10 % of *Tithonia* decreased the methanogenic bacteria and increased the cellulolytic bacteria, but to reduce the protozoa population level of 20 % level was necessary. The defaunating effect observed with some plants could be due to that it was found a quadratic relationship between the content of condensed tannins and the population of ruminal protozoa.

The results of methane production in relation to inclusion of 25 % of foliages of S. *saponaria, M. alba* and Trichantera using *P. purpureum* as pasture base, indicated that this foliages reduced methane production, significantly, in similar proportions to respect to pasture (Table II). *Trichantera* inhibit methane emissions in 41 % respect to *Pennisetum* (7,01 vs. 16.96 g/kg DM, respectively). The results suggest that the secondary compounds found in plants can to exerting defaunating effects on the rumen and also reduce the methanogenic bacteria when the adequate levels in the diet are utilized. The use of tree and shrubs, as strategies of supplementation is an adequate option to reduce methane production and improve the animal nutrition of ruminants.

TABLE I. EFFECT OF DIFFERENT LEVELS OF TROPICAL FOLIAGES ON RUMINAL MICROBIAL POPULATION

Plant Foliages, % DM	Cellulolytic bacteria, 10^7ufc/mL	Protozoa, 10^6 n/mL	Methanogenic Bacteria, 10^9cfu/mL
L. leucocephala;%			
0	2.92[a]	14.64[a]	-
20	3.67[b]	4.57[b]	-
60	6.83[c]	5.34[b]	-
SE±	0.28***	0.14***	-
Tithonia diversifolia			
0	24.9[a]	3.75[a]	45.2[a]
10%	55.8[b]	3.25[a]	27.5[b]
20%	29.2[a]	1.5[b]	16.8[c]
SE±	0.12*	0.02*	0.45**
Gliricidia sepium,%			-
0	6.31[a]	45.71[c]	-
15	7.94[a]	11.22[b]	-
30	13.90[b]	2.57[a]	-
SE±	0.14*	0.09***	-
E. cyclocarpum			
0	4.5	3.9	-
15	9.1	3.6	-
30	7.9	4.9	-
SE±	0.4	0.4	-

($P < 0.05$), (** $P < 0.01$), (***$P < 0.001$)

[a, b, c.] Means with different letters between columns differ at $P < 0.05$ (Duncan, 1955).

TABLE II. EFFECT OF DIFFERENT TROPICAL PLANT FOLIAGES IN METHANE PRODUCTION *IN VITRO* CONDITIONS

	Methane emission		
Treatments	mL	mL/g DM	g/kg DM
Pennisetum	13.48[a]	26.18[a]	16.96[a]
Sapindo	7.32[c]	14.01[cd]	9.10[de]
Trichantera	5.62[c]	10.82[d]	7.01[e]
Morera	7.52[c]	13.76[cd]	8.93[e]
Sap:Forage 25:75	9.13[c]	17.02[bc]	11.37[c]
Mor:Forage 25:75	10.30[ab]	19.10[b]	12.40[bc]
Trich:Forage 25:75	8.02[c]	18.02[bc]	11.60[c]

[a, b,c, d,e.] Means with different letters between columns differ at $P < 0.05$ (Duncan, 1955).

EFFECT OF REPLACING DIETARY SOYBEAN MEAL WITH TROPICAL LEGUMES ON THE PERFORMANCE OF LAMBS

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This study determined how supplementing bahiagrass hay (*Paspalum notatum* Flügge cv. Pensacola) with soybean [*Glycine max* (L.) Merr.] meal or warm-season legume hays affects intake, digestibility, and N utilization by lambs. Forty-two Dorper x Katadhin crossbred lambs (30.6 ± 5.5 kg) were fed ad libitum 6-wk regrowth bahiagrass hay (73.8% NDF, 8.1% CP) supplemented with nothing (Control), soybean meal, or hays of annual peanut [*Arachis hypogaea* (L.) cv. Florida MDR98; 46.2% NDF, 14.7% CP], cowpea [*Vigna unguiculata* (L.) Walp. cv. Iron clay; 62.2% NDF, 11.7% CP], perennial peanut (*Arachis glabrata* Benth. cv. Florigraze; 43.3% NDF, 15.2% CP), pigeonpea [*Cajanus cajan* (L.) Millsp. cv. Georgia two; 78.6% NDF, 12.2% CP], or soybean (cv. Pioneer 97B52; 59.0% NDF, 13.5% CP). Legume hays were supplemented at 50% of total diet DM and soybean meal was supplemented at a level (4.25% of diet DM) that matched the average dietary CP content (10.8%) of the legume hay-supplemented diets. The pulses were harvested at respective maturities that maximized both DM yield and nutritive value, and the peanuts were first cuttings. Diets were fed to six lambs per treatment for two consecutive 21-d periods. Supplementation with hays of annual and perennial peanut, cowpea, and soybean increased ($P \leq 0.002$) DMI, but apparent DM digestibility was only increased ($P \leq 0.03$) by supplementation with annual or perennial peanut hay. Nitrogen intake, digestibility, and retention were increased ($P < 0.001$) by all supplements and responses were greater when annual or perennial peanut hays were fed. Ruminal ammonia concentration was increased ($P \leq 0.01$) by all legume hay supplements. Microbial N synthesis and ruminally-degraded OM were increased ($P \leq 0.03$) by perennial and annual peanut supplementation, but efficiency of microbial synthesis was not different ($P \geq 0.52$) among diets. Annual and perennial peanuts were the most promising legume hay supplements for the lambs followed by cowpea and soybean.

EFFECT OF ESSENTIAL OIL FROM *CORDIA VERBEANCEA* ON THE FERMENTATION OF A HIGH CONCENTRATE DIET BY USING THE *IN VITRO* GAS PRODUCTION TECHNIQUE

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Studies with plant secondary metabolites as rumen fermentation modifiers have increased as an attempt to reproduce the effects of ionophores. *Cordia verbenacea* D.C. is a Brazilian bush with antimicrobial properties attributed to its essential oil (EO) [1]. The objective of this experiment was to determine the effects of *C. verbenacea* EO on the ruminal fermentation by using an *in vitro* gas production system [2].

Treatments were defined as: **Control** – without addition of monensin or EO; **MON** – addition of monensin (Sigma Aldrich Inc.) at 3 μM as a positive control; **COR37.5** – addition of 37.5 μL of EO in 75 mL of buffered rumen fluid; **COR75** – addition of 75 μL of EO in 75 mL of buffered rumen fluid. A complete randomized design was utilized with six replicates for gas production (mL/g OM_{incub}) and three replicates for all other variables. Two conditions were independently assessed: a) Coastcross (*Cynodon* sp.) hay (89.2% DM, 9.7% CP, 1.3% EE, 7.9% ash, 60.2% NDF, and 30.6% ADF) as substrate + inoculum of sheep on pasture; b) 80:20 concentrate:forage diet (20.0% Coastcross hay, 62.7% corn, 15.0% soybean meal, 1.0% limestone, and 1.3% mineral premix on DM basis; 91.5% DM, 15.7% CP, 3.3% EE, 4.3% ash, 20.3% NDF, and 8.8% ADF) as substrate + inoculum of sheep adapted to this diet. Two different inocula for each condition were used as source of variation. In each flask (160 mL), 0.5 g of substrate was incubated with 50 mL of incubation medium and 25 mL of rumen fluid at 39°C. Incubation time was 24 h for the hay and 16 h for the high-concentrate diet. Flasks without substrate (blanks) and flasks containing standard hay were also included.

According to the GC-MS analysis, the major compounds of *C. verbenacea* EO were: *trans*-caryophyllene (28.19%), alpha-pinene (23.58%), aloaromadendrene (6.90%), and alpha-humulene (4.54%). Considering both substrates, MON reduced gas and methane productions, increased propionate concentration, and decreased acetate:propionate ratio when compared with Control. Especially for hay, TDDOM was reduced by MON and COR75, which is at least for MON a limitation imposed by short-term *in vitro* trials. The most promising effect observed with EO inclusion was related to the inhibition of methanogenesis using hay as substrate. Methane produced per unit of OM_{incub} was reduced by 30% comparing COR75 with Control. Although not statistically different, methane production expressed as mL/g OM_{degrad} showed an intermediary value for COR75 (32.9) when contrasted with Control (38.9) and MON (25.8). The partitioning factor was reduced comparing COR75 with Control in the hay condition, which indicates lower microbial production efficiency. In general, no effects were observed with EO inclusion using the high-concentrate diet as substrate. In this condition, the doses seemed to be very low to manipulate rumen fermentation. In conclusion, the EO from *C. verbenacea* was able to modify *in vitro* ruminal fermentation. This study indicates that doses greater than 1 μL/mL of buffered rumen fluid may decrease methane production as much as monensin when hay is used as substrate.

TABLE 2. EFFECTS OF MONENSIN (3 μM) AND ESSENTIAL OIL FROM *C. VERBENACEA* (37.5 OR 75 μL IN 75 ML) ON IN VITRO RUMINAL FERMENTATION OF COASTCROSS HAY OR 80:20 CONCENTRATE:FORAGE DIET.

Item[†]	Treatments[‡]				SEM[§]
	Control	MON	COR37.5	COR75	
	Coastcross hay				
Gas, mL/g OM_{incub}	125.6^{a}	83.9^{c}	127.9^{a}	113.5^{b}	2.0
Gas, mL/g OM_{degrad}	252.7^{b}	230.7^{b}	278.5^{a}	284.9^{a}	5.9
CH_4, mL/g OM_{incub}	18.7^{a}	9.7^{c}	17.0^{a}	13.1^{b}	0.7
CH_4, mL/g OM_{degrad}	38.9^{a}	25.8^{b}	38.2^{a}	32.9^{ab}	2.0
TDOM, %	48.08^{a}	37.51^{c}	44.54^{b}	40.26^{c}	0.71
Partitioning factor	3.97^{b}	4.42^{a}	3.60^{bc}	3.57^{c}	0.10
Total SCFA, mM	73.81^{ab}	69.47^{b}	74.42^{a}	73.32^{ab}	1.13
Acetate, mM	54.29	50.96	55.13	54.36	1.11
Propionate, mM	9.88^{b}	10.16^{a}	9.57^{c}	9.27^{d}	0.05
Butyrate, mM	7.39^{a}	6.38^{b}	7.44^{a}	7.31^{a}	0.06
Acetate:propionate	5.50^{a}	5.02^{b}	5.76^{a}	5.86^{a}	0.12
NH_3, mg/100 mL	26.49^{b}	27.63^{ab}	30.46^{a}	27.91^{ab}	0.89
pH at 24 h	6.70^{c}	6.76^{ab}	6.73^{b}	6.77^{a}	< 0.01
	80:20 concentrate:forage diet				
Gas, mL/g OM_{incub}	223.0^{a}	209.0^{b}	222.4^{a}	216.0^{ab}	3.2
Gas, mL/g OM_{degrad}	290.5^{ab}	279.3^{b}	300.0^{a}	298.4^{a}	4.6
CH_4, mL/g OM_{incub}	32.5^{a}	23.9^{b}	31.7^{a}	33.4^{a}	1.2
CH_4, mL/g OM_{degrad}	42.3^{a}	32.4^{b}	43.1^{a}	47.2^{a}	1.9
TDOM, %	76.89^{a}	74.08^{ab}	73.68^{ab}	70.83^{b}	0.98
Partitioning factor	3.48^{ab}	3.59^{a}	3.35^{b}	3.36^{b}	0.05
Total SCFA, mM	90.43^{ab}	91.22^{a}	83.83^{b}	93.32^{a}	1.63
Acetate, mM	56.78^{a}	55.78^{ab}	50.33^{b}	58.26^{a}	1.43
Propionate, mM	17.08^{b}	21.05^{a}	16.55^{b}	17.36^{b}	0.34
Butyrate, mM	11.17^{b}	9.52^{c}	11.54^{ab}	12.04^{a}	0.16
Acetate:propionate	3.34^{a}	2.66^{b}	3.06^{a}	3.37^{a}	0.09
NH_3, mg/100 mL	45.19	54.26	53.52	52.77	2.64
pH at 16 h	6.55^{a}	6.53^{b}	6.57^{a}	6.56^{a}	< 0.01

[†]OM_{incub} = incubated organic matter; OM_{degrad} = degraded organic matter; TDOM = truly degraded organic matter; Partitioning factor = mg of OM_{degrad}/mL of gas); SCFA = short-chain fatty acids. [‡]Means followed by distinct letters within row differ by Tukey test ($P < 0.05$). [§]SEM = standard error of the mean.

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COST-EFFECTIVE AND ENVIRONMENTALLY FRIENDLY OPTIONS TO IMPROVE LIVESTOCK PERFORMANCE IN DRY AREAS

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Ruminant production is the main source of income for rural population living in dry areas. The lack of adequate year-round feed resources is the most important factor contributing to the low productive and reproductive performances of the farm animals. Rangeland degradation, increasing use of some concentrate feeds in biofuel industry, global warming, recent leap in the prices of concentrate feeds and the international economical crisis are seriously threatening the sustainability of livestock-based production systems. Some promising cost-effective and environmentally friendly options, which could overcome this situation, are discussed in this paper.

Rumen manipulation with secondary compounds - The possible use of natural plant products as a growth promoter provides cheaper, safer and more consumer acceptable alternatives to synthetic compounds. Recent studies showed that the association of a small amount of a tanniniferous legume shrub, i.e. *Acacia cyanophylla* Lindl., with soya bean meal (SBM) resulted in a significant increase in daily gain of lambs (67 *vs.* 43 g/d) on oaten hay [1]. This effect was obtained when total tannins to dietary protein ratio averaged 0.021 and SBM (200 g/d) was distributed immediately after the entire consumption of the Acacia leaves (100 g/d) by animals. Sulla (*Hedysarum coronarium*), a mediterranean legume, contains a moderate level of condensed tannins (CT) and is relatively high in crude protein. Due to these characteristics, sulla grazing lambs grew better (150 *vs.* 110 g/d) than those on the same pasture but drenched with polyethylene glycol, a tannin deactivating reagent.

The presence of gastrointestinal parasites (GIP) in ruminants decreases mainly protein utilization. This results in decreased growth of ruminants harbouring high number of parasites. Recent studies showed that the incorporation of CT-containing feedstuffs in the diet reduce GIP. Tannins might interfere directly with the biology of various nematode stages and they could indirectly improve the host nutrition by protecting the dietary proteins from ruminal degradation and thus decreasing nematode load. Consumption of tannin containing feeds such as sainfoin hay [2] and *Acacia cyanophylla* foliage [3] reduced faecal egg counts in kids and lambs, respectively.

The defaunating properties of saponins could lead to lowering of ammonia level in the rumen, which could improve the efficiency of microbial synthesis. Saponins are also known to increase permeability of the intestinal mucosal cells [4]. This probably leads to better absorption of nutrients from the intestine. The incorporation of small amounts (40 g/d) of Fenugreek (*Trigonella foenum-graecum* L.) seeds in the concentrate increased lamb growth (+ 52%) and milk production (+ 64%) in dairy ewes (unpublished data), most probably because of saponins (30 g diosgenin equivalent/kg dry matter seeds).

Cactus, a dromedary of the plant kingdom and Moringa - The popularity of cactus in numerous dry countries is increasing. Characterised by a remarkable tolerance to drought conditions, high water use efficiency, a rapid dissemination and growth, a high biomass yield and a multipurpose use, cactus is a promising range species that can promote livestock sector in dry areas and improve farmers' income. Ruminants do not need to drink water when receiving cactus cladodes (*ca.* 35 g dry matter/kg metabolic weight). Moreover, the association of cactus with low quality forages improves rumen digestion, thus increasing ruminant performances [5]. Importantly, cactus reduces the use of common concentrate feeds like barley and decrease feeding cost. In intensive plantations in Brazil, cactus has been shown produce up to 40 tons dry matter/ha. Similarly, another plant,

Moringa oleifera grown as intensive plantations has been shown to produce over 100 tons of dry green foliage/ha with protein content of *ca.* 25% and biological value of proteins as good as soya bean. In addition, this foliage has a number of antioxidants [4]. The feeding of this foliage has been shown to increase milk production and growth [6].

Better use of agro-industrial by-products (AGIBPs) – A wide range of AGIBPs could be used advantageously in livestock feeding. Studies [7] report numerous formulas of AGIBPs-based silages, feed blocks and pellets and their positive effects on livestock performances and on decreasing feeding costs. Recently, kernel meal from *M. oleifera* seeds has been shown to have cationic protein having rumen modulation properties. Supplementation of the defatted kernel meal in the diet at a level of 4 g per day increased body weight of lambs [8]. In addition, kernel meal from another plant, *Jatropha curcas,* capable of growing in harsh conditions has been shown to be an excellent substitute for fish meal in fish diets and for soya bean meal in the diets of farm animals. Kernel meal from the non-toxic *J. curcas* (available only in Mexico) could be used after heat treatment and from the toxic *J. curcas* (available in most tropical countries) after heat treatment and removal of phorbol esters [4].

It is concluded that a number of cost-effective and simple approaches are available for improving livestock performances raised under harsh conditions. There is a need to put in place an effective extension programme for taking the information from "laboratory to land".

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USE OF SUN-DRIED ON-FARM GENERATED POULTRY LITTER AS A FEED RESOURCE BY PIGS IN A POULTRY-PIG FARM ENTERPRISE: IMPLICATIONS ON CHEMICAL COMPOSITION OF FEED AND BLOOD CHEMISTRY

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Forty eight (48) growing-finishing pigs (36.11 ± 1.26 kg) were allotted to three dietary treatment groups of 0, 33.33 and 66.67% SOPL as a replacement for 30% maize in diets of growing-finishing pigs on weight for weight basis. Each treatment was replicated four times with 4pigs/replicate in a completely randomized design. The diets were formulated to contain 16-19% crude protein and the pigs were housed in concrete floored pens containing feeding and watering troughs for the duration of the study.

Proximate compositions of sun-dried on-farm poultry (SOPL) and the experimental feeds were done according to the methods of A.O.A.C. [1]. Blood sample was randomly collected from 2 pigs/replicate for the three treatment groups at the end of the feeding trial. All data generated on the serum metabolites and haematological parameters of the pigs were subjected to statistical analysis using the SAS Computer software package [2].

The result of the proximate composition of the SOPL (from growing pullets) used is as shown on Table I. Slight increases were observed in the crude protein and fibre contents of the SOPL-diets over the control diet. This could be because of higher protein content (23.76%) of the test ingredient (SOPL) over the maize it replaced. However, the crude protein of the litter used in this study was slightly lower than the range reported by Fontenot and Hancock [3] for broiler birds. Increases of about double were recorded in the ADF, NDF and ADL fractions of the diets because of the replacement levels of SOPL (Table I). This is expected, taking into consideration the fact that wood shavings, which is generally used as beddings in Nigeria is a highly fibrous material, being a by-product of the wood processing industry.

Haematology and blood biochemistry are routinely used in veterinary medicine to evaluate the health status of animals and poultry [4]. An increased level of up to 66% SOPL resulted in a depression ($P < 0.05$) in the values of PCV and WBC while the Haemoglobin values of the pigs across the groups were unaffected. Variations recorded in the values of the RBC did not follow any particular trend (Table II). The serum glucose, urea, creatinine and glutamate pyruvate transaninase values were unaffected by the treatment. However, the serum total protein, albumin and cholesterol values were increased while alkaline phosphatase did not follow any trend (Table III). With these results, it is obvious that with good management, feeding SOPL has no adverse effect on the health status of the pigs. Rather it has benefit for the poultry industry by helping to reduce the over-application of poultry litter to the soil, which possibly results in high levels of nitrogen and phosphorus in soil water [5].

From the above results, it could be inferred that growing-finishing pigs can tolerate the replacement of up to 66% of the 30kg maize fraction of the diet with sun-dried on-farm poultry litter (SOPL) without any adverse effect on the performance and the overall health status of the pigs.

TABLE I. PROXIMATE COMPOSITION OF SOPL AND EXPERIMENTAL DIETS

Parameters	0% SOPL	33%SOPL	66%SOPL	SOPL
Dry Matter (%)	88.59	88.65	88.72	88.61
Crude protein (%)	16.58	19.67	19.85	23.76
Crude fibre (%)	6.52	7.29	7.37	11.22
Ether extract (%)	3.51	3.69	3.75	3.49
Ash (%)	7.46	8.21	8.46	13.15
NDF (%)	34.97	49.77	51.28	42.68
ADF (%)	15.87	31.24	33.54	22.87
ADL (%)	5.12	13.58	13.76	8.77

TABLE II. HEMATOLOGICAL INDICES OF GROWING-FINISHING PIGS FED GRADED LEVELS OF SOPL

Parameters	0%SOPL	33%SOPL	66%SOPL	SEM (±)
Packed Cell Volume (PCV)	37.63^{a}	36.13^{ab}	34.50^{b}	0.61
Haemoglobin (Hb)	11.93	11.30	10.60	0.29
Red Blood Cell (RBC)	805.00^{a}	648.75^{b}	736.25^{ab}	31.67
White Blood Cell (WBC)	395.75^{a}	336.00^{ab}	251.25^{b}	24.93

TABLE III. SERUM METABOLITES OF GROWING-FINISHING PIGS FED GRADED LEVELS OF SOPL

Parameters	0%SOPL	33%SOPL	66%SOPL	SEM(±)
Total Protein (g/dl)	5.20^{b}	7.05^{a}	6.25^{ab}	0.38
Albumin (g/dl)	3.98^{b}	4.20^{b}	5.63^{a}	0.27
Glucose (mg/dl)	48.08	45.20	49.03	6.22
Cholesterol (mg/dl)	87.48^{ab}	72.78^{b}	101.20^{a}	4.84
Urea (mg/dl)	22.73	20.90	16.80	2.61
Creatinine (mg/dl)	0.88	1.18	1.48	0.13
GPT/ALT (IU/L)	7.33	6.18	6.25	0.38
Alkaline Phosphatase (IU/L)	36.35^{a}	22.73^{c}	28.40^{b}	1.90

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POSTER PRESENTATIONS

USE OF EDIBLE FISHERY BY-PRODUCTS AS SOURCES OF NUTRIENTS FOR FISH AND LIVESTOCK IN ZANZIBAR, TANZANIA

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Results show percentage fish waste (%ww) between individual genera and species varied significantly (10.47 ± 0.93; $P < 0.001$). Average annual fish waste produced is 2.040t out of total 1.84, 1.81, 1.79 and 2.72 thousand tonnes from the years 2001, 2002, 2003 and 2004 catches respectively. The body parts of fish significantly determined fat and mineral composition (LSM for gills = 10.85 ± 1.486 g/100 g DM EE vs. guts = 15.45 ± 1.486 g/100 g DM EE; $P = 0.033$ and LSM for gills = 23.49 ± 1.494 g/100 g DM mineral vs. guts = 14.27 ± 1.494 g/100 g DM Ash; $P < 0.001$), while treatment (boiling at 95 – 105^{o}C) had no effect on both fat, protein and mineral. Significant variation in overall protein content between species (LSM = 57.68 ± 2.33; $P < 0.001$) is observed. Slight dispersions between sites for mineral (LSM = 29.1 ± 2.01 g/100 g DM) and fat (LSM = 13.93 ± 1.05 g/100 g DM) showed up in bulky wastes. It was concluded that valuable quantity of fish waste is available to reduce the limiting protein supplementation problem in both livestock and fish farming in the western Indian Ocean region. Furthermore, effective utilization of fish waste could help in recycling of the by-product and clearing the environment. Additional on-station and on-farm studies are required to measure the intakes and effects of the fish waste supplement on milk yields and growth performances.

EFFECT OF ADDED *PUNICA GRANATUM* PEEL FRUITS AND *NIGELLA SATIVA* SEEDS ON IMMUNOLOGY AND PERFORMANCE OF SUCKLING BUFFALO CALVES

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The use of feed additives is considered as a good source to improve the immunity status of newborn calves instead of chemical products because the residuals of chemical products may have side effects on human health on the long-run. *Punica granatum* fruits peel (Pg-p) has been shown to possess a significant antioxidant activity also valued for their therapeutic properties and used to remove intestinal tapeworm. On the other hand, seeds of *Nigella sativa* (NS) are rich in fatty acids (Linoleic and Linolenic acid), essential fatty acids and non-starch polysaccharide. Recently, clinical and animal studies have shown that extract of the NS seeds have many therapeutic effects such as immunomodilative, antibacterial, hypotensive, hepatoprotective, and antidiabetic effects.

Thirty-six suckling buffalo claves (18 males and 18 females) were used in this study with average live body weight 38.44 ± 0.62 kg. The calves were divided into 3 groups, each group allotted to receive one of 3 tested starters throughout 105 d. The starter was offered without additive in the control group (G1), while it was mixed with 2% Pg-p in group (G2) and with 2% Pg-p plus 3% NS in group (G3). Weekly faecal swabs were collected from the experimental animals for bacteriological examination. Immunoglobulins (Ig) and blood metabolites were determined in blood serum. Three sets of digestion trials were carried out using 3 male calves of each group that fed the same tested rations.

Milk consumption was not significantly different among groups while, starter and clover hay intake was significantly ($P < 0.05$) higher in G3 than those in groups G1 and G2. The total weight gain achieved by group G3 was greater than that of G1 and G2 groups by 25.60% and 7.80%, respectively. Blood TP was significantly ($P < 0.05$) higher in G3 than that in G1. An albumin value was relatively higher in G1 compared with the treated G2 and G3. Meanwhile, blood urea nitrogen was significantly greater in G2 in comparison with G1 and G3. Concentration of creatinine was relatively less in the treated groups G2 and G3 than G1. Group 3 possessed greater IgG content than that in G2 and G3 serum. Addition of Pg-p and NS in ration limited the number of calves having symptoms of diarrhoea due to increased level of antibodies and antibacterial effect. The effect of Pg-p and NS in decreasing the number of diseased calves may be due to increase the level of antibodies and anti bacterial effect. The action of tannins against bacteria and yeasts can be established by a relation between their molecular structure and their toxicity, astringent properties or other mechanisms [1].

Digestibility of DM, CF and EE were significantly ($P < 0.05$) higher in G3 compared with G1 and G2. The improved nutrients digestibility with added NS seeds might be due to the role of medical plant as inhibitors of gram positive bacteria and improved ruminal fermentation by increasing bacterial activity, which in turn increases digestibility [2].

The Present results are revealing dietary supplementation of Pg-p in starter of buffalo calves was successful in improving growth rate of the animal. Moreover, Pg-p reduced the number of cases affected by microorganisms into the half number. Those effects were augmented by adding NS seeds in G3 as evidenced by improved immunity in treated animals hence achievement of better growth performance.

TABLE I. CHEMICAL COMPOSITION OF EXPERIMENTAL STARTERS AND DIFFERENT FEED INGREDIENTS.

Item	Experimental starters			Ingredients		
	S1[a]	S2[b]	S3[c]	Pg-p[d]	NS[e]	CH[f]
DM	89.24	89.21	89.38	87.84	94.78	88.74
DM composition, %						
OM	91.16	91.26	91.45	96.15	97.53	86.31
CP	20.33	20.01	20.09	4.71	22.72	12.40
CF	7.98	7.99	7.82	8.40	2.38	17.79
EE	3.43	3.43	4.45	3.36	37.58	2.01
NFE	59.42	59.83	59.09	79.68	34.85	54.11
Ash	8.84	8.74	8.55	3.85	2.47	13.69

[a] Starter containing soybean meal 23%, yellow corn 34%, wheat bran 30%, rice bran 20% and common salt 1%

[b] starter containing S1 plus 2% Pg-p on DM basis

[c] starter containing S1 plus 2% Pg-p and 3% NS seeds on DM basis.

[d] Punica granatum fruits peel

[e] *Nigella Sativa seeds*

[f] Clover hay ,

Milk containing (Protein% =4.54, fat%= 7.68, lactose% = 4.52 and ash% = 1.05)

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IDENTITY AND FUNCTIONAL ANALYSIS OF BACTERIAL POPULATIONS INVOLVED IN REDUCTIVE ACETOGENESIS

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Our current understanding of the microbial processes underpinning hydrogen utilization and methane production within the rumen is incomplete and the key to manipulating these emissions in the future will flow from fundamental improvements in our knowledge of methanogenesis and alternative hydrogenotrophic pathways. Reductive acetogenesis is an alternative hydrogen-utilising pathway to methanogenesis in the rumen and has potential as a strategy for reducing ruminant greenhouse gas emissions. The obligatory anaerobic bacteria responsible for reductive acetogenesis are known as homoacetogens. Homoacetogens use the acetyl-CoA pathway to reduce carbon dioxide to acetate, and most are able to use this pathway for growth on hydrogen and carbon dioxide as the sole energy source[1]. Homoacetogens are present in the rumen [1] and molecular tools are necessary to further investigate their ecology. The aim of the present work was to identify DNA sequences of genes present in homoacetogens that may be suitable as targets for the development of functional-group molecular tools for these microorganisms. The genes investigated were those of three key enzymes in the acetyl-CoA pathway: formyltetrahydrofolate synthetase (FTHFS), carbon monoxide dehydrogenase (CODH) and acetyl-CoA synthase (ACS).

Several batch fermentation systems inoculated with rumen microbes were established, with ^{13}C-carbonate as the principal carbon source, and in the presence or absence of methanogen inhibitors. Denaturing gradient gel electrophoresis (DGGE) was performed on samples to reveal minimal gross differences in community structure within the different systems. Metagenomic DNA was extracted and Isotope ratio mass spectrometry (IRMS) was used to confirm the uptake of ^{13}C, followed by the separation of ^{13}C-labeled DNA via isopycnic gradient ultracentrifugation. The ^{13}C-labeled DNA was used as a template for the production of 16S rDNA phylogenetic and formyltetrahydrofolate synthetase (FTHFS) libraries, as well as metagenomic (fosmid) libraries. Results from the 16S rDNA analyses identified several bacteria of interest in the methanogen inhibited cultures (*Actinomyces ruminicola, Desulfovibrio desulfuricans, Ruminobacillus xylanolyticum, Succiniclasticum ruminis Treponema bryantii*, *Ruminococcus productus* and *Enterococcus avium*). FTHFS sequence analysis from fermentations and cattle rumen samples supplemented with bromochloromethane (BCM) show clustering of sequences with the known homoacetogens. Putative CODH and ACS sequences were also generated from DNA obtained from a mixed enrichment culture of homoacetogens, from the rumen of cattle.

An extensive set of primers has been developed from the numerous phylogenetic and functional gene databases. These primers and probes are being used in targeting key enzymatic steps in hydrogen sequestering pathways for use in probing the metagenomic fosmid libraries and monitoring populations of hydrogen-utilising microorganisms in the rumen.

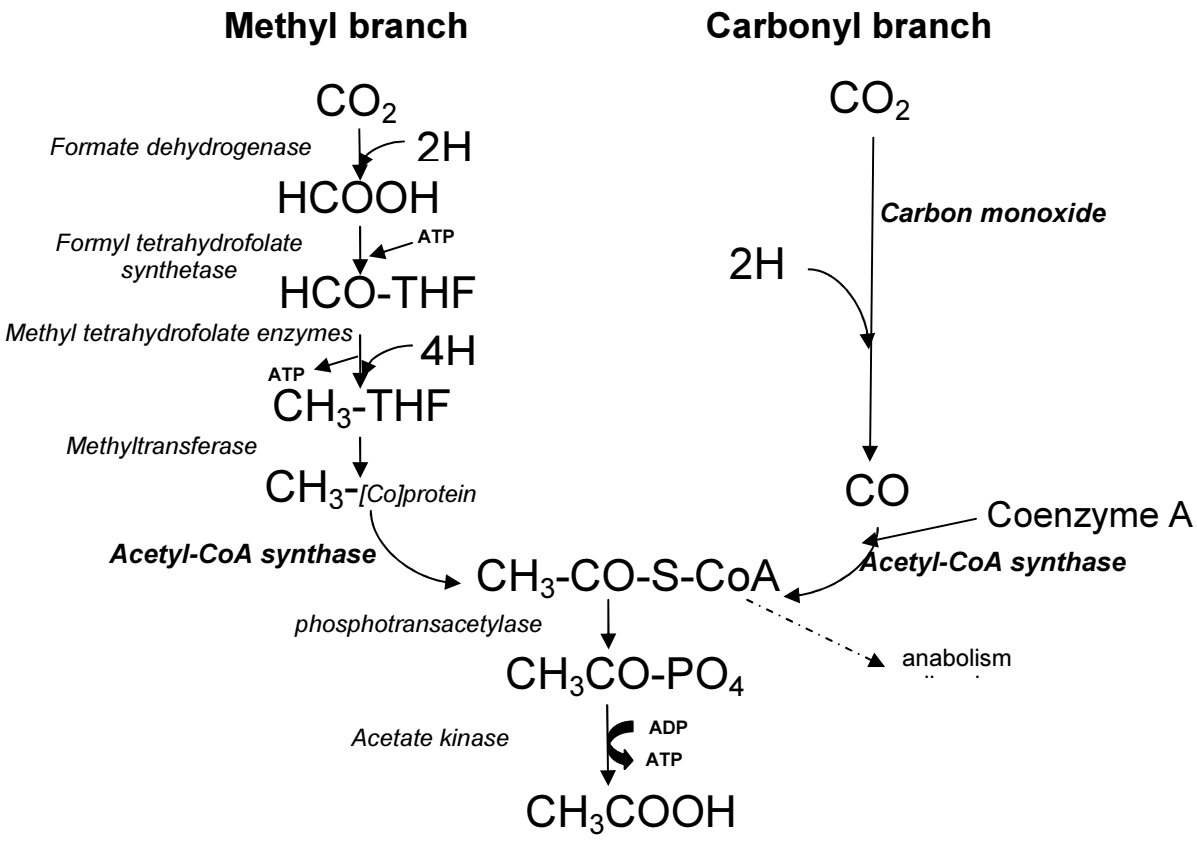

FIG 1. The Acetyl-CoA pathway of reductive acetogenesis. Adapted from Drake, [2].

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USE OF RADIOIMMUNOASSAY TO ASSESS THE REPRODUCTIVE PERFORMANCE OF SYRIAN AWASSI SHEEP AS A TOOL FOR SUSTAINABLE IMPROVEMENT

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Awassi, the fat-tailed triple purpose (about 20 million head in Syria), is the most important sheep breed in the Middle Eastern countries. Its desirable traits, such as the popularity of its meat and milk, high adaptability to different ecosystems, tolerance to extreme temperature, and endurance of adverse management and feeding conditions have encouraged breeders in many countries to raise Awassi sheep. Syrian Awassi sheep are seasonal breeders and normally the ewes lamb only once annually. Moreover, they have a poor reproductive performance and a low twinning rate.

A series of experiments has been carried out for the last 15 years to characterise, improve and enhance Awassi productivity through better reproduction tools. The main objectives of the current study were to determine the live weight and age at puberty in Syrian Awassi ewe lambs and the affecting factors; and to assess the response of multiparous Syrian Awassi ewes to the intravaginal sponges' insertion and equine Chorionic Gonadotropin (eCG) injection.

In the present study, two experiments were conducted on Syrian female Awassi sheep at Deir Al-Hajar area, 33 km south-east of Damascus. This is a dry area and resembles the Syrian steppe region where the majority of sheep population is raised. Blood samples were taken from the jugular vein of all animals twice a week starting at 5 months of age, continued for a period of 16 months for animals in the first experiment, and from an age of 21 until 29 months for those employed in the second experiment. Serum was prepared and stored at -20 ^{o}C until assayed for progesterone using RIA kits.

In experiment 1, 18 ewe lambs (9 singles and 9 twins) were used for a period of 16 months, starting at an age of 5 months and an average live weight ± SD of 24.6 ± 4.6 kg, and serum progesterone concentration of 0.27 ± 0.26 nmol/L. The average birth and weaning weights were 4.7 ± 0.8 and 22.5 ± 5.5 kg, respectively.

Based on the first elevation in serum blood progesterone to a concentration exceeding 3.18 nmol/L, as an indicator for active corpora lutea [1], followed by the appearance of regular oestrous cycles as a criterion for the attainment of puberty, it was found that ewe lambs attained puberty during the second breeding season after their birth, at an average age of 18 ± 1.1 months. There were no significant differences in the time at puberty between ewe lambs in terms of the month of birth, type of birth (single or twin) or weaning weight. The averages live weight and serum progesterone concentration of ewe lambs at puberty were53.7 ± 7.2 kg and 6.32 ± 3.69 nmol l^{-1} (FIG. 1), respectively. A positive and significant correlation ($r = 0.72$, $P < 0.001$) was found between serum concentration of progesterone and live weight of ewe lambs during the experiment.

In experiment 2, 16 multiparous cyclic Awassi ewes, 21 months of age, were used during the breeding season for an observation period lasting 8 months. Females were equally divided into 2 groups, experimental (E) and control (C) groups. Animals in both groups were treated with intravaginal sponges containing 40 mg of flugestone acetate for a period of 14 d. Only females in the E group were injected intramuscularly, at the sponge withdrawal, with 500 IU of eCG. Three fertile Awassi rams were introduced daily into all females in both groups after 24 hours of sponge withdrawal for oestrus detection and mating.

All females exhibited oestrus and were mated within 3 d of sponge withdrawal. Twinning rates were 37.5 and 12.5% for the females in groups E and C, respectively, with the difference between the two groups being significant ($P < 0.05$, Table I). There were no problems in delivery and the lambs born, together with the lambing ewes were healthy, and mortality rate from birth to weaning at 3 months of age was zero% in both groups.

It was concluded that Syrian Awassi ewe lambs attain puberty during the second breeding season of their life at an age of about 18 months and at an average live weight of around 54 kg at which they become capable of reproduction. In addition, no effects were observed either for the month of lambing or for the birth or weaning weight of ewe lambs on the time to attain puberty. It was also concluded that it is possible to improve the twinning rate of either the multiparous Syrian Awassi ewes in their first pregnancy using eCG with no adverse effects on the ewes lambed or the lambs born.

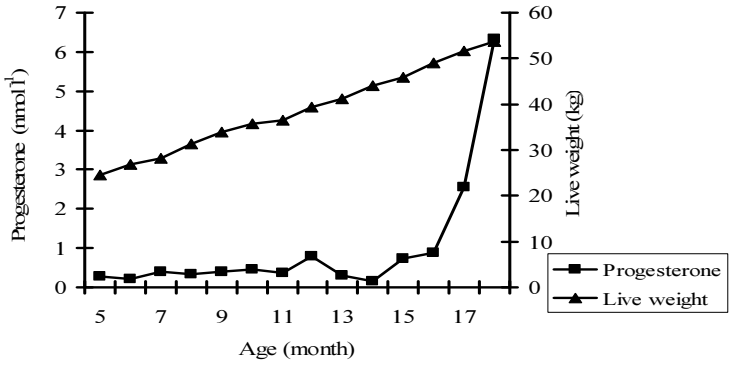

FIG. 1. Average blood serum progesterone concentration and mean live weight of Syrian Awassi ewe lambs.

TABLE I. THE EFFECT OF ECG INJECTION ON SOME REPRODUCTIVE PARAMETERS OF SYRIAN AWASSI EWES.

Parameter	Group E	Group C
Mating weight (kg).	56.0 [a]	55.5 [a]
Mating rate (%).	100 [a]	100 [a]
Weight after lambing (kg).	61.5 [a]	60.8 [a]
Duration of pregnancy (d).	151.4 [a]	151.0 [a]
Twinning rate (%).	37.5 [a]	12.5 [b]

[a,b] Means, within a row, followed by different small letters are significantly different ($P < 0.05$).

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SELECTION AND PRODUCTION OF BACTERIA WHICH DETOXIFY MIMOSINE: LEUCAENA LEAVES MAY BE USED AS RUMINANT FEED

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Mimosaceae are shrubs or trees, which grow abundantly in tropical regions. Their leaves contain high value protein which cannot be used as feed due to the toxic substance mimosine and its metabolites in the digestive tract These alkaloids cause diseases in ruminants, mainly loss of hair/wool, and may lead to death in higher concentration. This is the reason why the nutritive value cannot be exploited reasonably in animal production.

Experience has shown that there are some geographical regions where animals do not suffer by mimosine. It was found that they harbour ruminal bacteria, which degrade mimosine to non-toxic metabolites. In cooperation with other microbes in the digestive tract, the full dietetic value of Leucaena may be exploited.

To date several bacteria were isolated and may be used as feed additive, e.g., S. jonesii. However, their production and storage is expensive and complicated. A practical method is to orally inoculate animals and use their rumen fluid directly as feed additive. This rumen culture or cultured anaerobic bacteria in the laboratory may suffer during transport and storage; hence, they need a cold chain until the target animal.

Our institute has an outstanding experience to produce bacterial veterinary vaccines, probiotics, and biological fertilizers in tropical countries. Continuous culture in a bioreactor is the base for the success. It was the idea to use this technology for selection and production of mimosine degrading bacteria.

The presentation will give a short theoretical background of

- bacterial fermenter production
- continuous culture
- isolation of specific bacteria under selective environmental conditions
- mass production in a bioreactor to be used in tropical areas
- purification and concentration of the bacterial crop by rinsing and hollow fibre cross flow ultra filtration
- stabilisation of the product in alginate beads
- microbiota in the digestive tract.

The practical work started with ruminal content of German steers, which never had had contact with mimosine. Using a simulation of rumen digestion, this rumen fluid was used as starter culture. It was fed with a complex artificial medium 98-5, stabilized with artificial saliva, maintained at ordinary rumen temperature (39°C) under anaerobic conditions. Mimosine in increasing concentration was added continuously. After a “feeding period” of two weeks, almost pure cultures of bacteria were obtained which digested mimosine in the test tube. By standard bacteriological work, a pure culture of this isolate was obtained. By modern microbiology, it was identified as a apathogenic Klebsiella pneumoniae strain (no. 3948 of our Institute’s collection).

Mass production was tried under anaerobic and aerobic conditions. It was found that it was better to use aerobic fermenter conditions. Strain 3948 proved to be multiplied far better as an aerobe, but to digrade mimosine under anaerobic conditions. Continuous culture with standard brain heart medium yielded ~10^{13} cfu/mL. With a 500 mL fermenter and a dilution rate D= 0.1 h^{-1} 10^{16} cfu were

produced per day. The obtained bacteria were rinsed in water, and concentrated by cross flow ultra filtration. The obtained mud like suspension was incorporated in alginate beads and dried at 37°C within 24 h. The obtained chalk like beads had a diameter of approximately 1-2 mm. 1 g contained $5x10^{10}$ cfu.

For feeding trials under practical conditions, these dried bacteria were sent by parcel post to Myanmar. Accidently they were stored at ambient temperature (25-30°C) at customs in Yangoon for 8 weeks. Feeding tests with sheep finally proved that the bacteria were still active. A single dose of $5x10^{10}$ cfu or similar daily doses for 14 d were used. Sheep feeding on local Leucaena leaves lost their fleece and had to be saved by stopping the experiment, whereas the trial groups gained live weight and remained completely healthy. Preliminary results show that nitrogen retention was similar in groups feeding Leucaena leaves or standard diet. Hence, local available Leucaena could be used as staple feed

These results should encourage isolation and production of geo-specific or site specific bacteria to be used as probiotics and feed additive in tropical regions. The technology of production is well adapted to the situation in many tropical countries.

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STRUCTURE AND SEQUENCE VARIATION OF MINK INTERLEUKIN-6 GENE

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Aleutian disease (AD) is the number one disease threat to the survival and future of the mink industry in Nova Scotia and the world. Several ranchers have gone out of business in recent years in Nova Scotia as a direct result of AD.

Currently, the control measure for AD consists of testing and slaughtering of infected mink. This practice has not been effective in controlling the disease. Finding a means of controlling AD is the number one priority for the mink industry in Nova Scotia [1]. An effective control measure will have a long-term positive effect on the rural economy by improving production potential of mink and reducing production cost.

It has been shown that antiviral antibodies produced by activated immune system cells sometimes combine with interleukin-6 (IL-6) to form immune complexes that cause AD in mink [2]. There is evidence of a significant relationship between nucleotide variations in IL-6 gene and the onset of certain diseases in humans, which bears similar symptoms to AD [4]. Furthermore, pathological symptoms of AD resemble those of other conditions, such as systemic lupus erythematosus (SLE) and Castleman Diseases in humans, where overproduction of IL-6 coincides with the severity of the disease [3]. These findings suggest that IL-6 could be a candidate gene and warrant investigation vis-à-vis differences among mink genotypes in resistance or tolerance to ADV infection.

The sequence of the IL-6 gene in mink was done and identification of polymorphisms was used to evaluate the potential role of this gene in the immune system response to infections. The 4678 bp promoter region, five exons and four introns of the interleukin-6 (IL-6) gene were bi-directionally sequenced in four unrelated mink from each of the wild, black, brown, pastel and sapphire mink (Genbank accession number (EF620932). The 344 bp promoter region of the gene contained several transcription binding sites. One exonic and seven intronic single nucleotide polymorphisms (SNP) were detected by sequencing of the 20 mink and genotyping of an additional 82 animals from the five colour types. Only two intronic SNP were segregating at high frequencies, indicating that the level of polymorphisms in the mink IL-6 gene was low. A bi-allelic tetranucleotide repeat was detected in the promoter region, with the frequency of 0.0, 0.17, 0.25, 0.25 and 0.40 in the wild, black, pastel, brown and sapphire mink, respectively, suggesting that this locus may influence immune response to infection. A polymorphic $(CA)_{16}$ with 10 alleles was also detected in intron 2.

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ARRESTING EFFLUENT PRODUCTION IN SILAGE FROM HIGH MOISTURE AGRO-INDUSTRIAL BY-PRODUCTS: THE ROLE OF 'POTENTIAL WATER HOLDING CAPACITY' AND IMPLICATIONS FOR SILAGE EFFLUENT CONTROL STRATEGIES

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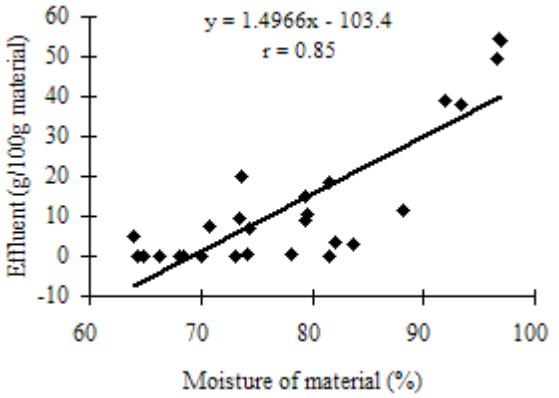
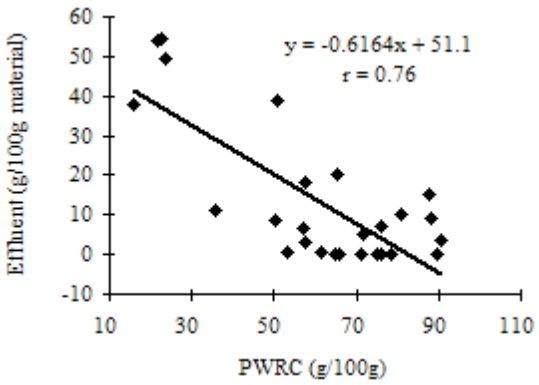
The role of 'moisture absorptive capacity' of a material used for silage and the relationship with silage effluent is assessed. The term 'water retention capacity' which is sometimes used in explaining the rate of effluent control in ensilage may be inadequate, since it accounts exclusively for the capacity of an absorbent incorporated prior to ensiling of a silage material, without consideration to how much the silage material can release. A new terminology, 'Potential Water Retention Capacity' (PWRC), which attempts to address this shortcoming, is proposed. Data from a series of experiments involving the ensiling of high moisture by-product feedstuffs (HMBF, n = 27) conducted separately over a period of ten years were used in this study.

An adaptation from the Water Retention Capacity (WRC) method of Robertson et al., [1], the procedure was modified as follows: one gram of air-dry weight of a HMBF material (dried at 60^{o}C for 48 h and milled to < 1 mm) was measured into a 50 ml centrifuge tube and hydrated with 30 ml distilled water containing 0.02 g sodium azide per 100 ml as a bacteriostat. The tube containing the sample was then equilibrated for 18 h at room temperature and its contents were transferred to a glass filter with a pore size of 100-160 μm, (1G P160, Sibata Company, Tokyo, Japan) and drained under a pressure of 2 g/cm^2 with a pressure pump (Compact air pump, NUP-1, AS-ONE Company, Tokyo, Japan) for 2 minutes. The glass filter containing the sample was weighed, oven-dried at 135^{o}C for two hours and weighed again. The PWRC of the material was calculated as the amount of water retained by the pellet (g moisture/g dry weight) after transfer to the glass filter and defined as 'the amount of moisture in grams that a HMBF or a silage material can retain per 100 grams of the fresh matter weight'.

The PWRC concept builds on the existing WRC calculations and approaches the theory of effluent retention in silages holistically, especially in HMBF, and the modifications follow observations made from a previous study involving the ensiling of a material with very high moisture content [2]. The high correlation between effluent and PWRC (r = 0.76) of ensiled materials indicated that it is an important factor in silage-effluent relationship. The theoretical moisture content and PWRC of silage materials necessary to stem effluent flow completely in HMBF silage in the study was 69.1 % and 82.9 g/100g in fresh matter, respectively.

Given the concurrence of the former figure to consensus, the latter is a novelty and a potential index in silage effluent mitigation strategies, with consequent positive effects on both the environment and animal feeding.





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REPRODUCTIVE PERFORMANCE FOLLOWING ARTIFICIAL INSEMINATION IN SANGA AND FRIESIAN × SANGA COWS IN THE ACCRA PLAINS OF GHANA

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The performance records of 126 Sanga, and 55 crossbred (Friesian x Sanga) cows bred at the AI Center of the Animal Production Departments' Amrahia dairy farm in the Accra plains of Ghana were assessed between the period January 1998 to December 2007. The Sanga cows were grazed from 08.00 to 15.00 h on natural pastures comprising *Panicum maximum*, *Stylosanthes haemata, Sporobolus and pyramidalis* and *Vertiveria fulvibarbis.* They had access to water from a dam twice daily in addition to water provided in the animal house *ad lib.* The crossbreds were zero grazed on *panicum maximum,* sorghum and spent malt, in addition to a concentrate mixture based on maize, wheat bran, palm kernel cake with or without soya bean meal. Salt lick is always provided. The crossbred had access water in the animal house *ad lib.* Oestrus (heat) was observed for the two groups of cows twice daily at 06:00h and 18:00 h G.M.T. A cow standing to be mounted (standing heat) was used as the main criteria for the cow to be assumed to be on heat therefore ready for insemination.

Parameters studied include interval from calving to first AI service, interval from calving to conception, calving interval and conception rate, The effect of season of calving preceding AI and season of AI on the above parameters were evaluated. The data was analyzed using the general linear models procedure of the Statistical Analysis Systems Institute (SAS).

The average interval from calving to first AI averaged 158.8 ± 8.9 d in the Sanga and 115.7± 19.2 d in the crossbred cows. This delay of first service after calving, particularly in the Sanga cows may be due to prolonged postpartum anoestrus. This is most likely a result of inadequate nutrition and suckling management [1]. The Sanga cows in this study were grazed sole on natural pastures. During the dry season, the limited pasture available on the Accra plains is of poor quality. In addition, there was lack of restriction on suckling by calves. Cows were allowed to suckle their young until they are weaned naturally. The low nutritional status of animals coupled with prolonged suckling stimulus could delay the resumption of ovarian cycles by interfering with the production and secretion of hormones important in ovarian follicular development and function in cattle [2].

The mean interval from calving to conception was 177.5 ±9.5 d in the Sanga cows and 138.6 ± 16.3 d in the crossbred cows. The calving interval averaged 517.9±13.8 d in the Sanga cows and 510.3 ± 41.0 d in the crossbred. These parameters were not affected ($P > 0.05$) by season of calving preceding AI or season of insemination. The prolonged interval from calving to first AI in the Sanga cows may account for the extended calving to conception interval compared to the crossbred cows. A major determinant of long calving intervals is a prolonged postpartum anoestrous interval

The CR at first service was 42.6 % and 46.2% for all services in the Sanga cows. The CR at first service for the crossbred cows was 54.5 % and for all services 53.5%. The major reason for this low CR may to due to poor heat detection and inappropriate timing of AI. Heat detection was not done very regularly at the 06:00 h and 18:00 h and also the concentrations of progesterone in the blood and milk of cows were not measured to provide information on the reproductive status as well as certain conditions of disorders that result in sub-fertility. The timing of insemination in relation to first detection of heat is critical for achieving high conception rates as well as factors relating to the transport, storage, handling and thawing of semen in the field [3].

In conclusion, improving the nutritional status of the cows through strategic supplementation coupled with effective heat detection mechanisms and appropriate timing of AI, as well as efficient methods of storage, transport and handling of semen should improve the reproductive performance of cows.

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A STUDY OF GROWTH TRAITS IN GOAT BREEDS OF NORTHERN AREAS OF PAKISTAN

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Pakistan has 49.14 million goats [1]. Goats are kept for milk and meat production and contribute significantly to the income of the rural farmers. Dairy goats are kept by the farmers belonging to poor socio-economic class of the society, mostly landless. Goat production is almost evenly distributed among all regions of Pakistan. The vast majority of small ruminant flock owners are small-scale farmers, sometimes landless. Mixed flocks are common, although separate flocks of sheep or goats can also be seen [2]. The Northern Areas of Pakistan provide many chances for goat raising on pastures and goats are the main stay of the poorest segments of rural society. The data on goat breeds of Northern Areas is still lacking. The present study was planned to provide baseline information of growth traits of goat breeds of Northern Areas. Six goat breeds, found in the Northern Areas of Pakistan, Pameri, Gojali, Balti (pashmina bearing), Dareli, Jerakheil and Koh-e-Ghizer were studied for growth traits like birth weight, weaning weight, adult weight, body length, heart girth and height. The mean birth (males: 2.81 ± 0.111 females: 2.36 ± 0.777 kilograms), weaning (males: 13.14 ± females: 11.11 ± 0.779 kilograms) and adult (males: 70.79 C females: 46.11 ± 0.786 kilograms) weight was highest in Dareli followed by Jerakheil, Koh-e-Ghizer, Pameri, Balti and Gojali. Among the various breeds studied Jerakheil displayed higher body length (males: 85.93± 0.875 females: 75.48 ± 0.975 cm) followed by Daeli, Koh-e-Ghizer, Gojali, Pameri and Balti. Dareli was found the tallest breed (males: 85.41± 0.424 females: 74.13± 0.750 centimetres) and Pameri the shortest (males: 70.36± 0.710 females: 57.91 ± 0.342 centimetres). The mean heart girth was also highest in Dareli (males: 95.67± 2.00 females: 86.89 ± 0.475 centimetres) and lowest in Pameri (males: 70.42± 0.498 females: 65.32 ± 0.603 centimetres). The differences between sexes and breeds were significant for all the parameters. The study has provided baseline information on goat breeds of Northern Areas, which would be useful for further studies on breed conservation and genetic improvement programmes.

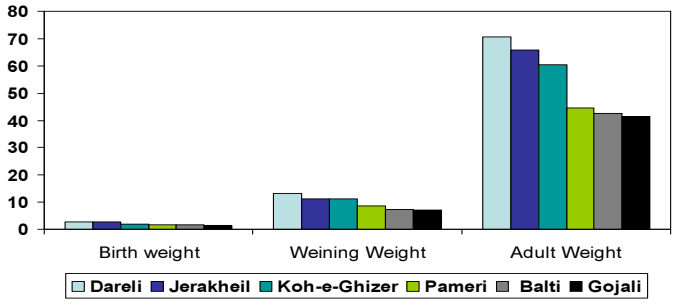

FIG 1: Birth, weaning, and adult weight in male goats

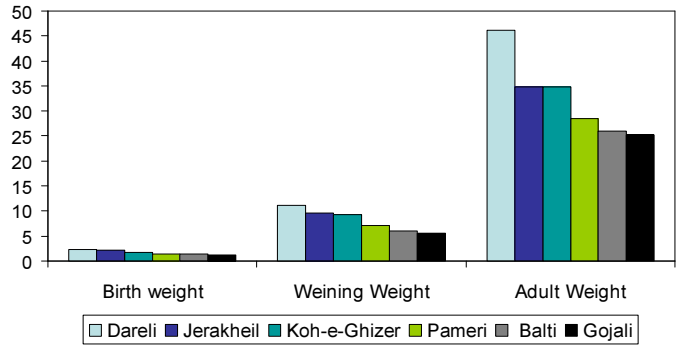

FIG2: Birth, weaning, and adult weight in female goats

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GENETIC POLYMORPHISM AT αs_1-CASEIN LOCUS IN MOROCCAN GOAT BREEDS

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The aim of this work was to investigate the genetic structure of the αs_1-Cas gene in 3 Moroccan goat breeds. A total of 150 goats from Draa, Atlas and North breeds were genotyped at αs_1-Cas locus using molecular techniques (PCR/RFLP and capillary electrophoresis). Six variants were found: A, B, C, E, F and O. The distribution of allele frequencies was as follow:

Allele	Draa	Atlas	North
A	0.2449	0.2449	0.0800
B	0.5714	0.6428	0.5600
C	0.0000	0.0204	0.0200
E	0.0918	0.0204	0.2600
F	0.0612	0.0408	0.0500
O	0.0306	0.0306	0.0300
A+B+C	0.8163	0.9081	0.6600

The O allele (zero content of αs_1-Cas in milk) was rare in the three breeds (0.03). It was the same for F allele (weak content of αs_1-Cas in milk; 0.04-0.06). The E allele (intermediate content of αs_1-Cas in milk), which is more frequent in European goat breeds, is rare in Atlas breed (0.02) and occurs in North breed and Draa breed at 0.26 and 0.09 respectively. A, B and C, considered as “strong” alleles since they are associated with high expression level of αs_1-Cas in milk (A+B+C), were predominant in Atlas breed (0.90) followed by Draa breed (0.81) and North breed (0.66). These results are close to those found by Tadlaoui Ouafi et al., [1] who reported allelic frequencies of 0.94 and 0.75 respectively in Atlas breed and Draa breed. This confirms studies reporting that Mediterranean and African goat populations present high frequencies of “strong” alleles, notably A and B [2].

The high frequency of « strong » alleles (A, B and C) at αs_1-Cas locus in Moroccan goats populations notably Atlas breed may be used to increase the incomes of farmers in mountainous areas by encouraging them to select their flock to produce goat's milk and make cheese. Similarly, it is strongly advised to establish a breeding program based on the selection of goats with A, B and C alleles.

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EFFECT OF TRANSIENT PREPUBERTAL HYPOTHYROIDISM ON SERUM TESTOSTERONE LEVEL AND SEMINAL CHARACTERISTICS OF IRANIAN INDIGENOUS CHICKENS

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Forty 6-week-old male Iranian indigenous chickens were randomly assigned into two equal groups, designated as control or propylthiouracil (PTU)-treated group. The goitrogen, PTU, was administered at a level of 0.1% (w:w) to the diet of PTU-treated group between the weeks 7 and 13 of age. From the week 13 to 26, both groups were fed with a PTU-free diet. The lighting schedule was 14 h-light:10 h-darkness. Blood sampling started at week 7 of age, and repeated every other week until the week 19 as well as body weighing simultaneously. Chicks were trained by the abdominal massage method and semen samples were collected from the week 21 and repeated once a week for seven weeks. Proc Mixed of SAS (6.03 edn.) was used to data analysis and body weight was considered as covariate in statistical model.

The effect of PTU treatment on serum thyroxine (T4) levels ($P < 0.003$) and body weight ($P < 0.035$) was significant, but it did not significantly affect the semen volume, sperm motility, percent live sperm concentration, total live sperm number and serum testosterone levels ($P > 0.05$). The effect of age on all parameters, including body weight ($P < 0.0001$), semen volume ($P < 0.0004$), sperm motility ($P < 0.005$), percent live sperm ($P < 0.0002$), sperm concentration ($P < 0.0001$), total live sperm number ($P < 0.0004$), and serum testosterone ($P < 0.0001$) and T4 ($P < 0.0006$) levels was significant. The effect of treatment x age interaction on semen volume, sperm motility, and percent live sperm was not significant ($P > 0.05$); but the interaction was significant for body weight ($P < 0.001$), sperm concentration ($P < 0.004$), total live sperm number ($P < 0.007$), serum testosterone ($P < 0.0001$) and T4 ($P < 0.0008$) levels. In both groups, there was a significant and positive correlation between testosterone levels and body weight (0.54 and 0.36 in control and PTU groups ($P < 0.01$), respectively) and also between T4 levels and body weight in the PTU group (0.23, ($P < 0.01$)), but not in the control group ($P > 0.05$). No significant correlation observed between testosterone and T4 levels in both groups.

GENETIC RELATIONSHIPS OF REPRODUCTION TRAITS BETWEEN PRIMIPAROUS AND LATER PARITIES IN SWINE

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This study was to investigate genetic relationships of litter size between first and later parities in Landrace (LR) and Large White (LW) sows by estimating genetic parameters; genetic and phenotypic correlations between number of piglets born alive in the first (NBA1) and later parities (NBA2+), heritability and repeatability. A total numbers of 9,120 and 7,539 Landrace and Large White litter records were collected from twenty four livestock research and breeding centres under the Department of Livestock Development of Thailand from 1993 to 2008 shown in Table I. The data were analyzed using bivariate animal mixed model with residual maximum likelihood methodology. The NBA1 and NBA2+ were treated as two different traits. Fixed effects included in the model were herd-year-season complex of farrowing sows, parity number and covariate effect of farrowing age. Random effects were the direct additive genetic of the sow and permanent environmental effects of repeated litter records of the same sows.

TABLE I. DESCRIPTIVE STATISTICS OF NBA1, NBA2+ AND NBA OF LANDRACE AND LARGE WHITE SOWS

Traits	Landrace			Large White		
	N	Mean	SD	N	Mean	SD
NBA1	1,786	7.9591	2.6916	1,509	8.2094	2.8311
NBA2+	7,334	8.8263	2.5552	6,030	9.2034	2.6652
NBA	9,120	8.6565	2.6052	7,539	9.0045	2.7282

N: number of litter records, SD: standard deviation, NBA: number of piglets born alive

It was found that the phonotypic correlation estimates between NBA1 and NBA2+ were low with the values of 0.0983 ± 0.0196 and 0.1171 ± 0.0205 in the LR and LW breeds, respectively (Table II). However, the estimates of genetic correlations between NBA1 and NBA2+ in both breeds were high with the values of 0.9987 ± 0.2499 and 0.9493 ± 0.1840, in the LR and LW breeds, respectively. The genetic correlation estimates were not significantly different from 1.00 by the log likelihood ratio test of the unity of the genetic correlation estimates. This indicates that NBA1 and NBA2+ are the same trait and the litter size records of all parities should be analysed using repeatability model for the studied population.

The literature estimates of the genetic correlations between NBA1 and NBA2+ were reported lower than the estimates from this study. For example, Wolf et al., [3] reported the genetic correlation estimates of 0.96 ± 0.05 and 0.88 ± 0.04 for the LR and LW breeds, respectively. Peskovicova et al., [2] estimated the genetic correlation of 0.83 ± 0.02 and Imboonta et al., [1] reported an estimate of 0.79 ± 0.16 in the LR breed. One explanation is that the age at first farrowing affected the relationships between NBA1 and NBA2+ in pigs. The older ages of the gilts at first farrowing the higher the genetic correlations between NBA1 and NBA2+. The farrowing ages of the sows at the first parity in this study (425.01 ± 5.19 and 391.76 ± 6.55 for the LR and LW breeds, respectively) were lower than those of commercial sows reported in the literature. Wolf et al., [3] reported ages at first farrowing of 369 and 375 d in the LR and LW breeds, respectively and Peskovicova et al., [2] reported age at first farrowing of 374 d in the LR breed.

TABLE II. ESTIMATES OF GENETIC CORRELATIONS (R_G) AND PHENOTYPIC CORRELATIONS (R_P) WITH STANDARD ERRORS BETWEEN NBA1 AND NBA2+

Trait	Landrace		Large White	
	NBA1	NBA2+	NBA1	NBA2+
NBA1	-	0.9987 ± 0.2499	-	0.9493 ± 0.1840
NBA2+	0.0983 ± 0.0196	-	0.1171 ± 0.0205	-

Genetic correlations in upper diagonal and phenotypic correlations in lower diagonal

The heritability estimates for NBA1 and NBA2+ were 0.1037 ± 0.02 and 0.0818 ± 0.0434 in the LR breed and 0.0630 ± 0.0203 and 0.0652 ± 0.0410 in LW breed, respectively. The repeatability estimates for NBA2+ were 0.1737 ± 0.0147 and 0.1739 ± 0.0136 in the LR and LW breeds, respectively. The heritability estimates for NBA1 and NBA2+ from this study were in the range of the literature estimates. The amount of genetic variations in the pig populations studied indicates that breeding improvement of the litter size trait by genetic selection is possible although the estimates of heritabilities were low. Selection to improve litter size using expected breeding values (EBV) is recommended in the pig population studied because the phenotypic correlations between NBA traits and the heritability estimates for NBA were low.

TABLE III. HERITABILITY (H^2) AND REPEATABILITY (R) WITH STANDARD ERRORS OF NBA OF LANDRACE AND LARGE WHITE SOWS

Trait	Landrace		Large White	
	heritability	repeatability	heritability	repeatability
NBA1	0.1037 ± 0.0200	-	0.0630 ± 0.0203	-
NBA2+	0.0818 ± 0.0434	0.1737 ± 0.0147	0.0652 ± 0.0410	0.1739 ± 0.0136

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MOLECULAR CHARACTERIZATION OF 10 TOLL-LIKE RECEPTOR GENES IN GOAT

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Toll-like receptors (TLR) play crucial roles in activating the innate immune system. To date, 10 [1] human and 13 [2] mice TLR have been identified to belong to the mammalian TLR family.

In this study, we have cloned partial CDs of goat TLR, TLR1、TLR2、 TLR5 for about 2kb, and the rest for more than 1kb. The cloned sequences were aligned to the corresponding regions of the relevant human, bovine and equip cabalas nucleotide sequences. As shown in Table I there is at least 79% identity between the relevant human and goat TLR nucleotide sequences. The identity of each cloned goat TLR to equus caballus and bovine reference sequence ranges between 81% and 98% (Table I). Translation of goat TLR nucleotide sequences and alignment to the human, bovine and Equus caballus TLR protein showed 69—97% of the amino acid sequence is conserved among the species (Table I).

A broad pattern of tissue expression was obtained for goat TLR mRNA in heart, liver, spleen, lung, kidney, lymph node, muscle and small intestine. TLR1 was found to be expressed in spleen and lung. Goat TLR2 were expressed in most tissues except muscle and small intestine. We detected TLR3 in the goat spleen and lung. TLR6 and TLR2 had similar expression patterns except in heart, while TLR2 was expressed but not TLR6. TLR7 was expressed in the liver, spleen and kidney. Expression of goat TLR8 was similar to TLR3, which is found in the spleen and lung. Analysis of TLR10 expression pattern in goat revealed that this gene only expressed in the spleen. All of goat TLR mRNA were expressed at reasonably high levels in the spleen that is a tissue with an organized immune compartment. This also agrees with human in where TLR are mostly expressed in the spleen [3].

To determine the overall role of goat TLR in mammal TLR evolution, a phylogenetic analysis was performed among mammal TLR (human, mouse, dog, cat, Pan-troglodytes, Macaca mulatta, Gallus gallus, equus caballus, bovine, pig, Mus musculus and Rattus norvegicus and goat). Phylogenetic and molecular evolutionary analyses of protein sequences of mammals TLR were conducted using MEGA version 4.0.The results demonstrates that goat TLR are the orthologues of mammalian TLR and are more closely related to bovine TLR than other mammalian TLR.

In conclusion, the characterizations of goat TLR, including sequence, expression pattern and phylogenetic tree were analyzed. The results indicated that goat TLR are the orthologue of mammalian TLR and more closely related to bovine TLR. This study should facilitate us do further work on the function of goat TLR.

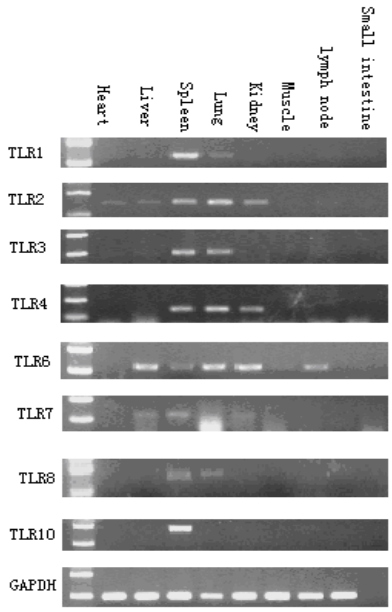

TABLE I. SIMILARITY OF GOAT TLR CDS AND PROTEIN SEQUENCES TO THE HUMAN, BOVINE, EQUUS CABALLUS AND OVINE REFERENCE SEQUENCES

TLR	Goat V human		Goat V Bovine		Goat V Equus caballus	
	Nucleotide (%)	Protein (%)	Nucleotide (%)	Protein (%)	Nucleotide (%)	Protein (%)
1	83	76	97	96	87	81
2	82	75	96	96	83	77
3	82	81	95	93	85	83
4	79	70	96	92	81	73
5	83	69	96	80		
6	85	78	96	92	86	79
7	85	82	98	97	88	87
8	81	74	95	93	83	76
9	85	83	95	94	87	86
10	84	79	96	95		

Fig. 1. Tissue expression of goat TLR mRNA.

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NON-INVASIVE MONITORING OF REPRODUCTION IN MALE LESSER MOUSE DEER (*TRAGULUS JAVANICUS*): ANALYZING HORMON TESTOSTERONE AND ITS METABOLITES IN FAECES USING HPLC AND ENZYME IMMUNOASSAY

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The lesser mouse deer (*Tragulus javanicus*, family Tragulidae) is a ruminant that inhabits the tropical rain forest of the South East Asia (Medway, 1969). The animal is regarded as the smallest ruminant and has been proposed to be a model in the biomedical and ruminant research (Kudo *et al.,* 1997). On the other hand, this animal is an interesting collection in the zoo and other conservation areas for the purpose of education and tourism, showed larger proportion of carcasses and therefore suitable as an alternative meat source.

Recently, the population of the lesser mouse deer is threatened by illegal hunting and habitat destruction. Therefore, conservation and management of the animal is becoming important. One success key on the conservation and breeding strategy of wild animal include the management and knowledge of the reproductive system. Only limited information are available on the reproductive physiology of the lesser mouse deer although several studies have been undertaken on the reproductive system of the male and female lesser mouse deer (Haron *et al.,* 2000; Kimura *et al.,* 2004; Agungpriyono *et al.,* 2005). Previous studies on the male lesser mouse deer revealed the sperm quality (Haron *et al*., 2000; Agungpriyono *et al.,* 2005), spermatozoa morphology and histochemical properties (Agungpriyono *et al*., 2005). Furthermore, it has also been reported that intermandibular scent gland may play important roles in the communication and sexual behaviour of mouse deer (Ralls et al., 1975; Agungpriyono et al., 2006).

Attempts at natural breeding of lesser mouse deer in captive are still unsuccessful. This may be due to the lack of information about animal reproduction, both male and female of lesser mouse deer, the unstable behaviour, aggressiveness, stress and less attraction and ability to mount during the captive breeding (Haron *et al* 2000, Kudo *et al* 1997).

Testosterone is the main hormone concerning the male reproductive biology that it indicates the age of sexual maturity and together with cortisol; it plays a role in the behaviour of dominance rank in their group. At the onset of puberty, testosterone is essential to maintaining sexual function, germ cell development and stimulates the development of male sex characteristics (such as external genitalia, accessory sex organs, hair growth, voice timbre, muscle and bone tissue mass), which it will maintain throughout life (Payne and Youngblood, 1995). In male animal, the pattern of reproductive activity can be observed by measuring the testosterone levels that it may indicate reproductive activity [1]. Many studies have been done to measure and observe the profile of testosterone to suggest the reproductive activity of male animal. In seasonal breeding animal, the testosterone level shows peak at the mating time. This information is not known in the male lesser mouse deer.

The most commonly used technique for analysis of the testosterone and its metabolites are High Pressure Liquid Chromatography (HPLC) with isocratic elution or mobile phase gradient.

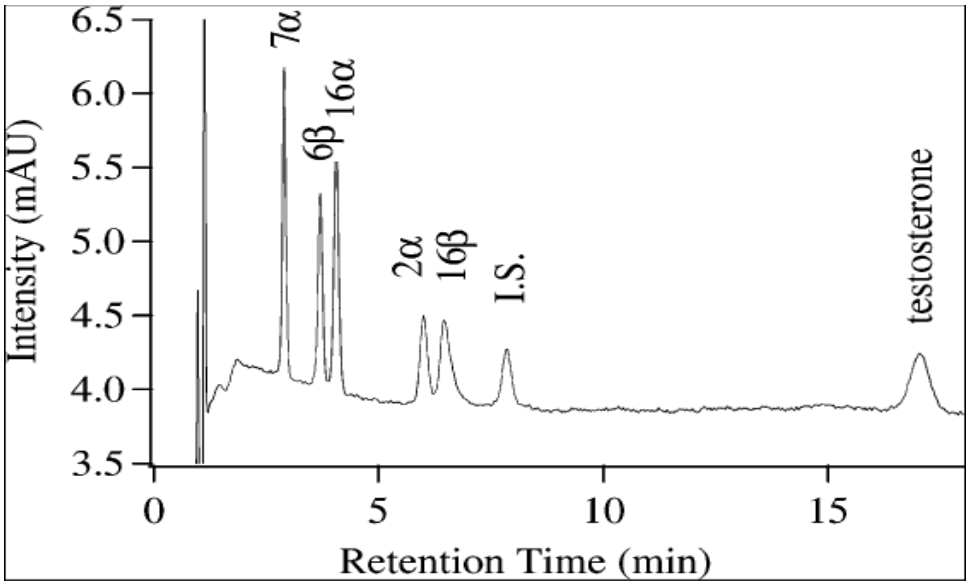

FIG 1. Separation of testosterone and its metabolites using HPLC flow-rate gradient (cited from Xing-Fang Li, et al., 2002).

Faecal concentration of immunoreactive testosterone or its metabolite (from the HPLC result) can be determined using enzyme immunoassays as described by Kusuda *et al.,* (2007). The enzyme-immunoassays were performed on microtitreplates coated with a goat anti rabbit IgG and using hormone-specific antibody as second antibody and enzyme or biotin-labelled hormones as competitive tracers. Sample extracts were diluted in assay buffer and duplicate 50 µl aliquots were taken to assay. A more detailed description of the result will be published elsewhere [2].

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INCREASE OF NUMBERS OF EGG CLUTCH AND REDUCTION OF MORTALITY RATE OF THAI NATIVE CHICKS

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Almost all Thai farmers in the villages raise Thai Native chickens. Chickens are the main protein source in the rural areas. The surplus of chickens can be the additional income for the farmer family. Raising of chickens is commonly in the backyard where chickens can feed on leftover or residuals from the family consumption. Generally people like to eat native chickens because their meat contains less fat and had a good taste. Native chickens are able to use low quality food efficiently and they are more resistant to tropical diseases. Thailand tries to improve native chickens for growth meanwhile maintain good characteristics; tolerance, feeding ability, fertility.

Thirty-six farmers in the North part of Thailand were selected for testing of raising chickens, by supporting 5 – 12 hens per family. Farmers were divided randomly into 3 categories; separation of chicks at birth, at 14 d and allowing chicks to be with hens naturally. Growth performance was recorded together with losses, mortality, consumption and sale of native chickens for a period of 1 year. Hens and chickens were feeding by themselves in the nature.

There were 273 hens at the beginning of the experiment and 210 hens at the end. The average body weight of the hens in the village at the first laying was 1.52 kg. The average number of egg clutch was 3.41. A total of 210 hens produced 8,550 eggs, 5,466 chicks in 1 year of the experiment. The hens whose chicks were separated from birth produced 14.8 – 16.4 more chicks per year, 1.2 – 1.4 more clutches of eggs and 19.0 – 22.0 more eggs than group 2 and group 3, respectively. On average, a hen laid 39.14 eggs and produced 24.84 chicks per year. Farmers in this study had 72.03 chickens for consumption per family and 84.47 chickens for sale, which incurred an income of 3,879.82 Baht per family. The expenses involved feed cost and electricity (for incubation) had a value of 1,797.94 Bath. The cash return on average was 2,081.65 Bath per family.

When averaging values per hen, number of chicks for consumption in the family was 9.54, number of chicks for sale was 10.88 creating the value of 503.48 Bath per year. Cost and profit from raising chicken were 236.26 and 273.90 Bath per hen per year, respectively.

TABLE I NUMBER OF CHICKENS FOR SALE AND INCOME FROM SELLING OF CHICKENS IN ONE YEAR

Details	Value
No. of eggs produced (eggs)	8,550
No. of chicks produced (heads)	5,466
Eggs per hen per year (eggs)	39.14
Chicks per hen per year (heads)	24.84
Consumption (heads)	72.03
Chickens for sale (heads)	84.47
Income from sale (Baht)	2,081.65
Return (Baht)/hen	273.90

There were 63 millions chickens in Thailand. There were three million families raised the chickens. If chickens are allowed to be with hens naturally a hen can produce 24.84 chicks per year. This will increase more income to farmers. The incomes from raising Thai native chickens very low and there

were no or little inputs from farmers. Native chickens can feed on leftover food from human consumption and feed by themselves in the nature from feedstuffs, which are not utilizable by human. This is an efficient way to convert low quality feed into a high quality protein in chicken meat. Moreover, raising native chicken for meat consumption can promote health because of lower fat in chicken meat than conventional broilers. Raising of native chicken can be developed to be a sustainable career for Thai farmers.

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PRELIMINARY STUDIES FOR USE OF OF *HARWICKIA BINATA* FODDER IN SENEGAL: BROMATOLOGICAL ANALYSIS AND FEEDING TRIALS ON DAIRY GOATS MURCIANO-GRANADINA

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This work is a part of studies, led by ISRA to promote the use of ligneous (local or introduced) feeds by livestock breeders, in semi-extensive or extensive breeding. The study was done during three months, with three (03) lots containing each five (05) *Murciano-Granadina* goats, multipares (which has been parturient many times), in beginning of lactation. It was held in a community farm in Gandiaye (near Kaolacl). All animals received as basic feed, *Panicum maxima* in green state, distributed *ad libitum*. This basic ration was completed by a commercial concentrated feed, distributed without weighing, for the pilot lot according to the practice of the farmer; whereas every goat of the lot 1 received a complementary feed containing 94.80 % of DM; 11.8% of crud proteins (CP); 2.6% of calcium (Ca); 0.36% of phosphorus (P) and 0.63 of milk fodder unit (UFL), composed by 500 g of *Hardwickia binata* dried leaves and 500 g of commercial concentrated feed, and each animal of the lot 2 received 250 g from of *H. binata* dried leaves and 750 g of concentrated feed, totalising 92.41% of DM; 13.9% of CP; 3.19% of Ca; 0.50%; and 0.71 UFL. After two (02) months of measure, animals of the lot 1 had consumed 278,256 ± 94.63 g of the complementary ration (concentrated and ligneous dried leaves), or 927.52 g by goat per day, those of the lot 2 had consumed little more: 292,627 ± 35.65, which represents 975.42 g by goat per day, that means almost the totality of a kg of complementary ration distributed. However, for milk production, goats of the lot 1 produced a quantity of milk slightly superior to that produced in the lot 2: 276.65 ± 0.24 L, against 274.4 ± 0.26 L respectively. It represents a daily production by goat of 0.2 ± 0.24 L for lot 1, which is not significantly different from that of lot 2, which is 0.91 ± 0.26 L. The milk produced was in good quality too. In addition, those productions are significantly more important ($P < 0.001$) that the pilot lot goat production (0.59 ± 0.26 L by goat per day). Bromatological analysis of *H. binata* dried leaves result reveals a moderate crud proteins rate 07.96 %; 1.36 % of Ca; 0.09% of P and 0.47 UFL. This study demonstrates the interest of the use of *H. binata* leaves to feed dairy females. It shows also, the benefit of good rationing management of dairy animals, according to the production level.

TABLE I. COMPLENTARY FEED COMPOSITION

Complentary fedd composition	Trials lots		
	Lot 1	Lot 2	Pilot lot
Industrial Concentred feed	500 g	750 g	NI
Harwickia binata died leaves	500 g	250 g	0 g
Dry matter rate	94.80 %	92.41 %	NI
Crud proteins rate	11.80 %	13.60 %	NI
Calcium rate	02.60 %	00.36 %	NI
Phosphorus rate	03.19 %	00.50 %	NI
UFL* (Milk Fodder Unit)	00.63 %	00.71 %	NI

*Unité Fougère Lait : Milk Fodder Unit ; NI : Not Identified

TABLE II. BROMATOLOGY OF HARDWICKIA BINATA DRIED LEAVES

Composition	Rate (%)	Composition	Rate (%)
Dry matter	99.50	Phosphorus	00.09
Crud Proteins	07.96	Cellulose	36.33
Fat matter	04.00	NDF	53.10
Calcium	01.36	UFL	15.35

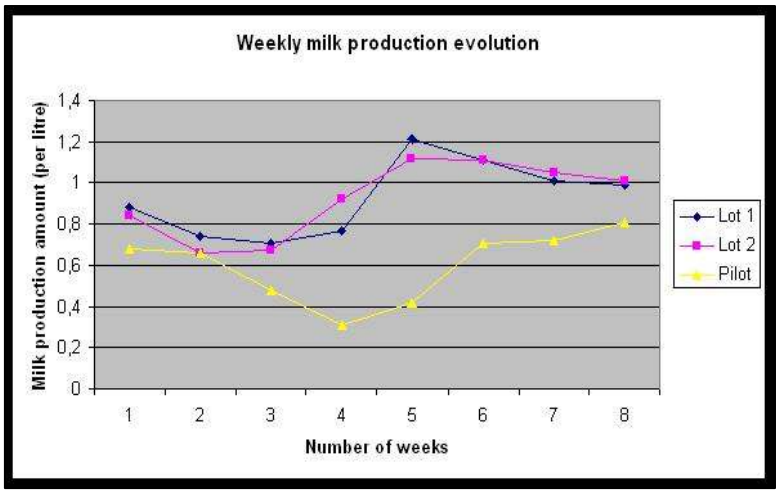

FIG 1. Weekly milk production progress

EFFECTS OF FERMENTED CASSAVA PULP ON DRY MATTER INTAKE, FEED DIGESTION, CONCEPTION RATE AND PERFORMANCES OF DAIRY HEIFER

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Sixteen crossbred Holstein Friesian heifers (307 ± 18.4 kg of body weight (BW)) were assigned to 4 treatments in randomized completely block design. Feed ingredients and compositions of experimental diets on DM basis are presented in Table I. Heifers were offered feed as TMR diets with a 10% refusal. Intakes and refusals were recorded daily. Experimental period consisted of 74 d, with 14-d adjustment period. During d 60 to 74, samples of feed offered were collected and BW was recorded. Diet composites were analyzed for CP, Fat (AOAC, 1990) NDF, ADF (Van Soest et al., 1991). Chromic oxide was used as indicator for feed. Blood samples were analyzed for BUN, Glucose. All heifers were induced by using the 2 injection protocol of Prostaglandin $F_{2\alpha}$ and Pregnancy check at day 60 after injection. All data were done using the GLM procedure of SAS (1988).

The experiment data suggested that intakes of DM per day were affected ($P > 0.03$) by amount of cassava pulp that heifer had lower feed intake as increased ratio of cassava pulp into diet (Table II). However, it did not effect body weight change among treatments and tend to improve feeding efficiency as using more cassava pulp. Diet DM and ADF digestion had no effects among treatments (average in 60.8% and 43.3%). But NDF digestion was significantly difference ($P < 0.03$) that increasing amount of cassava pulp increased NDF digestion. The average blood glucose and BUN levels during 1-3 h post feeding were not significant difference among treatments. Even blood glucose tended to decrease and BUN tended to increase. Number of oestrus heifers and pregnancy heifers were not significantly difference among treatments. Consequently, at the 50% of cassava pulp can use incorporative in diet without any effects on fertility.

Cassava pulp was one of the main by-product feed from Agro-industry. The study showed that when increased a ratio of cassava pulp to 50% TMR.DM, it improved feed conversion ratio and feed utilization without effect on heifer fertility.

TABLE I. FEED INGREDIENTS AND COMPOSITIONS OF EXPERIMENTAL DIETS ON DM BASIS

Ingredients and nutrients, %	T1	T2	T3	T 4
Rice Straw	24.0	23.0	22.0	20.0
Cassava pulp	12.5	25.0	37.5	50.0
Cassava meal	33.6	20.5	10.1	0.0
Soy bean meal	12.3	13.0	12.8	13.3
Corn meal	3.0	5.0	5.0	6.1
Palm kernel Cake	6.0	5.0	4.0	2.0
Cane sugar	6.0	6.0	6.0	6.0
Mineral	1.0	1.0	1.0	1.0
Urea	1.6	1.5	1.6	1.6
DM	59.3	44.2	35.3	29.3
CP	12.5	12.5	12.5	12.5
NDF	28.5	29. 5	30.1	29.9
ADF	17.3	18.1	18.9	19.1
TDN	69.2	68.6	67.6	67.2

TABLE II. EFFECTS OF EXPERIMENTAL DIETS ON HEIFER PERFORMANCES, FEED DIGESTION AND BLOOD METABOLITE

Items	T1	T2	T3	T4	SEM	*P*-value
Intake (kg/d)	11.4[a]	12.0[a]	10.9[ab]	9.9[b]	0.44	0.03
BW_{Chang} (kg/d)	0.7	0.6	0.7	0.8	0.10	0.69
Digestion (%)	51.9	61.0	66.1	64.0	5.41	0.43
Digestion of NDF (%)	31.7[a]	38.4[b]	50.8 [b]	47.4 [b]	2.92	0.03
Digestion of ADF (%)	41.5	40.6	48.1	42.8	6.66	0.32
Oestrous heifers,% of herd[1]	100	100	75	75	-	0.51
Pregnant heifers,% of herd[1]	50	75	50	75	-	0.26
Blood glucose (mg/dl)						
0 h –post feeding	62.0[a]	47.0[b]	61.0[a]	49.7[b]	2.44	0.02
1-3 h – post feeding	63.0	58.9	52.9	51.0	4.50	0.47
Blood urea nitrogen(mg/dl)						
0 h –post feeding	12.9	12.8	12.9	17.2	1.85	0.57
1-3 h – post feeding	16.5	15.8	16.8	18.9	2.06	0.18

*[a,b,] the different alphabets in the same row were significantly difference at $P < 0.05$; [1] Chi-square test

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IDENTIFICATION OF X-CHROMATIN AND DETERMINATION OF ITS INCIDENCE IN NIGERIAN GOAT BREEDS

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The numbers of x-chromatin body ("drumstick" appendages) in the interphase nuclei of three major breeds of Nigerian goats were studied. Each goat breed was derived from three different locations in the country based on the areas of its preponderance. Smears from buccal cavity and PMNS of each goat were developed using standard staining techniques. The mean values obtained per breed irrespective of sex were 1.92%, 1.65% and 1.60% for Sahel Goats (SG), Red Sokoto Goats (RSG) and West African Dwarf Goats (WADG), respectively. The mean value obtained for the bucks and does irrespective of breed were 0.13% and 3.07%, respectively. Those for males per breed were 0.15% for SG, 0.15% for RSG, and 0.10% for WADG and for does per breed were: 3.44% for SG, 3.10% for RSG and 2.67% for WADG. The results generally revealed that the frequency of 'drumstick' was statistically different ($P < 0.05$) between Bucks and Does; Bucks were statistically the same ($P < 0.05$) in 'drumstick' incidence, irrespective of breed and location, while the 'drumstick' incidence was statistically higher ($P < 0.05$) in Sahelian Does, followed by RS Does and least in WAD Does. This may account for higher prolificacy frequently observed in SG, followed by RSG. However, location exhibited an infinitesimal effect on the frequency of 'drumstick' within breeds, indicating that incidence of 'drumstick' is purely a genetic factor.

METHANE PRODUCTION BY SHEEP CONSUMING DIETS WITH DIFFERENT LEVELS OF EUCALYPTUS ESSENTIAL OIL

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Public concern over use of antibiotics in livestock production has increased in recent years because of possible contribution of antibiotics to emergence of antibiotic resistant bacteria, and their transmission from livestock to humans. Consequently, ruminant microbiologists and nutritionists have been exploring alternative methods of favourably altering ruminal fermentation thereby enhancing the efficiency of utilization of feed energy while decreasing methane emissions [1]. However, there have only been limited studies conducted to investigate the effects of essential oils on growth performance, digestive functions, rumen microbial activity and methane production in ruminants. The present study was designed to evaluate the potential effect of eucalyptus essential oils (EUEO, *Eucalyptus citriodora*) on intake, digestion, rumen fermentation and methane production in sheep.

The experiment was conducted during November and January (early summer) 2007-2008 at the Center for Nuclear Energy in Agriculture, Laboratory of Animal Nutrition, University of Sao Paulo, Brazil. Twelve Santa Ines sheep were allocated into 3 groups (59.7 ± 12.16 kg of live weight). The control group (CON) received only the basal diet (Tifton-85 hay-*Cynodon sp*, concentrate mixture, molasses and mineral mixture), the second group (EUEOI) received the basal diet plus 10 mL of EUEO/head/d while, the third group (EUEO II) received the basal diet plus 20 mL of EUEO/head/d. The EUEO was obtained from the Distillery Tres Barras Company, Sao Paulo, Brazil. All animals were fed the basal diet twice daily at 08:00 and 16:00 h. Four open-circuit respiration chambers were used [2]. Dry gas meters were ®tted in the pipe work between the chambers and the air suction pumps (60 l/min) to measure the total volume of gas passing through each chamber. Methane volume was adjusted to standard temperature and pressure. Data were subjected to analysis of variance using the General Linear Model procedure of the SAS [3]. The model used was: $Y_i = \mu + \alpha_i + e$, where μ is overall mean, α_i the treatment effect. The significant differences between individual means were identified using Tukey test [3].

The chemical profile of EUES analyzed by GC/MS is given in Table I. Nine compounds were identified in EUEO. The EUEO was dominated by citronella, which constituted 50.5%. Other major components of the EUEO were 1,8-cineole (15.9%), L-citronellol (10.5%), trans-caryophyllene (5.2%) and citronellyl acetate (3.8%). The methane production *in vivo* is presented in Figure 1. The supplementation of EUEOI and EUEOII suppressed methane production by 31 and 22% respectively in comparison to the CON diet. The DM intake, nutrients digestion coefficients, nitrogen balance, pH and NH_3-N concentration are shown in Table II. The results showed that both doses of EUEO decreased the DM intake but the difference between the doses was not significant.

TABLE I. MAIN CONSTITUENTS (%) OF THE EUCALYPTUS ESSENTIAL OIL

Compound	RT (min)	%
Terpinene	9.95	1.2
α- Pinene	10.40	1.3
L-Terpinene	11.46	1.3
L-Thujene	11.71	2.4
1,8-Cineole	13.34	15.9
Citronella	19.67	50.5
L-Citronellol	21.14	10.5
Citronellyl acetate	23.28	3.8
trans-Caryophyllene	25.12	5.2

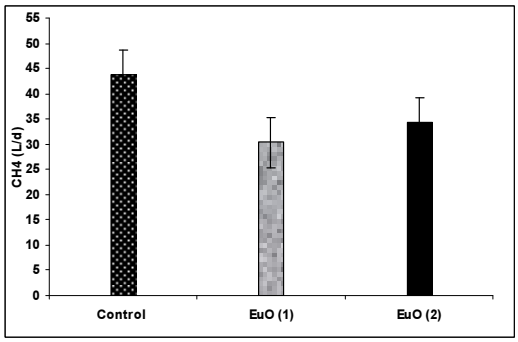

FIG 1. *Effect of eucalyptus essential oil supplementation on methane production in vivo*

The inclusion of EUEOI had no effect on the digestion coefficient of DM but EUEO II decreased the digestion coefficient of DM. The digestion coefficients of crude protein were 62.7, 67.8 and 71.4 for EUEO II, CON and EUEO I, respectively. The nitrogen balance was decreased ($P < 0.05$) with EUEOII while, the EUEOI improved the nitrogen balance but the difference was not significant. Both doses of EUEO had no effect ($P > 0.05$) on rumen pH before feeding. The ammonia concentration decreased ($P < 0.05$) with EUEO II but EUEO I no effect on ammonia concentration. It is concluded that the eucalyptus essential oil has the potential for the mitigation of methane production from ruminants.

TABLE II. EFFECT OF EUCALYPTUS ESSENTIAL OIL SUPPLEMENTATION ON DM INTAKE (DMI), NUTRIENTS DIGESTIBILITY, NITROGEN BALANCE (NB), PH AND AMMONIA CONCENTRATION

Parameters	CON	EUEO I	EUEO II
DMI (kg/h/d)	1.360	1.267	1.243
Digestibility coefficients (%)			
DM	56.0[a]	55.7[a]	53.9[b]
OM	56.1[a]	56.6[a]	53.6[b]
CP	67.8[b]	71.4[a]	62.7[c]
EE	58.7[a]	62.6[a]	61.8[a]
NDF	55.6	54.2	53.9
ADF	31.6	33.7	31.5
N balance (g/d)	9.99[a]	11.19[a]	7.92[b]
pH	6.30	6.38	6.36
NH_3-N(mg/100mLRL)	16.6[a]	16.3[a]	13.0[b]

[a,b] superscripts within the same row sharing the same letter are not significantly different;

DM = dry matter; OM = organic matter; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre.

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EFFECTS OF MILKING INTERVAL ON SECRETION AND COMPOSITION OF MILK IN THREE DAIRY GOAT BREEDS

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Twenty-four lactating goats (Alpine = 8, Damascus = 8 and Murciano-Granadina =8) in mid lactation were used to study the short-term effects of different milking intervals (8, 16 and 24 h) on secretion and composition of milk. Milk was analyzed for physical (pH, density and acidity) and chemical (Total solid, fat, protein and ash) parameters. Murciano-Granadina produced less milk ($P < 0.05$) than Alpine and Damascus breeds (1.30 ± 0.36; 1.68 ± 0.45; 1.64 ± 0.42 L, respectively). Milk secretion rate reached the greatest values at the 8 to 16 h milking interval in Alpine (97 mL/h) and Murciano-Granadina (75 mL/h) goats, but it increased in Damascus goat after 16 h. The pH of milk did not change with milking interval and averaged 6.6 ± 0.05. Density of milk increased with milking interval in all three breeds, and acidity increased only in Alpine and Damascus breeds. Milking interval affected milk fat content, which decreased markedly from 8- to 24-h, but no differences were observed in milk protein content which averaged 29.36 ± 2.72 g/L. Milk ash content decreased from 8 to 24 h in Alpine (8.9 ± 0.8 vs. 8.0 ± 0.5) and Murciano-Granadina (8.6 ± 0.9 vs. 7.8 ± 0.6) goats. This parameter was maintained constant in Damascus goat. No effect on the udder health was observed. In conclusion, this short-term study suggests that Alpine and Murciano-Granadina dairy goats could maintain high secretion milk rates during extended milking intervals.

PHENOTYPIC CHARACTERZATION OF GOAT BREEDS IN VIETNAM

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There are eight goat breeds in Vietnam with more than 1.5 thousand heads and about 95% of them are indigenous breeds. As one of the important livestock species, goat also plays an important role in income earning and poverty alleviation in the rural areas and is potential resource for supplying high quality meat.

The Vietnamese indigenous goat breeds were created many years ago by indiscriminate crossbreeding under natural selection or crossbreeding with imported breeds. Therefore, This study is being carried out to phenotypic characterize the goat populations of the country as part of Characterization of Small Ruminant Genetic Resources in Asia supported by Vietnamese basis research project in life sciences and IAEA. Methods: Survey and distribute the questionnaires for interviewing, collect data, morphology description of the breeds and their productivity performance, where available.

The research was carried out in five provinces in different geographic areas as follows: Northern Vietnam: HaGiang province; Red river delta: HaTay province; Western Vietnam: Son la province, center Vietnam: Thanh Hoa and Ninh Thuan Provinces.

The results showed that the goat population in the North is 72,5%, the south is 27,5%, in which 12,3% is in Tay Nguyen, 8,9% is in Central Coast, 2,5 % and 3,8% are in East and West Southern part consequently. The Goat population distributes mainly in the Northern Mountains, about 48% of all over country and 67% of the North (MARD).

Co native goats, the most common goat breed of the country, are reared under semi-intensive or extensive system by rural peoples. The Co goat population distributes all over the country, but concentrates mainly in the North Mountains and midland provinces; they are raised for meat. There are several kinds of Co goat breeds separate by colours and figures. The Bach Thao goat population is raised mainly in central coastal provinces for both meat and milk. Six breeds were introduced to Vietnam for milk and meat production such as Saanen, Boer and Alpille, Barbari, Jumnapari, Beetal and adapted well to the Vietnam environment and management and contributed significantly to goat production. They are subjected to cross with local ones to improve meat and milk production types.

The breed season is mainly seen after summer, from April to May and from September to October after onset of monsoon rain, when fresh green grass is available. The second breeding season is not prominent since the availability of grass and pasture is very limited. Feeding: Grass, leaves, sugarcane. Grazing is popular.

The limited data made during this study have allowed the preliminary description of the phenotypic characteristics of the Vietnam goat types. Genetic characteristics is needed

This study has also laid the foundation for the exploitation of the goat breeds described for the benefit of some of the world's most needy livestock keepers

TABLE I.CHARACTERISTICS OF DIFFERENT GOAT BREEDS IN VIETNAM

Characteristics	Co	Bach thao	Barbary	Jumnapari	Beetal	Alpille	Saanen	Boer
Body weight M/F (kg)	30/26	65/45	30/45	45/65	45/65	50/65	60/80	90/140
Milk yield (kg/year)	50-60	220-250	150-225	270-310	310-420	400-650	500-800	1000-1200
Age at sexual maturity (month)	6.0	7.5	7.5	7.5	12.5	9.5	7.8	16.0
Litter size	1.5	1.7	1.7	1.5	1.4	1.5	1.5	1.6
Number litter/year	1.4	1.6	1.6	1.3	1.5	1.2	1.1	1.2
Mating season (months)	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.	Apr - May & Sep-Oct.

There are some cross-formulas between local and some introduced goat breeds to improve goat productivities. For example: Bach Thao goat and Co goat, Barbary and Co, Bach Thao have good achievement results. The gain weight and grow of the crossbreeds are significantly better than the local breeds such as Bach Thao and Co Goat.

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CRYOPRESERVATION OF FARM ANIMAL GENETIC MATERIALS IN VIETNAM

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Vietnam is considered as one of the world's ancient animal domestication areas. In total, 62 local breeds have been found in Vietnam with a density of 1.520 species/km^2, high compared to the global overage of 0.098 species/km^2. However, due to undirected natural selection, market demands, importation of exotic breeds and crossbreeding, local breeds are being exposed to danger and extinction. Despite having low productivity, Vietnamese local breeds adapt very well to hot and humid ecosystem and local husbandry habits. These breeds contain potential genes deciding valuable characteristics such as egg and milk quality and resistance against diseases.

In this paper the focus is placed on *ex situ* conservation in Vietnam including: semen, embryo, somatic cell and DNA cryopreservation due to the ex-situ method is a very important components of the animal genetic conservation to prevent mishandling and mistakes. Cryopreserved germ cells can be saved permanently and, not withstanding any accidents in the storage system, remain available in exactly the same condition as at the time of their collections. This method could contribute not only to increase population size but also to avoid inbreeding depression in a species. Due to last some years, the procedures for preservation of semen, embryo and DNA, the techniques to extract DNA from blood, tissues and were improved. There have been several results as followed:

Sperm cryo-preservation

In Pig: In the general, pig sperms have not been preserved. After collected, sperms are mainly kept for within 2 or 3 d and taken straight to artificial insemination. 2300 pig sperm doses from I native Pig, Yorkshire and Landrace have been preserved so far and some of them has been used to create IVF embryos in laboratories and are also frozen in straws.

Embryo cryo-preservation

In general, embryo preservation is hard and rare. There have only been several results as followed:

- Frozen dairy cattle-HF embryo: 360 embryos of which 200 are IVF and 160 are invivo.
- Frozen beef cattle embryo: 142 IVF embryos.
- Frozen pig embryo: 189 embryos of which 139 are IVF and 50 are in vivo.

In general, embryo preservation is hard and rare. There have only been several results as followed:

DNA and somatic cell preservation

A total of 6169 cell samples of 32 livestock breeds are collected at different provinces. 5636 samples are extracted DNA, preserved and evaluated at the laboratory. Cell samples include 6169 samples from local livestock breeds and DNA samples include 5636 samples from rare breeds such as I, Mong Cai, Co, Ban pigs, Vang and Coc cows and Ri, Ho, Dong Tao, Mia, Ac and Choi chickens, etc.

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THE EFFECT OF SAVORY OIL (*SATUREJA HORTENSIS* L.) ON BROILER PERFORMANCE

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Natural plants and their extracts can represent one of myriad alternatives in response to the void created by the ban on antibiotic growth promoters in animal nutrition. Beneficial effects of botanicals additives in farm animals may arise from the improvement on feed intake and activation of digestive secretions, immune stimulation, antibacterial, coccidiostatic, antihelmintic, antiviral or antiinflamatory activity. The usage of plants and of their extract, essential oils, comes from phytoterapy, well known for the human being, and are perceived as natural and safe by consumers. This study was conducted to assess the use of savory oil in broiler nutrition as a natural growth promoting substance instead of antibiotics. Different levels of savory oil were added to standard diet, in order to determine its effect on weight gain, daily weight gains and feed conversion ratio, compared to control antibiotic group. One hundred day-old broilers (Ross-308) were divided into groups of 25 chickens each and randomly assigned to base diet. Experimental groups were as followed: E control group with 0.2 % antibiotic (Avilamycin), E1 group - 0.5 % savory oil, E2 group - 1 % savory oil, E3 group - 2 % savory oil. The best ($P < 0.01$) weight gain was observed at the E4 group (2919 g), and followed by the E2 one (2751 g), E1 group (2729 g) and E group (2698 g). The addition of savory oil within diets improved daily live weight gain by approximately 1% (E1), 2 % (E2) and 8 % (E3) compared to the control group. The addition of 2 % savory oil to the diet improved feed conversion ratio by approximately 5 %, compared to the control group with antibiotic. Consequently, the results revealed that essential oil of *Satureja hortensis L*. could be considered as a potential natural growth promoter in poultry feeding.

MANAGEMENT TOOLS FOR HEAT STRESS REDUCTION IN CROSSBRED DAIRY COWS IN A TROPICAL REGION

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There are three intervention management which can be considered to reduce the heat stress. These are genetic changes, environmental modification and nutritional strategies [2]. In this review some results from these three intervention practicing in Thailand are shown. By using cross breeding program, the performance of F1 crosses with Brown Swiss, Holstein or Jersey produced milk yield 265% of local breed [1]. In Thailand crossbred Friesian dairy cattle, average 50-87.5% Friesian, could produce 1987 kg to 5044 kg compare to 1973 kg to 6661 kg found in purebred Friesian [7].

TABLE I. DRY MATTER INTAKE (DMI), MILK PRODUCTION AND PRODUCTIVE PERFORMANCE OF CROSSBRED FRIESIAN HEIFERS IN ETCS AND NETCS (MEAN ± SD), ADAPTED FROM CHOKTANANUKUL ET AL., [3].

Parameter	ETCS	NETCS
BW, (kg)	393.14 ± 12.55	367.58 ± 13.93
DMI,(kg)	13.3 ± 2.01[a]	11.1 ± 2.43[b]
DMI / %BW	3.47 ± 0.30[c]	3.03 ± 0.28[d]
Milk yield, (kg)	16.9 ± 2.84[a]	12.5 ± 2.54[b]
4% FCM, (kg)	14.6 ± 3.58[c]	11.1 ± 2.29[d]
DMI / 4% FCM	0.70 ± 0.18	0.77 ± 0.16

Different superscript in the same row is significantly different [ab] ($P < 0.05$), [cd] ($P < 0.01$)

The second intervention, environmental modification, is generally applied in hot climate area especially in hot and humid countries. Evaporative cooling system is the method to reduce body temperature by using the water as a media. There are several methods in this system such as showering, sprinkler, sprinkler and fan, misting, fogging and evaporative tunnel cooling system (ETCS). ETCS has been used as a successful method to cool swine and poultry in hot climates and has been used for dairy cattle for many years. Study in purebred Friesian found that it could increase DMI 11-12% and MY was increased by 2.6-2.8 kg/cow/d [4]. Recently, ETCS has been utilized for raising dairy cow in Thailand and the good response has been found. Cows on ETCS could eat 19.8% more and produced 26% more milk than cow on open housing system (Table I).

The third interventions, nutritional strategies, are found to be effective if appropriate techniques have been chosen to use. Several feeding strategies have been developed to relief the deleterious effect of heat stress. These are increase density of nutrient such as fat supplementation, change in dietary mineral concentration, avoiding nutrient excess; increase feeding frequency; providing cool water or supplementation with some additives. In our experimental station various supplementation tools were chosen to reduce heat stress in crossbred Friesian. Positive effects of Monensin, Na_2CO_3 and bST supplementation on productive performances and physiological changes were found (Table II)

TABLE II EFFECT OF MONENSIN, NA_2CO_3 AND BST SUPPLEMENTATION ON PRODUCTIVE PERFORMANCES AND PHYSIOLOCICALCHANGE OF CROSSBRED LACTATING COWS.

Items	Cont. vs Monensin*	Cont. vs Na_2CO_3**	Cont vs bST***
DMI	na	13.8 vs 15.9	12.2 vs 13.3
DMI(%BW)	na	2.81 vs 3.19	3.26 vs 13.3
MY (kg/day)	7.3 vs 9.9	13.7 vs 14.9	13.9 vs 17.6
MY/DMI	na	1.0 vs 0.96	1.16 vs 1.22
Mammary blood flow (l/min)	3.74 vs 4.39	na	3.8 vs 5.3 A:P
ratio	4.0 vs 3.41	3.24 vs 3.29	na

Cont: control; na: not available; * Thammarachroen et al., [6]; ** Soothiluck [5]; *** M

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TROPICAL FORAGE MEALS: AN ALTERNATIVE FOR SUSTAINABLE MONOGASTRIC SPECIES PRODUCTION

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The utilisation of tropical forage meals for monogastric feeding is nowadays a priority in order to obtain ecological sustainable and low cost productions. The aim of this paper is to offer information of an integral evaluation of physico-chemical characterization and molecular composition of six tropical forage meals: three temporary legumes C*anavalia ensiformis* (canavalia), L*ablab purpureus*, (dolicho), *Stizolobium aterrimun* (mucune) and two shrubs (*Morus alba,* mulberry; *Erythryna poeppigiana*, erithrina) and a tree, *Tricahnthera gigantean,* trichantera. In addition the purpose was also to study the effects of these tropical forage legumes through the gastrointestinal tract of poultry, swine, rabbits and guinea pigs. The last aspect was confirmed with performance experiments under controlled condition for rabbits and guinea pig.

Twenty-nine experiments were designed in order to carry out these purposes. Seven were related to physico-chemical and structural characterization of forage meals, 15 corresponds with the study of the sources effects in digestive physiology of poultry (5), swine (3), rabbits (7) and guinea pig (5). Forage meals were elaborated as indicated by [1]. Chemical analyses were conducted according to AOAC (2000), fibrous fraction was determined by Goering and Van Soest [2] and molecular fibre structure was analyzed by infrared spectroscopy Phytochemical screening was carried out by the procedure of Miranda (2000). Extractable tannin content, protein and fibre bound tannin were quantified by Scull [3] as well as oligosaccharides present in foliage meals. Amino acids were determined according to Biochrom (1986).

There were utilised castrated growing pigs (22.5 ± 2 kg) Yorkshirex Landracex Duroc, hybrid HE_{21} roosters of 50 d of age and broilers of 21-42 d of same genetic line for digestibility and morphophysiological studies. Also there were employed growing hybrids rabbits New Zeland x Semi-giant for digestibility studies in vivo and their cecal inoculum for digestibility studies *in vitro*. Growing guinea pig Macabea) of 700 gr were used for digestibility and morphophysiological and performances studies. Tropical forage meals bromatological composition does not show their nutritional potential value that is limited by its fibrous fraction and secondary metabolites that could decrease digestive utilisation in monogastric species.

It was shown that determination of physico-chemical characterization (solubility, volume, water holding capacity, cationic interchange capacity, acid and basic buffer capacity are necessary for nutritive evaluation of fibrous feed for monogastric species. Forage meals spectra analysis confirmed the similarity between FDN structure and chemical composition. This could contribute to the utilisation and manipulation of these sources in order to formulate monogastric species rations.

Inclusions levels of tropical forage meals sere related to species digestive characteristics as well as with sensitivity to secondary metabolites present. Among forage legumes, dólicho was the most promising species due to its results in digestive utilisation of fibrous components and in the morphopysiology of gastrointestinal tract of poultry, swine and rabbits (Table I). It was shown that the complete replacement of alfalfa forage meal by mulberry in rabbits was possible taking into account its low lignin content, high digestive utilisation of fibrous components and its similar nutrient value (Table II). It is possible to replace until 30% of alfalfa meal by mulberry, trichantera and eritrina in concentrate for growing guinea pig without affecting nutrient consumption, morphophysiological indices, nor productive performances (experimental scale) and to obtain improvements in production costs.

The application of these knowledge will allow making decisions and taking choices in the formulation of diets based in forage meals for non ruminant species. These results proportionate

improvements in production performances with sustainable conditions for small and medium production scales.

TABLE I. DRY MATTER DIGESTIBILITY OF DOLICHO

Species	Levels	Digestibility
Poultry	10 %	78 %
Swine	20 %	84.8 %
Rabbits	30%	57 %

TABLE II. DIGESTIBILITY OF FIBRE FRACTIONS IN RABBITS

Indicators	Mulberry	Alfalfa
DMI, g/d	84.93	75.66
DADF, %	25.45	53.69
Cellulose, %	22.45	63.16
Lignin, %	9.35	21.06

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EFFECT OF TYPE OF FOOD SUPPLEMENT ON THE REPRODUCTIVE AND GROWTH PERFORMANCES OF CANE RATS (*TRYONOMYS SWINDERIANUS*)

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Cane rat (*Tryonomys swinderianus*) is a wild rodent hunted in West Africa for its meat and its estimated that 80 million are killed each year [1]. This specie will soon be extinguished if nothing is done to ensure its survival. Domestication attempts have not been as successful as desired due to paucity of information on its nutritional [2] and reproduction [3, 4] requirements.

An experiment was carried out in the teaching and experimental farm of the University of Dschang between July 2007 and April 2008, to evaluate the effect of food supplement type on the reproductive and growth performances of cane rats. Twenty-six female cane rats: 11 primiparous and 15 multiparous (1.5 to 3.0 kg Body weight) were used for this study. The 26 female cane rats were randomly distributed to four treatment diets i.e. T0 (permanently fed a mixture of maize and wheat bran), T1 (permanently fed a complete diet), T2 (fed maize and wheat bran until detection of gestation and a complete diet onwards) or T3 (fed maize and wheat bran for 50 d after detection of gestation and a complete diet onwards). These animals received in addition to the basal diet (*Pennisetum purpureum*), water and a piece of bone. Fifty-nine young cane rats from primiparous and 31 from multiparous were used to evaluate growth performances. The results are as follows:

As concerns growth performances, average feed consumption (25,641 ± 854 g) of treatment T3 was significantly ($P < 0.05$) higher than that of treatment T1. Body weight gain and feed conversion ratio were comparable for all treatments. As concerns reproductive performances, primiparous female cane rats T1 and T2 registered the highest fertility rate (100%) whereas for multiparous, fertility rate of 83.3% was registered with the T3 females and was significantly higher compared to that of T2. The average gestation period of 156.1 ± 3 d was comparable for all the groups. The prolificacy rate was 308.3 ± 14.4% for the primiparous and 327.7 ± 25.4% for the multiparous. Litter size for the primiparous was significantly higher in treatment T1. For the multiparous, higher litter size was obtained with treatment T3. Independent of sex, the lowest body weight at birth was registered in treatment T3 and the highest with the control (T0). However, there was no significant difference in body weight. The mortality rate of females at birth (41.1%) was significantly higher for the primiparous as compared to the multiparous (8.3 ± 14.4%). The pre weaning mortality was significantly higher ($P < 0.05$) for the multiparous compared to the primiparous during the same period. After weaning, young males from primiparous cane rats registered a mortality rate of 5.6 ± 9.5% while no mortality was recorded for the multiparous.

Feed consumption (forage, supplement and water) was comparable in all treatments. As concerns growth performances, the lowest average live body weight (9.6 ± 4 g) was obtained with diet T1 whereas the highest (12.4 ± 1.6g) was with the control diet (T0). Feed conversion ratio varied between 10.1 for T1 and 11.1 for T3. However there was no significant difference in feed conversion ratio among treatment groups. The average feed cost (1167.9 ± 651.4 CFA F) (1 euro = 655.96 CFA F) for the production of one kilogram live body weight in T0 was significantly ($P < 0.05$) lower compared to (2459.9 ± 474.6 CFA F) obtained in treatment T2. The shortest body length (36.1 ± 2.9cm), shortest tail (12.2 ± 2.1cm) and smallest thoracic measurement (19.8 ± 1.2 cm) were obtained in treatment T1 while the longest body length (40.5 ± 1.6 cm) and tail (15.0 ± 11 cm) were obtained with the control and the highest thoracic measurement (22.9 ± 1.5 cm) was recorded with treatment T3. A strong positive correlation was registered between body weight and body measurements. In general, regressions of body weight with body measurements were linear ($R^2 \geq$

0.95). It was concluded that supplementing the diets of cane rat breeders improves fertility rate and litter size in primiparous.

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ADDITION OF INSULIN-LIKE GROWTH FACTOR-I DURING *IN VITRO* MATURATION OF BOVINE OOCYTES UNDER HEAT STRESS AND THE CONSEQUENCES ON CLEAVAGE RATE AND APOPTOTIC CELL RATIO

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The aim of the present study was to investigate the effects of insulin like growth factor-I (IGF-I) during in vitro maturation of bovine oocytes under heat stress (HS) by evaluating cleavage rate and incidence of apoptosis by active caspase staining at 45 hpi. Selected oocytes were assigned to four groups for maturation in serum-free media supplemented with EGF (20 ng/ml): two control groups without the addition of IGF-I; control (normal maturation temperature 38,5°C for 22 hours) or HS-control (maturation temperature of 40.5°C for 12 hours followed by 38.5°C for 10 hours) and two treatment groups with the addition of IGF-I (100 ng/ml); IGF-I-normal (normal maturation temperature 38.5°C) or IGF-I-HS (maturation temperature of 40.5°C for 12 hours followed by 38.5°C for 10 hours). Matured oocytes were fertilized and cultured in SOF medium supplemented with 5% FCS until 45 hpi. Presumed zygotes were checked for cleavage rate and incidence of apoptosis by positive results of active caspases labeling. Linear mixed effect models with treatment groups as fixed factors and cleavage rate (CR) and apoptotic cell ratio (ACR) as random factors (CR and ACR were analyzed separately) were used to investigate possible differences in CR and ACR among tested groups. IGF-I supplemented to maturation media had no significant effects on cleavage rate, however, it significantly ($P < 0.05$) reduced apoptotic cell ratio (Fig. 1). There was no significant difference between both IGF-I-normal and IGF-I-HS groups, which may indicate that the low cleavage rate is not related to apoptosis in heat stressed oocytes, but the role of the cumulus cells need further investigation. We hypothesize that addition of IGF-I during in vitro maturation has a carry-over effect on the resultant zygotes and reduces ACR of embryos derived from heat stressed oocytes. However, heat stress seems to reduce cleavage rate through mechanisms independent of incidence of apoptosis.

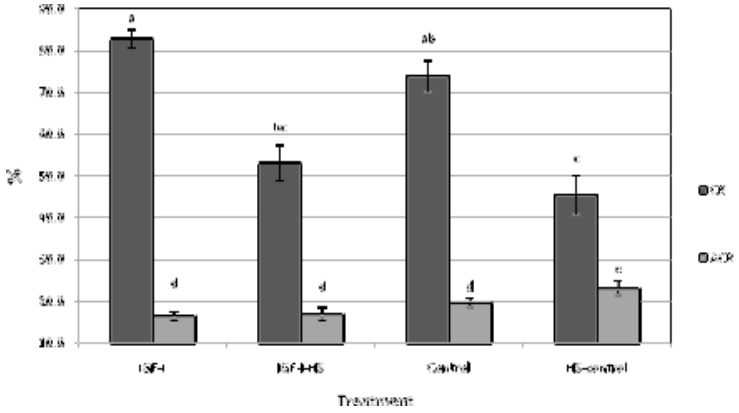

FIG. 1. Mean ± S.E.M. of cleavage rate (CR) and apoptotic cell ratio (ACR) at 45 hpi as affected by the addition of IGF-I under heat stress condition during maturation of bovine oocytes (^{a,b,c} Treatment groups with different superscript differ in CR ($P < 0.05$); ^{d,e} Treatment groups with different superscript differ in ACR ($P < 0.05$)).

EFFECTS OF ^{60}CO GAMMA RADIATION ON FOWL SPERM FOR INCREASE OF FERTILITY

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ZERO DAYS DRY – AN OPTION FOR HIGH-YIELDING DAIRY COWS?

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With the onset of lactation, the high yielding dairy cow is transitioning from the dry period to high peak milk yield during the first few weeks of lactation. In early lactation, the high yielding dairy cow is in a negative energy balance because of more or less extensive mobilization of body reserves, especially fat. Thereby, the risk for metabolic disorders and infection diseases increases. Accordingly, the majority of health problems and diseases occur in this short time frame. Alternative management systems are required. Omitting the dry period may be one option. The question was, whether continuous milking may reduce the metabolic imbalance associated with the onset of lactation due to lower nutrient output in early lactation combined with an improved subsequent fertility.

Multiparous cows were used with parity > 3 and previous lactation milk yield of > 8,000 kg. A total of 14 cows were assigned to two different management strategies concerning dry period (DP). Group I (control group; C, N = 7) was dried off 56 d before expected calving and milked twice a day after parturition. Group II (N = 7) was milked twice daily without drying off up to the day of calving and also post partum (continuously milking group; CM).

Milk samples were taken from the CM cows twice a week from wk -8 up to the day of calving. In the first week of lactation milk samples from all cows were taken daily from the afternoon milkings, up to 56 d in milk (DIM) twice a week, until 100 DIM once weekly, and biweekly up to 305 DIM. Milk samples were analyzed for fat using MilkoScan^{TM} FT6000- in Wolnzach MPR Bayern e.V. Forty days post partum, each cow was examined by the herd veterinarian by rectal palpation to check ovarian function. Cows with any reproductive dysfunction were inseminated after detecting the next oestrus. Otherwise, oestrous cycle was induced using exogenous PGF2α and GnRH for timed insemination.

During the last 56 d of gestation, cows of the CM group had average daily milk yield of 16.6 ± 6.5 kg, whereas cows of C were dried off. For the first 100 DIM, daily milk yield of C (35.9 ± 7.8 kg/d) was higher than that of the CM group (29.9 ± 6.9 kg/d), as in the second third (35.1 ± 5.3 vs. 29.2 ± 5.0 kg/d) and as in the last third of lactation (29.9 ± 5.6 kg vs. 21.0 ± 6.2 kg/d). Prepartum, average daily fat percentage was 5.6 ± 1.0 % for CM. During the first 100 DIM, milk fat percentage was 6.0 ± 1.4 and 5.8 ± 1.2 % for C and CM, respectively. Up to 200 DIM, average fat percentage was comparable for C (5.2 ± 0.8 %) and CM (4.8 ± 0.7 %) as up to 300 DIM (5.0 ± 1.0 vs. 4.9 ± 0.6 %). There was a tendency for number of open days to be higher for CM (67 ± 19 d) compared to C (59 ± 6). Furthermore numbers for calving to conception interval (91 ± 29 vs. 113 ± 61 d), services per conception (1.83 vs. 3.14) and calving to calving interval (370 ± 29 vs. 383 ± 44 d) were lower for cows assigned to CM than for cows assigned to C.

Results indicate that omission of the dry period for dairy cows resulted in significantly lower milk yield in the following lactation (approximately 20 %). However, considering that continuously milked cows are in lactating state with 16.6 kg/d while cows of control group are dried off, total produced milk yield is only 12 % lower than that of cows having a dry period. No differences were found for milk fat concentrations. Continuously milking promoted earlier resumption of ovarian cyclicity, mediated through improved nutritional status at the cost of reduced milk yield.

ASSESSING THE EFFECT OF FARMERS' SUPPLEMENTATION STRATEGY ON FEED INTAKE AND LIVE WEIGHT OF GOATS GRAZING NATURAL RANGE AND CROP FIELDS OF ZAMFARA RESERVE IN SEMI–ARID NIGERIA

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In the semi-arid Nigeria goats are raised extensively on natural grazing lands, crop residues, farm weeds and sometimes supplemented with industrial crop by-products. Under the extensive production system, supplementation has frequently been advocated as the main solution to the nutritional constraints livestock face during the long dry season (2 and 3). In order to design experiments to assess the optimum level of supplementation for the local producers, there is the need to assess their current practices. This experiment was therefore designed to test the farmers' feeding practices so as to ascertain its potential. This would serve as a baseline for designing future supplementation experiments with grazing animals.

This on-farm study was conducted in Zamfara reserve northwestern Nigeria between July, 2002 and June, 2003 to assess feed intake and live weight of 12 indigenous Red Sokoto castrated bucks, separated into two groups of six, supplemented and unsupplemented respectively. The unsupplemented group grazed natural pasture and crop stubble of crop fields, whereas the supplemented group grazed natural pasture, crop stubbles and concentrate supplementation. Concentrate supplement (wheat offal) was fed at 1% of the metabolic weight of the animals, mean of the farmers offer [1]. The total faecal collection method and grab samples of feed were used to estimate total intake of dry matter (DM), organic matter (OM), crude protein (CP) and metabolisable energy (ME) according to 3. Live weight of the animal was recorded at five weekly intervals. Results of the study indicated that nutrients intake of supplemented animals were generally higher than those of the unsupplemented group, but not significantly different ($P > 0.05$) Table I. However, supplementation significantly ($P < 0.05$) affected the live weight of the supplemented goats during early dry season in December, Figure 1. During this period feed became more available to the grazing animals from crop residues. It was therefore concluded that supplementation with wheat offal at 1% metabolic weight may not be enough to counteract weight loss of grazing goats during the other periods of the dry season in this environment.

TABLE I. MEAN NUTRIENT INTAKE OF SUPPLEMENTED (S+) AND UNSUPPLEMENTED (S-) GOATS

Parameter	S+	S-	SEM
Dry matter (g $kg^{-0.75}$ day^{-1})	55.5	54.3	1.18
Roughage from range and crop fields (g $kg^{-0.75}$ day^{-1})	48.5	54.3	1.90
Supplement (g $kg^{-0.75}$ day^{-1})	7.0	0	0.40
Organic matter (g $kg^{-0.75}$ day^{-1})	38.0	37.4	0.81
Crude protein (g $kg^{-0.75}$ day^{-1})	6.8	6.7	0.14
ME (KJ $kg^{-0.75}$ day^{-1})	396.1	391.0	8.3

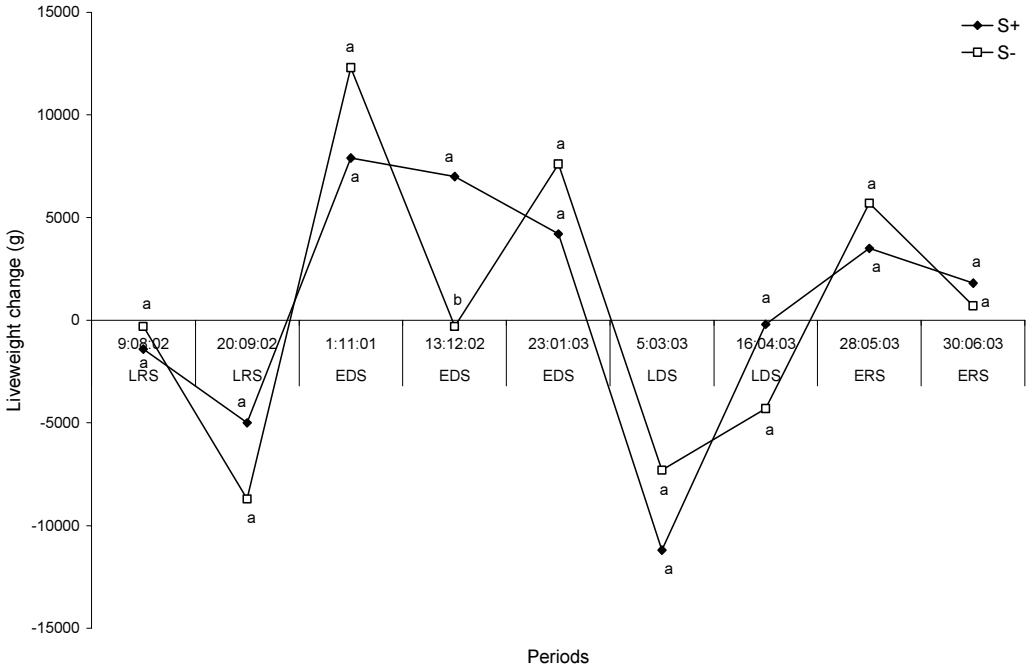

FIG 1. Mean live weight change (g) of supplemented (S+) and unsupplemented (S-) goats at different periods

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TRENDS IN FEED SUPPLY OF THREE BROWSE SPECIES IN A SEMI-ARID ENVIRONMENT OF NIGERIA

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The feed supply trends of three important browse species, *Anogeissus leiocarpus, Balanites aegyptiaca* and *Sclerocarya birrea* was monitored in the Zamfara reserve of north-western Nigeria (6^o 45'-7^o 10' E and 12^o 00'- 13^o 10'N), between peak period of production (September) and peak period of the dry season (April). 36 trees, 12 of each species were randomly selected and marked on a 3000 × 500 m transect. On each tree 12 twigs of one centimeter diameter thick were randomly selected and their leaves harvested and weighed fresh. After weighing, samples from each species were thoroughly mixed and 100 g sub samples were collected for oven drying and dry matter (DM) determination. This procedure was repeated at an interval of five weeks between 2002 and 2003. The experiment was laid as a Randomized Complete Block Design. Data were subjected to analysis of variance (ANOVA), where there were significant differences between treatment means, Duncan Multiple Range Test method was used for comparison [2]. Figure 1 presents the result of the DM production of the different browse species at the different time intervals. *Anogeissus leiocarpus* produced the highest ($P < 0.001$) amount of DM at all the periods, except in the month of March. Dry matter yield of the specie declined progressively from September (33.0 g) to March (9.0 g). Dry matter yield of *Balanites aegyptiaca* and *Sclerocarya birrea* was not consistent throughout the study period, however the former produced more yield ($P < 0.001$) than the latter (13.3 vs. 8.4 g respectively). In the month of March, *Sclerocarya birrea* produced no leaves at all.

On the overall, the mean DM yield of all the species was highest in November (23.3g) and lowest in March (6.3 g). The decline in the DM yield of all the species during this period could be due to physiological stress resulting from lack of moisture. It could also be due to competition of the grazing animals that switch from grazing to browsing during this period, which was earlier reported by [1] in the region.

It can be concluded from the result of the study that although *Anogeissus leiocarpus* species produced the highest DM yield throughout the periods except in March, *Balanites aegyptiaca* species was fairly more consistent in DM production throughout the periods. It could also be concluded that Anogeissus *leiocarpus* and *Balanites aegyptiaca* seems to be more ecologically adapted to this semi-arid environment and to the animal browsing activities.

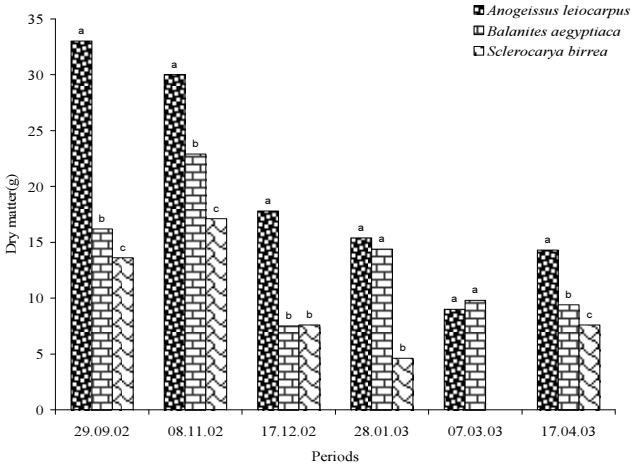

FIG 1. Dry matter yield (g) of Anogeissus leiocarpus, Balanites aegyptiaca and Sclerocarya birrea species at different time interval

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GENERAL ASPECTS OF PARTICIPATIVE RESEARCH AND ITS ROLE IN SMALL RUMINANT RESEARCH IN ARID REGIONS OF MEXICO

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This study was carried out in Casa de Cerros in the municipality of Panuco, Zacatecas, México, located between 23º 05' 36'' y 22º 50' 40'' N and 102º 19' 54'' and 102º 39' 51'' W. A Municipality is the political division of the Mexican States. The Panuco Municipality has a surface of 555.36 km2 and a population of 13,985. The participative research site was selected, representing the agro-ecologic in which small ruminants are raised and the prevailing farming systems in Semiarid North-Central Mexico.

Participatory research methods take into account the householders opinion, which is collected in workshops. The product of the workshop was a list of main activities and problems associated to them. The group of participants was householders, which are Panuco ejido members. Groups of householders were separated according to number of small ruminants they possess and the ranges were less than 50, between 50 and 100, between 100 and 150 and more than 150. Some indicators of the human resources associated to the size herd are showed. The average age is 51.3 ± 12.1 years and only 76% of them are able to read and write, which locate this group below of the total illiteracy of the Municipality, which is only 5% and the group surveyed have 24%. Those indicators can be associated with the way to deal with herd and their interest for change some technology components.

Some problems found in the survey were corroborated by the workshop results. Beans and corn were the main crops grown in the system as well as goats and sheep meat production and market were the main activities which define the production system of the region. Additionally, some of the problems were considered as social problems which are mainly the lack of domestic services or restrictions on them. After the problems definition, the householders were asked for ranking each problem included. Problems classified as market problems were ranked in the top, followed by organization, technology and equipment, respectively.

A possible solution for market problems mentioned by householders is the transformation of primary products in order to add value. Goat milk transformation can be considered. Milking goats is not a common practice and imply a change in the crop production subsystem (Table I). However, new crops to substitute beans as the main crop of the micro-watershed were mentioned. Such crops shall help to reduce the market problems and able to grow and adapt to rain fed conditions. Young goat males and adults females were the preferred stages for sale. That is defined by the market, which asks for meat to cook a traditional dish. The young stages as young females, yearlings or kid goats were sold seldom. However, householders were asked directly about their interest for selling kid goats, which may represents a substantial change in their production system, householders answer positively (68% of the total) and a 30% have sold goat and sheep at that age, but in small amounts and not as a market demand.

Participatory research methods take into account the householders opinion, which is collected in workshops especially designed. The workshops include six steps, which are: presentation of the participants, review of aerial photography, main activities and problems definition, tendency, prioritization and compromises. All participants are asked for participate and a summarization of results are presented. A linkage between secondary information from a macro system level and primary information from a survey and demands expressed directly by householders ensure a better understanding of the importance and magnitude of problems. Macro level studies and secondary information define the starting point before technological intervention which may be used as ex-ante evaluation for further impact evaluation and the output of a list of research activities.

TABLE I. STRATEGIES TO ADDRESS THE PROBLEMS

Assessing the current status of production systems and market potentials
• The establishment of grazing system in the watershed rangeland. • Improve herd management practices • Test of new crops to increase forage yield and substitute beans and consequently improve animal feeding • Technology to increase goat milk production • Technology to transform milk to cheese. • Differentiation of householders according to their technological and investment level which may be exposed to different technological components and production goals is suggested as a strategy to arise a more intensive goats and sheep production system Production improvement with a market-orientation

RESEARCH ON THE PRODUCTION PERFORMANCES ACHIEVED BY PIG BLOODLINES USED WITHIN A SWINE INTENSIVE HUSBANDRY UNIT IN THE NE OF ROMANIA

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Through this paper, we proposed to establish the production performances of breeder lines belonging to one of the most important providers of genetic material in Romania, respectively the Pig Improvement Company (P.I.C.), exploited within a top unit in Moldova in swine husbandry - S.C.SUINPROD S.A. ROMAN. The researches goal was to study the reproduction performances (sperm volume, spermatozoids concentration in sperm, the amount of spermatozoids and of produced doses, etc.). There have been analysed the reproduction performances achieved by three PIC boars lines (PIC 1075, PIC 402, PIC 408). The insemination material, issued from the 1075 boars, is used to artificially inseminate the PIC 1050 sows from the hybridisation farm of the unit. The insemination material, issued from the boars of PIC 408 and PIC 402 lines, is used to artificially inseminate the Camborough sows, resulting piglets exclusively designed for slaughtering. Both boars and sows used as biological material have been examined from the reproductive life onset toward their culling.

Ejaculate volume at the three bloodlines of boars we studied, was comprised within the limits specified in the references. It reached values between 224 and 235 ml during 8-12 months old, between 310 and 366 ml during 13-24 months old, between 330 and 348 ml between 25-36 months old and between 304 and 404 ml during 37-42 months old. Significant and distinguished significant values occurred both between boars and age periods. The level of sperm concentration, as influenced by boars' age, was found high, in all lines, during the 25-36 months old period, the differences compared to the other periods being statistically significant. Comparing the values achieved in each boar line, we could find differences between PIC 1075 (372 x 106 spermatozoids / ml =100%) and PIC 402 (311.5 x 106 spermatozoids / ml), of 16.28%, or compared to PIC 408 (302.3 x 106 spermatozoids / ml), of 18.76%.

The average spermatozoids mobility within the crude semen had mean values comprised between 76.92 % and 79.4%, but not significantly influenced by boar's age. Expressed in relative values, the differences between the average level observed in PIC 402 line (79.4%=100) and those found in the other lines, were comprised between 3.13% (comp. to the PIC 1075 line) and 0.57% (comp. to PIC 408 line). The amount of doses per ejaculate subscribed to the trend presented in the last field researches. The maximum amount of doses/ejaculate has been achieved in both bloodlines during 25-36 months old period, while the poor amount during the reproduction activity onset (8-12 months old period). The highest doses amount (21.12) has been produced by the PIC 402 line. No significant differences occurred between groups. Expressed as relative values, the differences were of 3.17 % (compared to PIC 1075 line), respectively of 7.20 % (compared to PIC 408 line).

It could be stated, basing on the researches we carried on, that, due to the high sperm concentration, meaning high spermatozoids amounts per ejaculate during the whole exploitation period, the reproduction usage intensity of studied PIC boars could be improved. Thus, the period between two ejaculates could be shortened to 3 or 4 d, compared to the actual used interval, of 5 d.

In the studied PIC boars, the sperm production level allows the exploitation of a reduced amount of males, generating thus favourable financial and zootechnic consequences.

EFFECT OF A MULTIPLE MIXTURE OF HERBACEOUS LEGUMINOUS AND *LEUCAENA LEUCOCEPHALA* IN THE POPULATION PROTOZOA AND OTHER ECOLOGICAL GROUPS OF RUMEN IN YEARLING CATTLE CROSSBREEDS HOLSTEIN × CEBÚ

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Twenty four yearling cattle crossbreeds Holstein × Zebu of 134 kg of PV, were managed with the objective of comparing the effect of a multiple mixture of herbaceous leguminous or *Leucaena leucocephala* in the population protozoa and their relationship with other ecological groups and their fermentative products. The treatments were: (To) *Leucaena leucocephala* (T_1) mixes of herbaceous legumes (*Neonotonia wightii, Pueraria phaseoloides, Macroptilium atropurpureum and Centrocema pubensis)*. The stocking rate was 2 animals per ha and the duration of the experiment was 150 d between May to October. *L. leucocephala* was associated in 100% of area with nature pasture with a population density and botanic composition of 6,000 plants per ha and *Sporolobius indicus, Dechantisum anmorlatum y Paspalum notatum,* respectively. Chemistry and phytochemical screening were carried out.

The total area was 12 ha; 6 ha per treatments. The animals also had free access to water and mixed minerals composed of (g/kg) $(PO4)_2$ Ca_3, 500; NaCl, 400; $ZnCO_3$, 20; $CuSO_4$, 10; $FeSO_4$, 27; $MgSO_4$, 23; $CoSO_4$, 0.1; Sodium Selenite, 0.02 and 19.86 mixed maize.

Dry matter digestibility, expressed as stocking grass, was of 12.9 kg DM.100 kg de PV^{-1} with legumes mixes and 9.9 kg de DM. 100 kg de PV^{-1} with Leucaena. Rumen liquor samples were taken at 10:00 a.m. with oesophageal tube every month to determine ruminal bacteria, protozoa and fungi populations, pH, ammonia, VFA, and methane production. The technique of culture in roll tubes was used under conditions of strict anaerobiosis.

There were not significant differences in the ruminal protozoa population when *L. leucocephala* or legumes mixtures were used in the diet. In both treatments the protozoa populations were lower in respect to diets without legumes. This result suggests that multiple mixtures or Leucaena produce defaunant effect in the rumen.

With the use of multiple mixtures of herbaceous legumes, the ruminal cellulolytic bacteria population was triplicate (11.1 and 34.2 x 10^6 ufc. /mL to T_0 and T_1, respectively). The total viable and proteolytic bacteria populations were higher in the rumen of animals that intake multiple leguminous mixture in relation to *L. leucocephala*. The ammonia concentration was 13.58 and 18.26 mmol./L for L. *leucocephala* and herbaceous legumes, respectively. The mixture of legumes increased in 12% the concentration of total VFA. The concentration of acetic acid was 84.78 and 102.68 mmol. /L and propionic, 20.22 and 14.13% to T_0 and T_1 treatments, respectively.

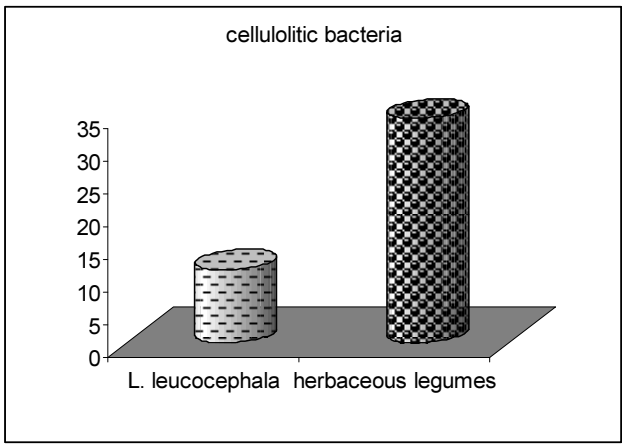

FIG1. Effect of multiple mixture of herbaceous leguminous and Leucaena leucocephala in the cellulolytic bacteria population (10^6cfu.mL^1)

TABLE I. EFFECT OF MULTIPLE MIXTURE OF HERBACEOUS LEGUMINOUS AND *LEUCAENA LEUCOCEPHALA* IN THE MICROBIOLOGICAL POPULATION AND FINAL FERMENTATIONS PRODUCTS IN YEARLING CATTLE

Indicator	*L. leucocephala*	Multiple mixture of herbaceous leguminous	EE ± SIG.
Bacterias viables totales, 10^{11} ufc/mL	2.04 (32.51)	2.95 (51.81)	0.15*
Bacterias Proteolíticas, 10^6 ufc/mL	1.17 (6.52)	2.28 (40.23)	0.12**
Hongos celulolíticos, 10^4 uft/mL	1.85 (10. 01)	1.98 (11.00)	0.14
Protozoos, células/mL	1.01 (6.32)	1.82 (9.98)	0.26
pH	6.65	6.96	0.02
NH_3, mmol/L	13.58	18.26	2.10*
$AGCC_t$, mmol/L	117.48	131.77	3.45*
Ácido Acético, mmol/L	84.78	102.68	2.23*
Ácido Propiónico, mmol/L	23.75	18.62	1.19*
Ácido Butírico, mmol/L	6.89	7.08	1.03
Ácido Isovalérico, mmol/L	1.41	1.45	0.87
Ácido Valérico, mmol/L	0.65	1.94	0.05

Datos transformados según Ln X; medias originales entre paréntesis; a, b, medias con letras diferentes en la misma fila, difieren a $P < 0.05$ (Duncan, 1955) * $P < 0.05$; ** $P < 0.01$

It is concluded that multiple mixture of herbaceous produced increase in the cellulolytic bacteria and improve the microbial rumen population in relation to Leucaena leucocephala, but two systems are appropriate for rumen in yearling cattle.

CHEMICAL AND NUTRITIONAL FEATURES OF THE MEAT ISSUED FROM TWO CHICKEN BROILER GENOTYPES

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Nutrition quality, consequently the nutrients level and quality in food, is a main factor influencing the consumers' health status. The food industry should consider all possible ways to improve nutritional value of aliments. Despite this, the data related to some quality indexes of the poultry meat, that could contribute to establish an overall image of the nutritional and dietetic facts of this product are poorly represented in the scientific literature, mainly when the consumer might be interested by the meat produced and commercialised nowadays in Romania. It imposed to organise some studies related to certain qualitative features of the skeletal muscles issued from chicken broilers, knowing that the technical specifications of the companies producing hybrids do not issue about them, mainly focusing on the meat yield parameters.

The original researches have been organised in two experimental series, which had as main goal the analysis of the qualitative meat production at two of the most used commercial chicken hybrids in our country - „COBB-500" (60 chickens, sex ratio 1:1) and „ROSS-308" (60 chickens, sex ratio 1:1), reared in similar technological conditions. This paper emphasises on those results dealing with meat chemical composition and caloricity. There has been sampled five pair of skeletal muscles, from those carcass areas with market importance: *Pectoralis superficialis* (PS) and *Pectoralis profundis* (PP) – breast, *Biceps brachialis* (BB) - wings, *Semimembranosus* (SM) - thighs and *Gastrocnemius medialis* (GM) - drumsticks. They were processed in accordance with the analytical chemistry laboratory methods in order to assess dry mater and water content, proteins and amino acids content, lipids and fatty acids content, these values leading to caloricity computation.

The achieved results are briefly listed below:

- for both studied hybrids, it was observed that the males pectoral muscles comprise a higher quantity of dry matter, compared to the pullets; when the wings, thighs and shanks muscles are analyzed, the ratio conversely presents;
- the lipids constituted the compound with the highest variation amplitude between the muscles studied in both hybrids: in "COBB 500" chickens, the minimal value (1.12%) was found in the cockerels PS and in the pullets PP, while the maximal one (9.24%) was found in the females SM; a similarly situation occurred for the "ROSS-308" broilers, but with wider interval, meaning a minimum of 0.95% (PS in males) and a maximum of 9.92% (SM in females);
- meat cholesterol content varied within the 56 mg/100g (breast muscles) – 83 mg/100 g (thigh muscles) interval for the samples issued from "COBB-500" hybrids, respectively between the minimal value of 57 mg/100g and the maximal one of 83 mg/100 g, in the samples taken from the "ROSS-308" chickens;
- the ratio between polyunsaturated and saturated fatty acids in "COBB-500" muscles has been calculated at 1.06:1 (PP), respectively at 0.79:1 (SM); for "ROSS-308", the ratio was better in the PP (1.1:1) and less convenient in the SM (0,8:1) and GM (0,86:1) muscles.
- the chemical assessments revealed protein contents within muscles between 17.94% (SM females) ÷ 24.10±0.25% (PS females) ("COBB-500"), respectively within the 17.13% (SM males) ÷ 23.80 (PS females) limits ("ROSS-308");
- protein quality in the analysed muscles, considered by its content in essential amino acids, is presented below:
  a)in "COBB-500", broilers, lysine oscillated between 3.23 g/100g (PS males) – 6.75g/100 g (SM females); methionine had a lower variation amplitude (3.24-3.79 g/100g), while the

phenylalanine was found between the minimal of 2.74 g/100g (SM males) and the maximal of 5.54 g/100g (PS females);

b) for the "ROSS-308" hybrids, the lysine quantity varied between 3.31 g/100g (PS in males) – 6.92g/100 g (SM females), the methionie varied within the 3.03-3.95 g/100g limits and the phenylalanine oscillated between 2.81 g/100g (SM males) and 5.78 g/100g (PS females);

– the calculated energetic value revealed, once again, the dietetic features of the white meat (pectoral muscles), compared to the red meat (limbs muscles). In the samples in the 1st experimental series ("COBB-500") the minimal value for the gross energy reached 138.26 Kcal/100 g product (PP from pullets) and the maximal one reached 191.73 Kcal/100 g product (SM from pullets). During the 2nd experimental series, the samples caloricity varied between 138.93 Kcal/100 g product (PP in females) and 202.28 Kcal/100 g product (SM in females).

The pectoral muscles could be considered as qualitative superiors, mainly due to the low energetic value, to the low cholesterol content and high polyunsaturated fatty acids content. Nutritionally speaking, although the white meat proved to be richer in proteins, their quality was lower, due to the reduced content in essential amino acids, especially in lysine. This fact could be explained by high collagen content in the structure of the pectoral muscles fibrous connective tissue.

The achieved results are in accordance with the values in the scientific References**:** 20.1% proteins in chicken broiler meat [1] 23.8-24.5% proteins in broilers meat [6]; 22.5-23.0% proteins in pectoral muscles and 18.0-18.5% proteins in thighs muscles at the „ROSS-308" hybrid [5]; 23-24% proteins in „ISA" broilers meat [4]. Meantime, the cholesterol and the polyunsaturated fatty acids levels were closet o those specified in other researches [2, 3].

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EFFECTS OF PREBIOTIC AND MYCOTOXIN BINDER FEED SUPPLEMENTATION ON THE QUALITY OF THE EGGS PRODUCED BY THE HENS IN THE END OF THEIR LAYING PERIOD

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The eggs yield decreases while the hens approach the end of their laying cycle. On the contrary, the weight of the eggs increases. When laying hens become older, the egg volume and the yolk proportion increase, and the albumen proportion and the shell thickness decrease [9]. These undesirable situations are related to some factors as shell thickness and shell stiffness, which decrease proportionally with hens ageing [2, 5]. Some previous researches proved that the Bio-Mos® prebiotic product, which contains mannan oligosaccharides issued from the cell wall of the *Sacharomyces cerevisiae* yeast, could generate beneficial effects, such as combat against intestinal pathogen germs in birds and mammals, through the immune response modulation and through the improvement of the intestinal mucosae structural integrity [7, 8]. Prebiotics also improve the absorption of the nutrients, including macro and microelements, through the intestinal wall [6, 1], increasing meantime the degree of their availability to be used for organism's maintenance and regeneration, as good as for production. Egg production could negatively affected, quantitative and qualitative speaking some mycotoxins exist in feed. It was proved that aflatoxin and ochratoxin produce a 20% depression of the serum Ca level in laying hens, leading to some osteogenesis and eggshell formation troubles. The mycotoxins harmful effect could be prevented or eliminated by using some feed additives, which have the property to selectively bind the mycotoxins and to carry them out of the organism, without binding other beneficial elements, as vitamins or minerals [4]. Mycosorb® is such a product, based on gluco-mannans extracted from the yeasts cell wall, which have a higher capacity to bind a series of important mycotoxins, comparing with other detoxifying agents [3].

The present study aimed to evaluate the effects of Bio-Mos® and Mycosorb® on the performance and egg quality of commercial laying hens approaching the end of the laying cycle. The trial was conducted at the Animal Husbandry Faculty's Experimental Farm in Iasi, Romania, during a period of 4 weeks. A total of 90 hens, 57-week-old ISA Brown layers were allocated to a completely randomized experimental design with three treatments, with 30 birds each. Dietary treatments consisted in feeding hens a corn soybean meal basal diet with supplementation of 0.1% Bio-Mos® (treatment A1) and of 0.2% Mycosorb® (treatment A2). The parameters studied included: living weight and feed intake dynamics, feed conversion ratio, laying intensity, egg mass production, egg weight, eggshell weight, eggshell thickness, shell index, Haugh Index. Both groups received fodder additives given superior results, concerning the production performances, the internal and external egg quality, as shown in Table I.

Major influence on the laying hens' health and production is given by the relationship existing between intestinal bacterial population, gut morphology, immune system and nutrients absorption, as previously stated in the reference literature. Some production indexes were improved due to the supplementation of the laying hens feed with Bio-Mos® (+ 0,1%) and Mycosorb® (+ 0,2%):

- egg mass production increased with 1.67%, respectively with 3.00%;
- feed conversion ratio decreased with 1.85%, respectively with 6.61%.
- Some egg quality indexes were also improved:
- shell thickness increased with 3.86% at the eggs respectively with 2.84%, these effects being mostly observed since the 4th week of usage;

- the amount of eggs with unconformities decreased. Thus, the amount of integer shell eggs was 5.60% higher in A1 treatment, respectively 3.98% higher in A2 treatment, as compared to the control group.

TABLE I. EGGS YIELD AND EGGS PRODUCTION QUALITY AT THE HENS APPROACHING THE END OF LAYING PERIOD, WHOSE FEED WAS SUPPLEMENTED WITH PROBIOTIC AND MYCOTOXIN BINDER

Variable		Groups		
		Control	A1 (+ 0.1% *Bio-Mos*®)	A2 (+ 0.2% *Mycosorb*®)
Mean egg production (eggs/day/group)		25.97	26.14	26.11
Laying intensity (%)		86.55	87.14	87.02
Egg-mass production	(g/hen/day)	53.83	54.73	55.45
	% (C = 100)	100.00	101.67	103.00
Feed conversion	(kg feed/kg egg mass)	2.103	2.064	1.964
	% (C = 100)	100.00	98.15	93.39
Egg weight (grams)		65.09	64.38	65.50
(%) (C = 100)		100.00	98.91	100.63
Eggshell weight (grams)		6.04	6.26	6.25
(%) (C = 100)		100.00	103.64	103.48
% shell in whole egg weight		9.30 [a]	9.75 [b]	9.56 [b]
(%) (C = 100)		100.00	104.84	102.80
Shell thickness (mm)		0.387	0.402	0.398
(%) (C = 100)		100.00	103.86	102.84
Shell index		7.99 [a]	8.34 [b]	8.26 [b]
(%) (C = 100)		100.00	104.38	103.38
Haugh Index (H.U.)		85.96	86.83	87.81
(%) (C = 100)		100.00	101.01	102.15
Eggs with intact eggshell (pieces)		679	717	706
(%) (C = 100)		100.00	105.60	103.98
Eggs with unconformities (pieces)		48	15	25
(%) (C = 100)		100.00	31.25	52.08

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MARINE PLANTS: A NEW ALTERNATIVE FEED RESOURCE FOR LIVESTOCK

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Under the Tunisian conditions, and particularly during the drought periods, the feeding management remains a limiting factor to improve livestock productivity. Consequently, the farmers use more and more concentrates whereas these are mostly imported. Therefore, searching for other feeding alternatives will be more profitable for farmers.

Among these resources, the marine plants as algae were studied as to their adding to the concentrate formula, producing rabbit and sheep meat. The study dealt with five types of marine plants where 3 were kept, based upon their chemical composition and organic matter digestibility (OMD).

The result show that crude protein (CP) content of the marine plants (Ulva, spp., Chaetomorpha, spp. and Ruppia, spp.), varied from 10% DM to 20% DM, and OMD varied from 60% to 75%.

The marine plants were added at different ratios (10, 20, 30 and 40%) and with different concentrates formula and performance trials on young rabbits and lambs were realized. The concentrates based on marine plants were conserved in pellets and were distributed to the different groups of animals, in comparison to the commercial concentrate. The pH and NH3 concentrations in the rumen were satisfactory.

The addition of marine plants until 30% in the concentrate formula had no effect on OMD and N degradability. The average daily gain of rabbits and lambs were 25 g and 175 g respectively. The results showed that the carcass lipid content decreased when animals received marine plants concentrates.

The results confirmed the possibility of use of marine plants in animal feeding systems.

REPRODUCTIVE WASTAGE THROUGH SLAUGHTERING OF PREGNANT EWE AND GOAT IN OUAGADOUGOU, BURKINA FASO

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Data collected from 3190 small ruminants (2408 ewe and 782 goats) have been examined after slaughtering, from January to June 2005 in Ouagadougou refrigerated abattoir. 26.70% of sheep and 34.91% of goats (Table I) were more in advanced gravidity with losses estimated to 0.33 lamb and 0.44 kid per slaughtered sheep and goat. Animals slaughtered during the second half of their gravidity (2 - 5 months) represented 53.19% of sheep and 58.24% of goats although at this period, the fœtus becomes palpable and detectable. This survey attracts the attention on the losses due to the slaughtering of pregnant females, as the deficit in animal proteins doesn't stop worsening. Urgent measures must be considered to decrease the extent of these losses and it is recommended to detect pregnancy females in abattoir and breeders must be trained to simple methods of gestation detection.

TABLE I. REPARTITION OF PREGNANT SHEEP AND GOATDES PER GESTATION LENGTH.

Durée de gestation (mois)	Femelles gravides abattues			
	Brebis		Chèvres	
	Nombres	%	Nombres	%
0 - 1	118	18,35	30	10,99
1 - 2	183	28,46	84	30,77
2 - 3	335	52,10	159	58,24
3 - 4	7	1,09	-	-
Total femelles gravides abattues	643	26,70 %	273	34,91 %

EFFECT OF UREA-TREATED SORGHUM STOVER SUPPLEMENTED WITH LOCAL PROTEIN SOURCES ON THE PERFORMANCE OF SHEEP

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Feeding trials were carried out on-farm to examine the effect of supplementing urea treated sorghum stover (UTSS) with sesame cake (SC) or fishmeal (FM) on the body weight of sheep. Twenty-one male sheep were divided into three groups of seven sheep in each treatment. All the sheep used in this experiment were from the same breed (Gerej), with the same age and initial body weight from the same area.

The experiment was conducted in Gash Barka, western lowlands of Eritrea. All the animals were fed on UTSS for an adaptation period of 15 d. The control diet consisted of UTSS fed *ad libitum.* The second and third treatments consisted of UTSS fed *ad libitum* supplemented daily with 80 g/head of SC and 60 g/head of FM, respectively. The experimental period lasted for 90 d. Feed intakes and body weights were recorded regularly. The dry matter intake (DMI) in sheep was significantly different ($P < 0.05$) between the control and SC supplemented groups, but not between the other treatments. It was highest for the SC supplemented group at 847 g/head/day followed by the FM supplemented group and the control at 826 and 821 g/head/day, respectively. Sheep supplemented with SC had the highest significant ($P < 0.05$) body weight gain (BWG) (134 g/head/day) followed by the group supplemented with FM (115 g/head/day). The BWG for the control was 66 g/head/day. Feed conversion was best on SC (6.92) followed by FM (7.70) supplementation. The lowest cost of feed per kg of BWG (16.91 Nfa) was attained by supplementing with SC. It can be concluded that feeding UTSS alone or supplementing with small amounts of sesame cake or fishmeal can increase the live weight of sheep at a reasonable cost.

ESTS FROM SKIN AND PBMC cDNA SUBTRACTIVE LIBRARY OF ALPACA

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As an effort to map and identify genes and genetic markers that influence the fibre quality in alpacas, cDNA subtractive libraries of Alpaca (*Vicugna pacos*) were constructed in order to find differentially expressed genes in skin. Skin and blood samples were removed from six adult Alpaca (1.5 year old). Total RNA was extracted using Trizol (Invitrogen) and mRNA was purified using the Gene Elute mRNA purification kit (Sigma). Suppression PCR was used to construct the library using mRNA from skin as a tester and the mRNA from PBMC as a driver. The subtracted PCR products were inserted into the TA cloning vector and the ligation reaction was transformed into TOP10 *E. coli* cells.

Randomly selected clones were sequenced and a total of 2280 high quality 5' end sequences were generated. Clustering analysis using StackPACK version 2.2.0 resulted in 1075 unique transcripts, consisting of 347 consensi and 728 singletons. BLAST analysis of the generated sequences revealed skin associated transcripts such as hair keratin 6A, keratin 10, keratin KA27, keratin 34, wool keratin microfibril type I, and collagen. A total of 27 microsatellite loci were also uncovered.

Further work is in progress to generate more sequences in order to build an EST database of differentially expressed genes from Alpaca skin and PBMC, and for the generation of genetic molecular markers such as microsatellites and SNP for Alpaca.

PARENTAGE TESTING AND MOLECULAR SEXING IN ALPACAS (*VICUGNA PACOS*) USING MICROSATELLITE MARKERS AND ZFY/ZFX GENES

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The aim of this study was to assess and apply a microsatellite multiplex system for parentage determination and sex identification test by analysis of the ZFX and ZFY genes in alpacas. A panel of 10 microsatellites were evaluated for parentage testing in a population of 329 unrelated alpacas and 121 related alpacas from a pedigree book (Mallkini Breeding Center – Puno). All microsatellite markers, which amplified in two multiplex reactions, were highly polymorphic with a mean of 14.5 alleles per locus (six to 28 alleles per locus) and an average expected heterozygosity (HE) of 0.8185 (range of 0.698 to 0.946). The total parentage exclusion probability was 0.999456 for excluding a candidate parent from parentage of an arbitrary offspring, given only the genotype of the offspring, and 0.999991 for excluding a candidate parent from parentage of an arbitrary offspring, given the genotype of the offspring and the other parent. In a case test of parentage assignment, the microsatellite panel assigned 53 (from 53 cases) offspring parentage to 15 sires with LOD scores ranging from $2.19 \bullet 10^{+13}$ to $1.34 \bullet 10^{+15}$ and D values ranging from $2.80 \bullet 10^{+12}$ to $1.34 \bullet 10^{+15}$ with an estimated pedigree error rate of 13.2%. The performance of this multiplex panel of markers suggests that it will be useful in parentage testing of alpacas.

Primers from ungulates conserved ZFY and ZFX loci were used to amplify 450 bp of the ZFX/ZFY loci by PCR from female and male alpaca DNA. Both strands of the PCR products (417 bases) were sequenced on a ABI3130 Genetic Analyzer (ABI). Eleven gender-specific single nucleotide polymorphisms were observed and used to design sex-specific primers (Figure 1). Three specific primers were designed for the differential PCR amplification of the ZFY and ZFX sequence in alpacas. Primers were tested with thirty unrelated alpacas (15 female and 15 males); female alpaca DNA produced a 238 bp single fragment and male alpacas showed two fragments (238 and 127bp) by PCR (Figure 2). Both fragments were cloned in pCR®4-TOPO® and sequenced to confirm sequence identity. This multiplex PCR might be useful for molecular sexing of alpacas.

```
ZFX_alpaca  GTCAGAAGAC AAACGTCACA CTTGAATGGC ATCTCTTTAC TATGCTTAGT TTTTACGTGT GTTTTCAAGT TAGAAGAGTC
ZFX_alpacal .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpaca  .......... ...T...... .......... .......... .......... ......A... .....T.... ..........
ZFY_alpacal .......... ...T...... .......... .......... .......... ......A... .....T.... ..........

                                                                                  ATATG AGCTCTGAGG

ZFX_alpaca  TGCAGACCTG TATTCGCAGT ACTGGCACTG GTACGGCTTC TCCCCGGTAT GGATTCGCAT GTGCTTTTTG AGCTCTGAGG
ZFX_alpacal .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpaca  .......... .......... .......... ......T... .....A.... .......... .......... ..........
ZFY_alpacal .......... .......... .......... ......T... .....A.... .......... .......... ..........

            GGT (ZFX-F)

ZFX_alpaca  GGTGTCGAAA ACCTTTACCG CACTCCACGC ATATATGAGG AAAGTTCTTG CTGTGGACCG CCAAGAGGTG GCGATTCAGT

ZFX_alpacal .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpaca  .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpacal .......... .......... .......... .......... .......... .......... .......... ..........

                        TCAGAG TATAAGTGTC GG (ZFY-R)

ZFX_alpaca  AACCCCTGTT CAGCGGTCTC GTATTCGCAG AATTTACACT TGTGCATTTT GTTGGCTCCT TTTTCCTTAT GCACCATTTT
ZFX_alpacal .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpaca  .....T.... ....A..... A.....A... .......... .......... .......... .......... ..........
ZFY_alpacal .....T.... ....A..... A.....A... .......... .......... .......... .......... ..........

                                                        TAGCG TGAGCTACCG GAA(ZFX-R)

ZFX_alpaca  GTGAGTAAAC AAAGCCCCAG CATGAGAGAA ATGCTTCCCA CACTCATCGC ACTCGATGGC CTTCTCTGCC TTGCTGGTCA
ZFX_alpacal .......... .......... .......... .......... .......... .......... .......... ..........
ZFY_alpaca  .......... .......... .G........ .......... .......... .......... .......... ....C.....
ZFY_alpacal .......... .......... .G........ .......... .......... .......... .......... ....C.....


ZFX_alpaca  GCTTGTGGCT CTCCATG
ZFX_alpacal .......... .......
ZFY_alpaca  .......... .......
ZFY_alpacal .......... .......
```

FIG. 1. Sequence alignment of polymorphisms between ZFX and ZFY using MEGA software. All sequences are presented in the original 5'-3' format of the Zinc Finger Protein gene. Dashes indicate the nucleotide identity according to the Taiwan water buffalo sequence. Primer sequences are underlined.

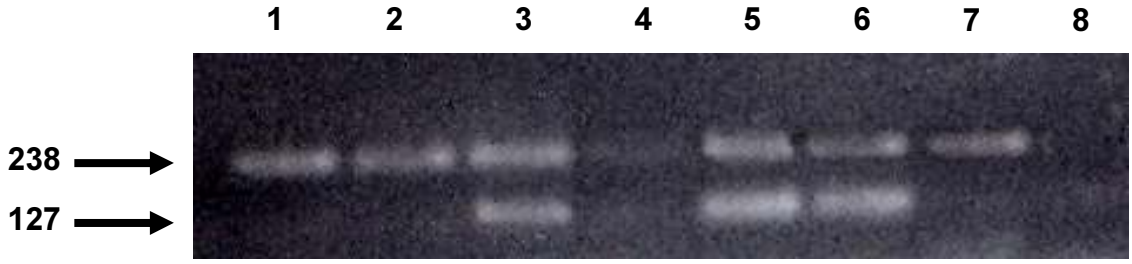

FIG. 2. The ZFX and ZFY amplification products after electrophoresis in 2% agarose TBE 0.5X gel. Lane 1, 2 and 7 alpaca female (ZFX/ZFX fragments), Lane 3 5 and 6 alpaca male (ZFX/ZFY fragments), Lane 8 PCR blank.

EFFECT OF PHYTOCHEMICAL AND COCONUT OIL SUPPLEMENTATION ON RUMEN ECOLOGY AND METHANE PRODUCTION IN RUMINANTS

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Four, native beef cattle were used to determine the effect of phytochemical and coconut oil on rumen ecology and ruminal methane gas. The animals were housed in individual pens and fed with rice straw and concentrate containing 7% coconut oil (CCO) or supplemented with 100g mangosteen peel powder (MSP, *Garcinia Mangostana*), 100g soap berry tree fruit (SBF, *Sapindus rarak*) or with no supplement (NS). Feeding regimes lasted for 3 weeks and samplings of feeds, rumen fluid and gas measurements were done during the last 3 d.

Animals received SBF had lowest ($P < 0.05$) feed intake (%BW and $BW^{0.75}$), however, CCO supplemented group had highest ($P < 0.05$) feed intake per $BW^{0.75}$. The OM and NDF digestibility were found lowest ($P < 0.05$) when animals received CCO supplemented group. Total VFAs results corresponded with the OM and NDF digestibility in which the CCO supplemented group had significantly lowest values ($P < 0.05$). MachmÜller et al. [1] reported that coconut oil was found to depress ruminal fiber degradation and linearly decreased in digestibility in response to increasing levels of coconut oil. MSP and SBF supplementation groups had reduced acetate and increased propionate ($P < 0.05$). Moreover, MSP supplemented group significantly lowest ($P < 0.05$) in proportion of butyrate production. Ratio of acetate and propionate (C2:C3) were affected by MSP and SBF supplementation ($P < 0.05$).

Ruminal methane production per body weight (CH_4 : BW, L/kg) was significantly highest ($P < 0.05$) in CCO supplemented group and lowest ($P < 0.05$) in MSP and SBF supplemented groups. However, when considered by % NDF digestibility and methane gas production per body weight ((CH_4:BW) : %NDF digestibility), it was found that MSP supplemented group had lowest value but were not significantly different when compared with control and SBF supplemented groups. These results were similar with methane gas production per % NDF digestibility (Table1).

Quantification of the three predominant cellulolytic bacteria by using real time PCR technique are shown in Table I. It was found that CCO supplementation influenced on three species ($P < 0.05$) and resulted in lower ($P < 0.05$) total cellulolytic bacterial population. These results reflected on lower OM and NDF digestibility. However, MSP supplemented group had greatest in cellulolytic bacteria population. Supplementation had resulted in variable population of cellulolytic bacteria ($P < 0.05$), which corresponded with Ngamsaeng et al. [2], who reported that MSP supplementation increased ($P < 0.05$) bacterial population and total count of bacteria and was highest at 150 g/hd/d. Quantification of *Methanogenes* population was found that SBF and CCO supplementation could decrease the population, particularly, SBF supplementation had resulted in lowest value ($P < 0.05$). Based on this study, use of phytochemicals showed potential effect on rumen manipulation especially supplementation with 100 g of mangosteen peel powder.

TABLE I. EFFECT OF SUPPLEMENTED TREATMENTS ON RUMEN FERMENTATION, DIGESTIBILITIES AND RUMEN MICROBES

Items	Control	CCO	MSP	SBF	SEM
Feed intake, %BW	1.5[a]	1.6[a]	1.5[a]	1.3[b]	0.046
Feed intake, $BW^{0.75}$	61.8[b]	68.5[a]	64.4[ab]	55.9[c]	1.892
Digestibility, %					
OM	67.2[a]	47.9[b]	67.8[a]	65.8[a]	2.404
NDF	57.4[a]	35.2[b]	59.8[a]	56.9[a]	4.320
Total VFAs (mM)	102.5[b]	96.6[c]	106.1[a]	102.9[ab]	0.816
Acetate (C2)	66.4[a]	66.1[a]	65.2[b]	64.2[c]	0.150
Propionate (C3)	21.6[b]	21.4[b]	23.0[a]	23.5[a]	0.061
Butyrate (C4)	12.0[bc]	12.5[a]	11.8[c]	12.3[ab]	0.094
C2:C3	3.1[a]	3.1[a]	2.8[b]	2.7[b]	0.035
CH_4:BW (L/kg)	0.665[ab]	0.723[a]	0.639[b]	0.631[b]	0.193
(CH_4:BW) : %NDF digestibility	0.014[ab]	0.017[a]	0.011[b]	0.012[ab]	0.181
CH_4: % NDF digestibility	3.8[ab]	5.3[a]	3.4[b]	3.8[ab]	0.364
Real time PCR technique, copies/ ml rumen fluid					
R. albus, x 10^8	15.1[b]	10.2[c]	22.0[a]	3.0[d]	0.606
R. flavefaciens, x 10^8	11.8[b]	6.1[c]	14.1[a]	5.6[c]	0.277
F. succinogenes, x 10^7	64.4[bc]	0.4[c]	172.0[a]	111.0[ab]	23.10
Total cellulolytic bacteria[1], x10^8	11.1[b]	5.4[c]	17.7[a]	6.6[c]	0.780
Methanogenes, x 10^8	23.4[a]	8.3[c]	24.9[a]	1.6[d]	0.830

[a-d]Values in the same row with different superscripts differ ($P < 0.05$).

[1]The values are included with *R. albus*, *R. flavefaciens* and *F. succinogenes*.

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PRODUCING CONSUMER ACCEPTABLE WOOL - A CHALLENGE FOR AUSTRALIAN SHEEP FARMERS FACING ANIMAL WELFARE BOYCOTTS

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Livestock farming is a complex and demanding business and now is further complicated by consumers expecting greater care for the welfare of livestock and the environment. Meeting the expectations of customers while at the same time trying to make a living on a family farm is becoming far more difficult. Gone is the time when farmers, wherever they are, could rely on the image of idyllic rural scenes as a way of fulfilling the 'promise' of clean, green and ethically produced product. Australia, for many, still conjures up the image of a wide brown land with stockman droving contented sheep to shady riverbanks. However, this is changing as consumers become aware of current sheep management practices such as mulesing, which is portrayed as a barbaric act by animal welfare lobby groups.

Since the early 1850's Australia has produced high quality apparel wool from Merino sheep, but early in the 20th century problems with sheep dying from 'flystrike' (infestations of maggots from the Australian sheep blowfly *Lucilia cuprina*), reached epidemic proportions, which threatened the industry with collapse. A history of selecting sheep with heavy fleeces and many skin wrinkles to produce more wool made them easy targets for the blowfly. This is because with extra wool around the tail area, then soiled with faecal material, the chances of infestation increase substantially.

Sheep in Australia are run in big flocks on large properties (500 – 100,000ha) at low stocking rates (1-10 ewes/ha) and are usually managed by a single family. This means that it is very difficult to monitor sheep individually, on a daily basis, and also means that any control technique requires minimal intervention, and cannot be recurrent.

To deal with these problems, research and development organisations began investigating methods of control and by the early 1950s, a surgical procedure called 'mulesing' was developed. Mulesing involves stripping the skin away from the area around the anus and tail at three weeks of age. The area that grows back is bare of wool and therefore reduces the amount of wool and conditions favourable to fly strike. This method showed much promise in reducing mortality in sheep flocks.

Many farmers saw mulesing as a bloody and unpalatable technique, and so it took extensive information programs run over 20 years to finally convince farmers it was worth doing to improve the welfare of their animals by reducing deaths from flystrike.

In the late 1990s, extensive campaigning by People for the Ethical Treatment of Animals (PETA) led to a growing awareness of this practice by retailers in Europe and the USA. Believing it to be an invasive and painful practice, particularly without pain relief, some companies threaten to boycott Australian wool. In reaction to the growing pressure the Australian wool industry in 2004 agreed to phase out the practice of mulesing by 2010. The wool industry is now intensively seeking a viable and practical alternative to mulesing. Research into various technologies including intradermal injections and modification of the blowfly genome is continuing, and it is likely that a combination of technology and breeding will provide the ultimate solutions. In the short term, control will mean increased applications of chemicals and greater intervention and monitoring. This however, leaves the farmer with more complex decision-making, high labour costs and greater exposure to chemicals and residues.

Understandably, some farmers are angry and upset by the reaction of the international retailers, as they believe they are doing the best thing to protect their sheep from a slow and painful death. This

situation has lead to small numbers of defiant farmers stating they will continue to mules until a viable alternative is available. This is not the first time Australian farmers have faced challenging times. Australian famers have an enviable reputation as innovators, and a capacity to adapt, with many recognising the importance of meeting consumer demands by ceasing mulesing. In 2009, at least 35% of Merino lambs born will be unmulesed.

Even with this dramatic change in practice well before the agreed deadline, retailers such as Hugo Boss, Pierre Cardin and H&M have publicly stated that they will no longer source any Australian wool. This response indicates that even when the product is of high quality, markets will still be affected by the perception of the product in terms of how it is produced. Australia's experience shows that developed, or developing, countries intending to market animal products are not immune from global consumer perception. For this reason, livestock producers must consider issues such as animal welfare, and its impact on their potential customers, as well as biophysical limitations to production.

FIG. 1. Typical Australian sheep farm

References:

[1] www.wool.com.au/2010
[2] www.agric.wa.gov.au/mulesing
[3] www.abc.net.au/news/stories/2008/11/19/2424496.htm

CONTRIBUTION OF LIVESTOCK RESTOCKING TO THE RESETTLEMENT OF DISPLACED RURAL FARMING COMMUNITIES IN POST-CONFLICT SIERRA LEONE

S. Kanu

Sierra Leone

THE EFFECT OF MANAGEMENT PRACTICES ON PREVALENCE OF MASTITIS IN LARGE SCALE DAIRY FARMS

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The study was conducted to investigate the impact of management practises on udder health status of dairy cows in Thuringia-Germany. 48 dairy farms were randomly selected, 64542 milk samples from 10741 dairy cows were collected and subjected to bacteriological investigation. The prevalence of the infection was 27.57% of the quarters, and 49.66% of the composite milk samples were positive. *Staphylococcus aureus* and Coagulase Negative Staphylococci (CNS) were the most frequently isolated contagious pathogens with an udder and quarter prevalence of 28.70/35.50% and 26.60/32.70% respectively. Whereas, *Streptococcus daysgalactia* and Esculin Positive Streptococci (Environmental pathogens), showed a prevalence in the udder and quarter samples of 12.90/13.90% and 9.0/10.60% respectively. Incidence rate was found to be 32.75% in small herds and 31% in large ones. Housing and milking systems, feeding and udder cleaning methods were significantly influenced the mean incidence rate of mastitis. Ignorance of inter-milking sanitization resulted in a higher incidence rate (33.50%), which was lowered by practicing sanitization (31.50%). Application of teat dipping reduces incidence rate of mastitis to 32.25%, whereas, the non-use resulted in an incidence rate of 33%.

A HIGH-RESOLUTION OF PORCINE RADIATION HYBRID PANEL HELPS TO DEVELOP USEFUL SNP MARKERS WITH BIOINFORMATICS AND RE-SEQUENCING METHODS

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A Pig-Human comparative map established by a Pig RH map was used to localize single nucleotide polymorphisms (SNP) in the pig genome. SNP are abundant form of genetic variation, which are useful for gene mapping, association and evolution analyses. NCBI pig SNP database has deposited over 5,450 SNP and the 5,450 sequences were consolidated into 465 unique sequences (189 singleton sequences and 276 contigs). These 465 sequences contained 1787 putative SNP and had strong sequence homology to 433 human protein coding genes based on BLAST analyses. These genes were assigned to the pig QTL maps (http://www.animalgenome.org/QTLdb/pig.html) via the human and pig comparative maps established by a pig radiation hybrid (RH) map. In order to determine identity and allelic distribution among commercial pig breeds, in total, 428 pairs of primers including 1,338 putative SNP on SSC1-18 were re-sequenced in Berkshire, Landrace, Duroc, Yorkshire and Korean native pig (KNP) breeds, and 908 SNP were identified in at least one pig breed. Of the 908 SNP, 228 novel SNP (25.1%) were found. According to the SNP location in the predicted gene sequences, these SNP were categorized to intronic SNP (86.34%), synonymous SNP (sSNP, 10.35%), and non-synonimous SNP (nsSNP, 3.41%). The nucleotide substitution frequency of the SNP revealed that 70.26% were transition (33.5% A/G, 36.8% C/T), 24.01% were transversion, and 5.51% were deletion/insertion mutation. The SNP information from comparative RH map between pig and human will be useful for association studies using high-throughput genotyping and help to differentiate the commercial pig breeds on DNA level.

THE EFFECT OF FEEDING ENSILAGES OF POULTRY LITTER WITH LEFT OVER BREAD ON THE BODY WEIGHT OF BARKA CATTLE

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Poultry litters from replacement birds, layers and broilers were collected; sun dried and stored for silage making. The poultry litters were a mixture of bird excreta, wasted feed, feathers and bedding materials. The litters were rid of other foreign material when they were sun dried. Samples of the collected litters were analyzed for the content of crude protein (CP), crude fibber (CF), ash and fat. The CP content (% DM) for the replacement, layer and broiler litter was 21.15, 18.59 and 18.03, respectively while the ash content (% DM) was 14.54, 38.56 and 16.79 for the respective litters. Bread left over was collected from cafeterias, restaurants, educational institutions, military camps and other places. The litters were ensiled with bread left over in a ratio of 45.5:54.5 on dry mater basis. Water (80 liters/100kg mixture) was added to raise the moisture content to about 50%. The mixtures of poultry litter and bread left over were firmly packed and pressed into the plastic containers. A plastic sheet was inserted between the lids of the containers and the ensiled material to ensure airtight sealing. The silage was prepared daily and each container was opened after a period of at least 21 d.

The process of ensiling resulted in a product that had a higher CP content. The CP content (% DM) for the initial mixtures of bread left over with the replacement, layer and broiler litter was 16.44, 15.27 and 15.02, respectively. This was elevated to 21.36, 19.47 and 17.94, respectively, after ensiling. The higher content of CP after fermentation has also been reported by Rasool *et al*. (1997). The ensilages were of wholesome appearance, palatable and safe and were used in a feeding trial on Barka cattle.

A trial was conducted to examine the effect of ensiled different kinds of poultry litters with bread left over on the body weight of cattle. Sixteen Barka cattle having the same weight and age were divided into groups of four cattle in each treatment and a 90 d trial was conducted. The four groups were randomly allocated to either the control group or the silage group. The control group (T1) received a commercial type ration consisting of 30% wheat bran, 36.3% bread left over, 2.4% fish meal, 30.3% taff straw and 1% salt on dry matter basis. The other 3 silage groups (T2, T3 and T4) received 36.3% bread left over, 2.4% fish meal, 30.3% taff straw, 1% mineral and 30% replacement, layer or broiler litter, respectively ensiled with bread left over (36.3%). The feeding system was restricted and all the groups consumed all the feed that was offered to them (7.44 kg of DM per cattle per day). Average body weight gains (ABG) for T_1, T_2, T_3 and T_4 were 1.093, 1.019, 0.673 and 0.966 kg/day, respectively. ABG for T_1, T_2 and T_4 were not significantly different from one another ($P > 0.05$), whereas cattle fed on T_3 were significantly different ($P < 0.05$) from ABG of T_1, T_2 and T_4 (Table I). The body weight gains obtained in this study are similar to results obtained by Odhuba [2] fed steers ensiled layer litter with sorghum forage.

TABLE I: EFFECT OF FEEDING THE DIFFERENT TREATMENTS ON THE BODY WEIGHT GAIN OF BARKA CATTLE.

Parameter	T_1	T_2	T_3	T_4	LSD
Number of animals	4	4*	4	4	
Live weight (Kg) - Initial	293.9	293.4	291.7	290.9	
Live weight (Kg) - Final	392.3	390.7	352.3	377.8	
Feeding period (d)	90	90	90	90	
Average daily gain (ADG) (kg/day)	1.093^a	1.019^a	0.673^b	0.966^a	0.2264

T_1 = Wheat bran + bread left over + fishmeal + taff straw (Control diets).
T_2 = Ensilage of bread left over with replacement litter + fish meal + taff straw.
T_3 = Ensilage of bread left over with layer litter + fish meal + taff straw.
T_4 = Ensilage of bread left over with broiler litter + fish meal + taff straw.
Number in a row with different superscripts differ significantly ($P < 0.05$).
LSD = Least significant difference
* One animal was lost from the group and was not replaced.

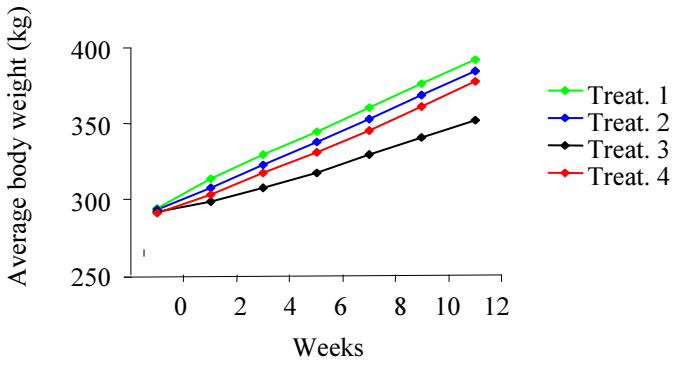

FIG 1. Weekly average body weight of cattle fed a conventional feedlot type control diet or ensilages of poultry litters with bread left over.

Wheat bran can be completely replaced by replacement and broiler litters in rations for Barka cattle. The ensiling process is an effective, simple and low cost technique. Feeds containing poultry silage can improve the cost of feed for farmers engaged in fattening of cattle.

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ASSESSING INORGANIC CONTAMINANTS IN ALTERNATIVE PHOSPHORUS SOURCES USED IN ANIMAL NUTRITION – A PARTICULAR FEATURE FOR THE AGRICULTURAL POLICIES

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Since feed and fodder are the major limiting factors in enhancing animal husbandry productivity, improvements in feeding and nutrition should aid in making animal production more profitable. Phosphorus is one of the most important elements in man and animal nutrition, especially in tropical conditions. There are many phosphorus-containing products to satisfy any P recommendation in animal diets. It is mandatory to predict the presence of any hazardous element before indicate phosphate as supplemental phosphorus in animal nutrition, as long their hazardous contents are quite variable and these elements may cause several problems in animal and man health and nutrition. The first goal of this study was to assess inorganic and radiological aspects of eight different phosphorus sources: calcinated bone meal (FAR), dicalcium phosphate (BIC), super triple phosphate (FST), super simple phosphate (FSS), monoammonium phosphate (FMA), sulphur ammonium phosphate (FSA), ammoniated calcium polyphosphate (POLI) and a bovine mineral supplement (SMB).

The multielemental analysis of P sources and muscle tissues were carried out using the nuclear technique named Neutron Activation Analysis. Irradiations took place at the IPR-R1 Triga Reactor from the CDTN/CNEN, Belo Horizonte, Brazil. Some toxic elements (Al, As, Ba, Cd, Mg, Mn, Th and U) were identified in some products, especially in the sulphur ammonium phosphate. Natural radiation from the following radionuclides ^{226}Ra, ^{228}Ra, and ^{40}K present in the products were assessed by the Gamma Spectrometry technique using a hyper pure germanium detector (HPGe). The results are examined in the light of standards for exposure adopted in some countries including from Brazil. Some products present radioactivity in high levels, especially super simple phosphate. The second aim of this project was to evaluate the zootecnic responses of using these products in feeding growing rabbits. To accomplish this goal, it was undertaken an experiment using White New Zealand rabbits. Young rabbits (48 males and 48 females) were taken in randomized blocks with 12 repetitions. Treatments were consisted on 98% of a basic diet plus 2% of each P source. Rabbits were fed from 30 to 72 d.

Nutritional requirements include: Calorie requirements for growth: 2500 kcals/kg food, a good quality protein ration, containing a number of essential amino acids including arginine (0.6%), methionine and cystine (0.6%) and lysine (0.65%). Rabbits require a dietary intake of essential fatty acids for growth, hair coat condition and normal reproductive performance, and fats (or oils). Fibre is an important component of a ration for a rabbit and it is recommended that 12%-22.5% of a food should be crude fibre. Only about 18% of this fibre is digestible in rabbits, which is half that of other domestic herbivores, but it is important for maintaining healthy bacteria in the intestinal tract. The precise requirements for some of the vitamins are not well known. 580 IU Vitamin A is adequate for growth in rabbits. Rabbits require about 1mg Vitamin E/kg body weight per day. Each one of the eight different feeds has the same 98 percent (dry basis) of fibber, energy, amino acids. The formulation is based in raw materials as following (in percent, dry basis): Alfalfa (lucerne meal) 34.63, Soybean oil 01.00, Whole sugar cane powder 02.00, P-source 02.00, Salt 00.50, Lysine 00.25, Methionine 00.04, Calcium 01.00, Mineral mixture 00.40, Maize 06.05, Wheat straw 25.00, Soybean meal 12.13, Maize's by products 15.00. All products (in powder formula and cereal products after grinding) are mixed to homogenize the different material included in the feed. The final pellet shape was of 3.00 mm to permit a good balance between pellet quality and good intestinal motility. Animals receiving fluoride high level contents (sulphide phosphate of ammonium and bovine mineral supplement) presented worst results of weight gain - BIC: 1449.5g; FAR:

1446.6g; FST: 1409.6g; POLI: 1370.0g; FMA: 1366.7g; FSS: 1320.5g; SMB: 1016.9g; FSA: 1009.1g - food consumption and conversion, and also the lighter liver weights amongst all animals from the experiment - FSS: 75.2g; BIC: 70.1g; POLI: 69.9 g; FAR: 69.9g; FMA: 64.1g; FST: 60.3g; FSA: 47.0g; SMB: 46.9g what confirms the high toxicity that fluoride presents to this organ. Furthermore, no one of those potentially toxic elements present in phosphates and feeds - i.e. aluminium, arsenic, barium, cadmium, cooper, thorium, uranium, vanadium, zinc - was not identified above normal elemental level in the rabbit muscle from any animal tested.

In conclusion, it is mandatory to assess the fluorine content in rock and industrial alternative phosphorus to produce a farm animal feed, since fluorine is the most notorious - limiting factor in order to enhance animal meat productivity.

DEPLETION OF SELENIUM IN WHOLE BLOOD, LIVER AND MUSCLE TISSUE OF BEEF HEIFERS THAT HAD PREVIOUSLY GRAZED FORAGES CONTAINING HIGH AMOUNTS OF SELENIUM

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Environmental regulations in California have become more restrictive to prevent contamination of waterways by agricultural runoff, particularly nitrogen and minerals such as selenium (Se). Understanding Se dynamics in cattle can be useful in determining future environmental policies to address movement of trace minerals in the environment. Our objective was to investigate the dynamics of Se mobilization in heifers, previously loaded with Se by grazing high Se containing pastures, from whole blood, liver and muscle tissue.

Angus heifers (n = 20) that had previously grazed either 'Jose' tall wheatgrass (TWG) (*Thinopyrum ponticum* var. 'Jose') or creeping wild rye (CWR) (*Leymus triticoides* var. 'Rio') were used to evaluate Se mobilization from whole blood, liver and muscle tissue. The CWR and TWG were grown in saline soils irrigated with saline drainage water, and were therefore naturally rich in trace minerals, particularly Se (i.e., > 2 mg/kg). Heifers were moved to a feedlot facility after the end of the grazing season in October 2008 and fed diets that contained low levels of Se (i.e., < 0.25 mg/kg). Heifers were first fed alfalfa hay *ad libitum* for 20 d and then moved to a total mixed ration that contained 45% alfalfa hay, 4% wheat straw, 36% rolled corn grain, 13% almond hulls and 2% mineral mixture.

Blood was sampled at the end of grazing (d 1), and at d 23, 41 and 81 after grazing. Samples of liver tissues were collected at the end of grazing and at d 23 and 81 after grazing, whereas muscle tissue was obtained at the end of grazing and at d 81 after grazing. The concentrations of Se in blood, liver and muscle were determined by ICP-AES. Data were analysed as repeated measures with the experimental unit (heifers) nested within subplots using the MIXED procedure in SAS software.

At the end of grazing, CWR and TWG heifers had similar body weight (BW), but the concentration of Se in blood, liver and muscle were markedly different. Concentrations of Se decreased over time after grazing ($P < 0.01$), but the rates of Se mobilization from blood, liver and muscle differed. Even though the concentration of Se in liver was 25% higher in TWG heifers at the end of grazing, after 20 d of being fed a diet with lower levels of Se, both groups had similar concentrations of Se in liver (i.e., 0.92 vs. 0.85 mg/kg), demonstrating that mobilization of Se that had accumulated in liver occurred quickly. The Se concentration in blood decreased at a much slower rate than in liver, possibly because Se mobilized from liver and muscle continuously entered the blood pool. At 81 d of post-grazing feeding, concentrations of Se were reduced by 77% in liver, 49% in blood, but only 31% in muscle. Overall, muscle tissue had the slowest rate of Se mobilization after grazing high Se pastures, but it is the largest pool of Se in the body. The differences in the muscle Se content for CWR and TWG grazed heifers also demonstrate that the content of Se in forage is critical to maximizing Se levels in muscle. Beef production systems that target marketing of Se enriched beef may have to rely on feeding strategies that allow finishing of cattle in no more than 60 d after Se loading, but ideally in as short a time as possible.

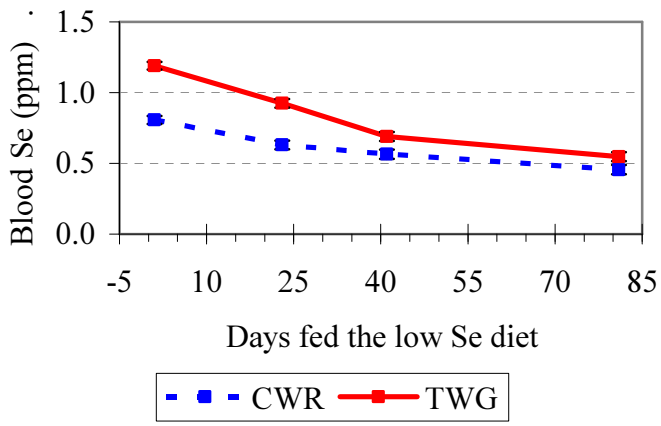

FIG 1. Concentration of Se in whole blood of heifers that previously grazed CWR (dashed) or TWG (solid) [blood concentration of Se was higher in TWG heifers during the first 40 d of feeding a low Se diet (forage by time interaction; P < 0.01)].

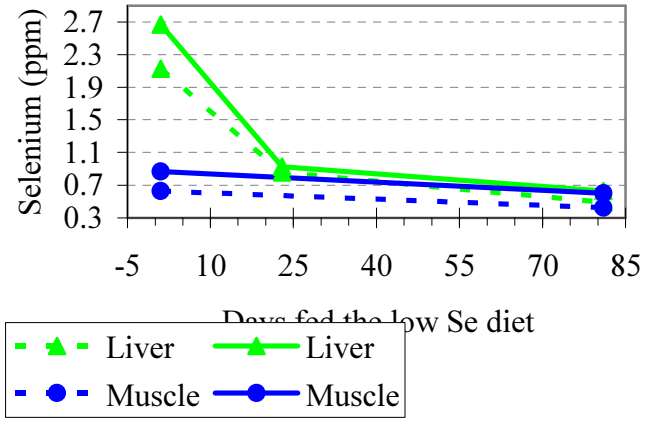

FIG 2. Concentration of selenium in liver (▲) and muscle (●) of heifers that previously grazed CWR (dashed) or TWG (solid) [heifers that grazed TWG had higher concentrations of Se in muscle (P = 0.01), but only a trend for the concentration of Se in liver (P = 0.08)].

PERFORMANCE AND SELENIUM INCORPORATION IN BEEF HEIFERS GRAZING PASTURES GROWING IN SALINE SOILS CONTAINING HIGH LEVELS OF TRACE MINERALS

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Soil salinity increases in parts of the Westside of the San Joaquin Valley of California has led to cultivation of salt tolerant forages in order to evapo-concentrate saline drainage water (DW). Our objective was to determine tissue selenium (Se) accumulation, health and performance of beef heifers grazing DW irrigated forages that contained excessive amounts of Se (>2 mg/kg).

'Jose' tall wheatgrass (TWG) (*Thinopyrum ponticum* var. 'Jose') and creeping wildrye (CWR) (*Leymus triticoides* var. 'Rio') were utilized for grazing during 2007 and 2008. Each grazing area of ~9 ha of TWG or CWR, physically adjacent to each other, was divided into four paddocks, with each subdivided into 2 sub-paddocks that were rotationally grazed at 14 d intervals. Different groups of twenty Angus heifers were allocated to two grazing areas (i.e., 1.1 heifers/ha) in each grazing season (i.e., 2007 and 2008). Heifers grazed the experimental pastures for 190 d during 2007, from May to November, whereas during 2008 the grazing period was slightly shorter, 165 d, from May to October. Blood and liver were sampled before grazing and at 190 d of grazing. At ~45 d intervals during grazing, additional samples of blood were collected and body weight (BW) was recorded before grazing and at 135, 150 and 190 d of grazing in 2007. A similar sampling scheme was repeated in 2008, but with additional samples of liver and muscle collected at 91 d of grazing, whereas blood was sampled at 20 and 70 d of grazing. Concentrations of trace minerals in whole blood or serum, and in liver tissue were determined in 2007, whereas during the 2008 grazing season, blood or serum, liver and muscle were collected to evaluate the change in Se concentration over the grazing period. BW was measured every 45 d in 2008. Data sampled over time were analysed as repeated measures with the experimental unit (heifers) nested within subplots utilizing the MIXED procedure in the SAS software package.

Chemical composition of forages varied between years, with Se concentration similar for CWR and TWG (i.e., 4.5 vs. 3.9 mg/kg DM) in 2007 but, during 2008, the concentration of Se in the forage was considerably higher for TWG (i.e., 4.0 mg/kg DM) than for CWR (i.e., 2.4 mg/kg DM). The CP levels were higher in 2007 (11.0 vs. 12.2% DM) than in 2008 (9.3 vs. 9.5% DM) for CWR and TWG, respectively, but ash free NDF values were consistently higher for CWR than TWG in 2007 (69.4 vs. 64.0% DM) and 2008 (68.0 vs. 63.3% DM). Mean BW before grazing was similar in CWR and TWG groups in 2007 (190 kg) and 2008 (268 kg). Heifers grazing TWG gained more BW (0.59 vs. 0.27 kg/d; $P < 0.01$), and these BW gains were higher than expected. However, during 2008 the BW gains were lower and did not differ in heifers grazing TWG or CWR. Average concentration of Se in blood (0.095 mg/kg), liver (0.23 mg/kg), and muscle (0.06 mg/kg) tissues were similar between CWR and TWG during 2008, as well as the concentration of Se in blood and liver in 2007. Accumulation of Se in blood occurred quickly, and blood concentration was 300% higher by 20 d of grazing in heifers grazing TWG in 2008, and almost 200% for CWR (Figure 1). Grazing TWG resulted in higher concentrations of Se in blood then CWR during the early grazing season during both years. Heifers that grazed TWG in 2007 had similar Se concentration in blood by 190 d of grazing (0.87 vs. 0.94 mg/kg), which differed from 2008 when TWG heifers had higher levels of Se in blood, liver and muscle throughout the 165 d grazing period (Figure 2). Despite higher concentrations of Se in blood during the early grazing season in 2007 for TWG heifers, CWR

heifers had higher concentrations of Se in liver tissue (i.e., 3.9 vs. 2.1 mg/kg) at 190 d of grazing, which suggests that heifers grazing TWG started to mobilize Se from liver during the last months of grazing, possibly as a consequence of lower daily intake of Se during the 2007 grazing season. However the findings from two grazing seasons are consistent in demonstrating the safety of TWG and CWR forage irrigated with saline DW in grazing beef cattle production system, in spite of the high concentrations of Se in the forage, thereby creating an economically viable alternative for utilization of saline soils that have limited use to cultivate high value salt sensitive crops.

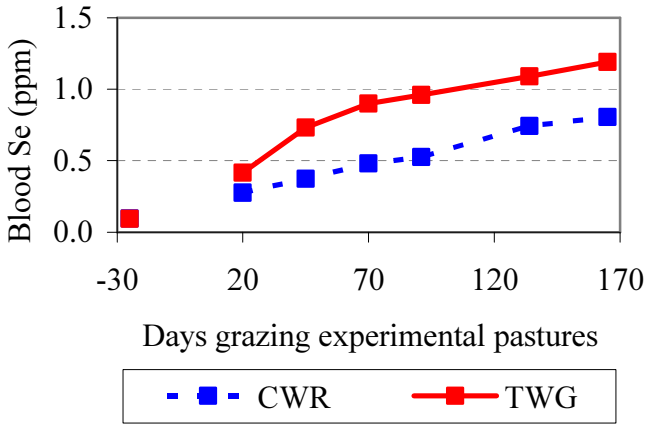

FIG 1. Concentration of Se in whole blood in heifers grazing CWR (dashed) or TWG (solid) pastures in 2008 [heifers that grazed TWG had higher concentrations of Se throughout the grazing period (forage by time interaction; P < 0.01)].

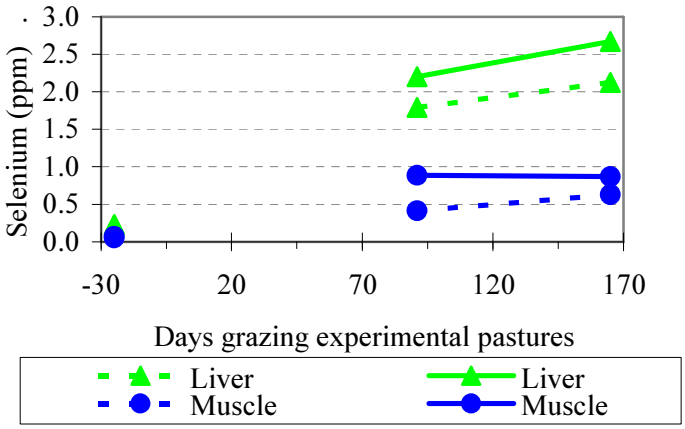

FIG 2. Concentration of selenium in liver (▲) and muscle (●) from heifers grazing CWR (dashed) or TWG (solid) pastures in 2008 [heifers that grazed TWG had higher concentrations of Se in liver (P < 0.05) and muscle (forage by time interaction; P < 0.01) throughout the grazing period].

BIRTH WEIGHT AND WEANING WEIGHT OF BOER KIDS UNDER AN INTENSIVE MANAGEMENT SYSTEM

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In Malaysia, mutton (as chevon is called locally) is one of the main red meat consumed. The total mutton demand in 2007 was 20,000 mT [1]. However, its output for the year was only 1750 mT, a mere 8.75% self-sufficiency. The demand is met by import of mutton and live animals, especially from Australia. However, with stimulus provided under the National Agricultural Policy and the recent introduction of the Boer goats with excellent body conformation, growth rate and carcass quality [2], the goat industry is rapidly developing. Many medium to large Boer goat farms are being established with purebred Boer goats and crosses imported from Australia and South African as breeding animals. Many of the farmers are new to the farming industry and have acquired knowledge and skills through short courses, publications, government extension workers, local and foreign stock suppliers, or hired consultants. To ensure the economic viability of the industry, good management systems, quality and cost-effective feed resources, and highly productive animals are essential.

However, there is little information on the growth and reproductive performance of Boer goats in Malaysia. In addition, the effects of genetic and non-genetic factors influencing the traits of interest have yet to be evaluated. A project was undertaken to evaluate the growth performance of the Boer goats under intensive management system. This reports the preliminary results, focusing on the effects of year of birth, sex of kid and litter type on birth and weaning weights in one of the newly established Boer goat farms.

The data is from 397 purebred Boer kids, offspring of 174 does and 16 bucks imported from Australia, in a medium sized (300 – 1000 heads), commercial farm. The kids were raised with their dams in group pens in raised, wooden sheds. They were fed fresh Napier grass, provided twice daily, supplemented with concentrated feed. The kids were weaned at about 90 d of age. The GLM procedure of SAS 9.1 was used to analyse the data.

Except for litter x parity effect, which was significant ($P < 0.05$) for birth weight, the interaction effects were not significant for both the traits. Year and litter effects were significant ($P < 0.01$) for both traits, but effect of sex was significant ($P < 0.05$) only for birth weight. The least square means for birth and weaning weights of the Boer kids are displayed in Table I. Kids born in 2008 had significantly ($P < 0.01$) higher birth and weaning weights than those born in 2007, probably due to more experienced management. Males were 7.5% heavier than the females at birth but showed no significant difference for weaning weight. Similarly, single born kids were heavier than twin born kids only at birth (by 15%). Surprisingly, first parity, multiple birth kids recorded higher birth weight than the twins of the same parity. This may be due to supplemented foster feeding of kids born as triplets and quadruplets.

The effects of the non genetic factors considered in this study were generally as expected and reported by many researchers. However, the weight achieved by the Boer kids at weaning was below what was expected. One possible reason could be that the optimal nutrition requirement of the Boer kids to display their growth potential was not met. This requires further investigation.

TABLE I. LEAST SQUARE MEANS FOR BIRTH AND WEANING WEIGHTS OF BOER KIDS

	Birth weight (kg)	Weaning weight (kg)
Year of birth		
2007	2.83 ± 0.09[a] (191)	11.82 ± 0.78[a] (101)
2008	3.25 ± 0.07[b] (201)	15.75 ± 0.89[b] (19)
Little type		
Single	3.37 ± 0.08[a] (138)	14.91 ± 1.01[a] (50)
Twin	2.93 ± 0.06[b] (212)	12.65 ± 0.71[a] (70)
Multiple births	2.83 ± 0.14[b] (42)	-
Parity		
First	3.10 ± 0.06[a] (285)	13.58 ± 0.56[a] (110)
Second	2.98 ± 0.11[a] (107)	13.99 ± 1.21[a] (10)
Sex		
Male	3.15 ± 0.09[a] (186)	13.96 ± 1.02[a] (54)
Female	2.93 ± 0.07[b] (206)	13.61± 0.72[a] (66)
Parity x Litter type		
First parity, single	3.28 ± 0.08[ab] (105)	14.80 ± 0.62[a] (46)
First parity, twin	2.83 ± 0.07[cd] (150)	12.35 ± 0.68[a] (64)
First parity, multiple birth	3.18 ± 0.16[a] (30)	-
Second parity, single	3.45 ± 0.15[a] (33)	15.03 ± 1.92[a] (4)
Second parity, twin	3.02 ± 0.11[bce] (62)	12.95 ± 1.47[a] (6)
Second parity, multiple birth	2.48 ± 0.24[d] (12)	-

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SEASONAL VARIATION IN PROTEIN PROFILES AND HSP70 OF HOLSTEIN CROSSBRED BULL SEMEN

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Since HSP70 is the stress response protein, the impact of heat stress on semen quality may be displayed through the expression of protein profile and HSP70. This study investigated the seasonal effects on the protein profiles and HSP70 in spermatozoa and seminal plasma of 10 Holstein crossbred bulls from an AI centre located in Lopburi, Thailand. Bull semen was collected weekly for 8 consecutive weeks during rainy (average THI 79.34), cool (average THI 75.27), and summer (average THI 80.10) seasons. Protein was extracted from both spermatozoa and seminal plasma using Laemmli's sample buffer [2].

The protein profiles of spermatozoa and seminal plasma were subjected to one-dimensional SDS-PAGE with 12% (w/v) acrylamide gel and 4.0% (w/v) acrylamide stacking gel for 120 min. at 8 mA. To visualize the protein profiles, gels were fixed in acetic acid: ethanol: H_2O (7: 40: 53), stained with 0.125% (w/v) Coomassie blue R-250 in acetic acid: ethanol: H_2O (7: 40: 53) for 60 min., and distained with acetic acid: ethanol: H_2O (11: 26: 63) until the background was clear.

Western blotting, as described by Kamaruddin et al. [1], was conducted to determine HSP70 using anti-HSP70 monoclonal antibody. Proteins in the polyacrylamide gel were electrophoretically transferred, for 90 min. at 156 mA, to a PVDF membrane. The membrane was rinsed in PBS and blocked overnight in a blocking solution (advanced ECL blocking; Amersham Life Science Inc., Oakville, ON, Canada). The membrane was then incubated for 1 h at room temperature with monoclonal anti-HSP70 (H5147 Sigma Chemical Supplies CO., LTD), incubated with anti-mouse IgG horse radish peroxidase conjugated for 1 h at room temperature, and then detection for immunoreactive bands using ECL detection reagents (Amersham Life Science Inc.) on scientific imaging film.

It was found that the profiles of protein were not different among seasons in both sperm and seminal plasma. The profiles of spermatozoa protein range from 10 to 220 kDa (Figure 1) while most of proteins found in seminal plasma (Figure 2) were low molecular weight (14-30 kDa). The HSP70 was found in both sperm (Figure 3) and seminal plasma (Figure 4). However, the amount of HSP70 in winter appears to be greater compare to those found in summer and rainy seasons.

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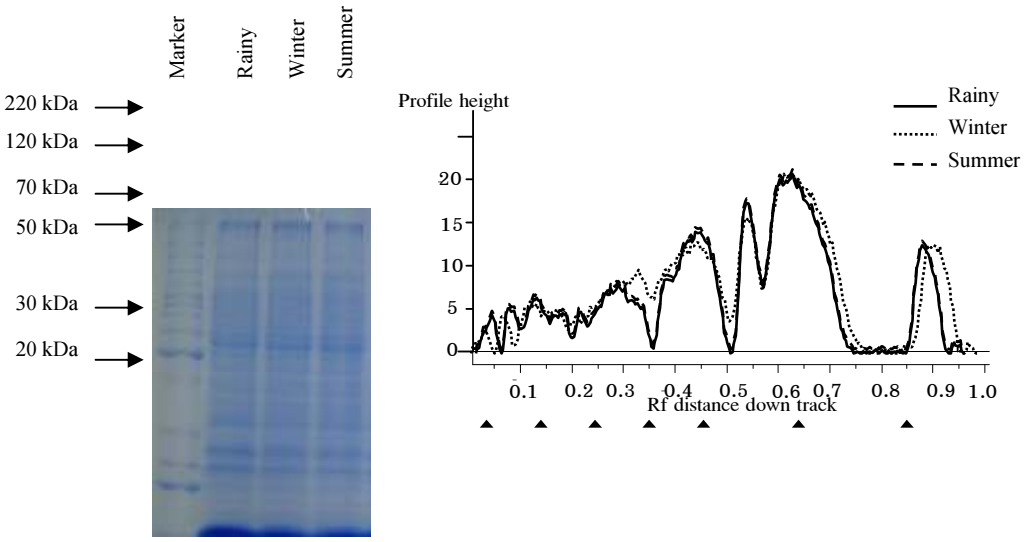

FIG 1. Protein profiles and computer generated lane profiles of sperm

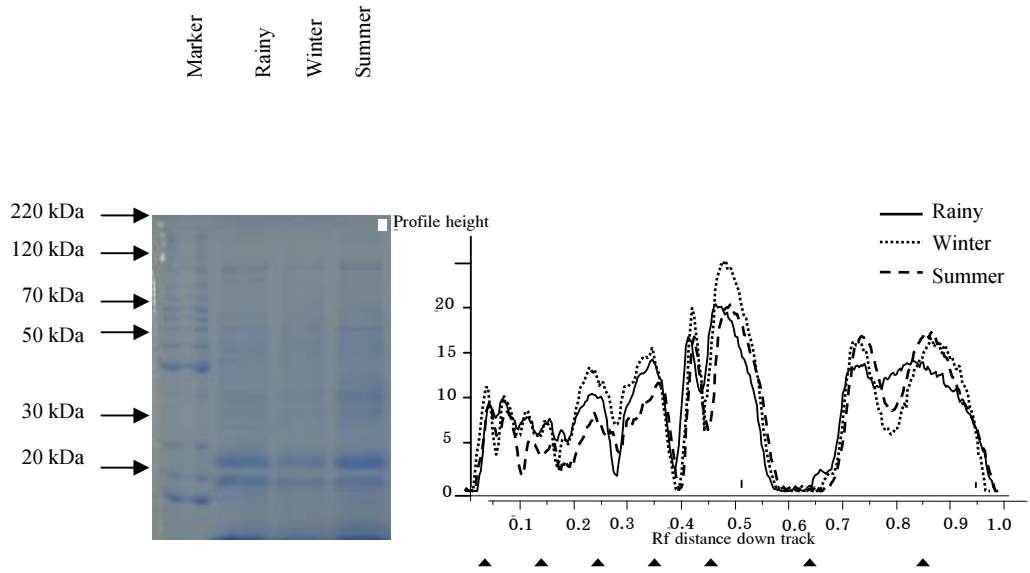

FIG 2. Protein profiles and computer generated lane profiles of seminal plasma

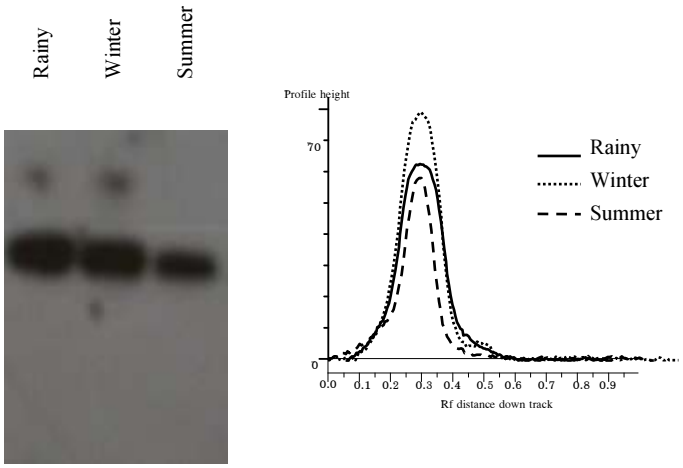

FIG 3. The western blot and computer generated lane profiles of HSP70 in sperm.

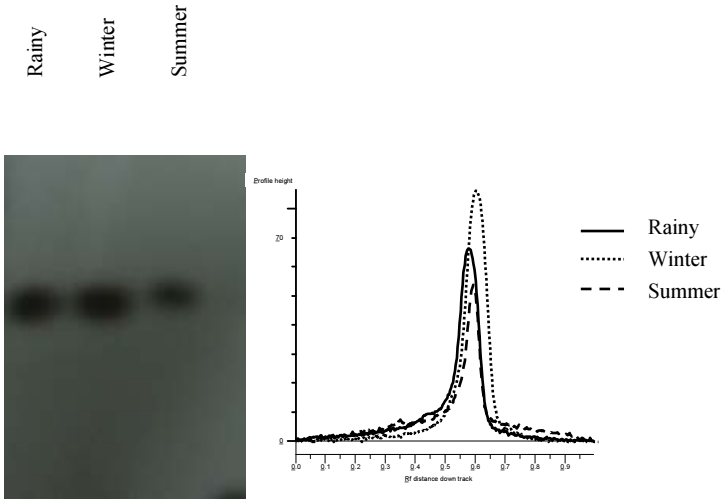

FIG 4. The western blot and computer generated profiles of HSP70 in seminal plasma.

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NUCLEOTIDE SEQUENCE AND POLYMORPHISM IN 5'FLANKING REGION OF HSP*70-2* GENE IN HOLSTEIN FRIESIAN BULLS

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This study determined the nucleotide sequence and polymorphism in 5'flanking region of HSP70-2 gene in semen samples from 10 Holstein Friesian bulls. The Bos Taurus HSP70-2 gene sequence deposited in Gene Bank under the accession number BTU02892 (http:/www.ncbi.nlm.nih.gov.) was used to design PCR primers using software GeneFisher. The forward primer: 5'CTGTTTCCTCCAGCGAA3' and reverse primer: 5' GCTTTTTCGGCTCCGAA 3' were designed for PCR reactions to amplify specific regions of the 5'flanking region of the HSP70-2 gene. The PCR reactions carried out in the 25 µl. of 1X Taq buffer (Promega), 2mM $MgCl_2$, 0.1mM dNTP, 0.5 µM Primer F, 0.5 µM Primer R, 40 ng genomic DNA and 1 unit Taq DNA polymerase (Promega). The amplification conditions were as follows: initial denaturing at 95°C for 5 min, followed by 40 cycles of 1 min denaturing at 95°C, primer annealing at 55°C for 1 min, at 72°C for 1 min, and final elongation at 72°C for 5 min.

The resulting PCR products were purified using Wizard SV gel and PCR clean-Up system (Promega). Purified PCR products were ligated into the pGEM-T Easy vector (Promega), transformed into E. coli JM109 cells and plated on 2 × YT (16 g l–1 tryptone, 10 g l–1 yeast extract, and5gl–1 NaCl) agar plates containing 100 µg/mL ampicillin. Plasmid DNA was isolated from ampicillin-resistant transformants using the alkaline lysis method. The presence of inserts was determined by Eco RI restriction analysis. The nucleotides sequences were determined by automate DNA sequencer (MegaBACE 1000 and ALFexpress sequencers). The diversity and identity of the sequences of the cloned from individual bull were analyzed with software Clustal W. Nucleotide substitutions, deletions and insertions were analyzed as well.

Alignment of the DNA sequence (DNAstar MegAlign) of the 5'flanking region of the HSP70-2 gene of ten HF bulls (Figure 3) showed polymorphism in 3 bulls. Two polymorphic sites were found: one nucleotide deletion (G) on position 61 and nucleotide substitution (A→ G) on position 241.

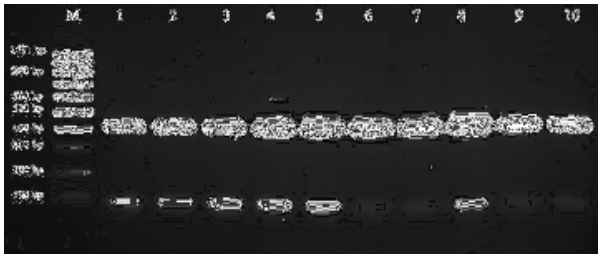

FIG 1. PCR products of the 409 bp of 5'flanking region of the HSP70-2 gene. Lane M, DNA size marker; Lane 1-10 are ten bulls

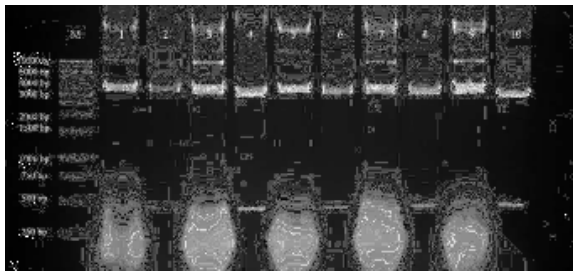

FIG 2. The presence of insert in plasmid DNA (lane 2, 4, 6, 8, 10) confirmed by EcoRI restriction analysis. Lane M: DNA size marker; lane 1, 3, 5, 7, 9: uncut plasmid DNA.

```
     G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  Majority
                       70                  80                  90
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  129.seq
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  179.seq
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  244.SEQ
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  248.SEQ
61   - C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  249.seq
61   - C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  258.seq
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  259.SEQ
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  262.SEQ
61   G C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  274.SEQ
61   - C G C T G A T T G G T T C C A G A A A G C C A G G G G G C  276.seq

     A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  Majority
                      250                 260                 270
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  129.seq
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  179.seq
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  244.SEQ
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  248.SEQ
240  G A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  249.seq
240  G A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  258.seq
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  259.SEQ
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  262.SEQ
241  A A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  274.SEQ
240  G A C T T A C G G C T T A G T C C G T G A G A G C A G C T T  276.seq
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FIG 3. Nucleotides sequences of the 5'flanking region of the HSP70-2 gene of 10 HF bulls. Two polymorphic sites: A nucleotide deletion (G) at position 61 and substitution (A→ G) at position 241 are boxed.

EVALUATION OF SOME NATURAL PASTURES IN THE GEZIRA STATE, SUDAN

M. Elimam

Sudan

NON CONVENTIONAL LIVESTOCK FEEDS OF ARID ZONE OF INDIA: POTENTIAL NEED TO TAP EFFECTIVELY AND EFFICIENTELY

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Arid region is characterized by low & erratic rainfall coupled with high temperature. Consequently, agriculture is a gamble, leaving livestock as a potential option for livelihood of marginal & poor farmers [1]. However, availability of feedstuffs remains major challenge. Increasing demand for potential food grains for human population allows little scope for sparing required quantity of cereals and other food grains for feeding of productive animals.

In such situation, exploration of non-conventional resources and their effective incorporation in regular feeding regime seems promising solution to address deficit of nutrients. Various fodder grasses viz. Sewan (*Lasirus sindicus*), Blue panic (*Panicum antidotale*), Dhaman (*Cenchurus ciliaris*), Karad (*Dichanthium annulatum*) etc. are very rich in native nutrient contents and grows in rainfall ranging from 200-400mm. Various agri-industrial by-products viz. oil cakes such as Tumba (*Citrullus colocynthis*), Taramira (*Eruca sativa*), Matira (*Citrullus lanatus)* etc. are abundantly available in desert region and can be utilized as non-conventional protein replacer for livestock in the region [2]. Various shrubs and trees can serve as a potential source through their pods and leaves. Prominent among them are Babul (Acacia Arabica), Ardu (*Alianthus excelsa*), Siras (*Albizia lebbeck*), Neem (*Azadirachta indica*), Khejri (*Prosopis cineraria*) and Vilayati babool (*Prosopis juliflora*).

Suggested futuristic model:

These are futuristic potential feed resources to solve nutrient crisis, however nutrient utilization from these feed resources poses a serious problem due to presence of incriminating factors. It has been realized that in spite of good macro & micro nutrient contents, those feed resources could not find optimum place in regular livestock feeding. Various factors, importantly, socio-economic and lack of awareness are responsible for that. A strategic model has been hypothesized for organized & corporate farming in desert with bulk processing & detoxification facility. Since most of those plants are having ethno veterinary importance, so there is dire need to explore hidden medicinal attributes in those resources, which can supplement nutritional value of feeds. Combining traditional biomass with recent technology can bring forth a potential option for their effective incorporation and efficient utilization. This new focus on their nutritional values and medicinal & industrial use will prompt & attract future conservation & propagation of these resources. This in turn can solve nutrient crisis for livestock in arid zones of India along with improving socio-economic status of the poor and marginal farmers.

Feedstuff	Feed Type	Nutrient	Important Remarks
Eruca sativa	Oil seed meal	28% CP	Present glucosinolates *Cruciferae* family, Lactagouge
Citrullus colocynthis	Oilseed cake	20% CP	Alkaloids presence. Bitter taste
Citrullus lanatus	Oil cake	20% CP	Wildly grown
Cymopsis tetraganaloba	Protein source	40 % CP	Commercial gum production
Acacia Arabica	Fodder Tree	15 % CP	Useful for pods. DCP 5.14 % in pods.
Prosopis juliflora	Fodder Tree	21.4 % CP	Pods having high sugar contents
Prosopis cineraria	Fodder Tree	14 % CP	Improves soil fertility, Tannins
Albezia lebbec	Tree leaves	16.8% CP	Requires strong light. Goat feed
Aleanthus excelsa	Fodder Tree	19.9%	Fast growing & high palatability
Azadirachta indica	Fodder Tree	14.5%	Not relished by cattle but camel & goat browse well. Anathematic
Zigiphus nummularia	Fodder tree	11.7 %	Wildly grown. Goat relishes.
Lasirus sindicus	Pasture grass	10.19	"King of desert". Good prod. Under climate & soil stress.
Cenchurus ciliaris	Pasture grass	9.18	Dominant, relished by all livestock
Cenchurus setigerus	Pasture grass		Hardier & draught resistant
Dicanthium annulatum	Pasture grass	10.06	Excellent. Carrying capacity 2 sheep/ha
Panicum antidote	Pasture grass	5.25	Excellent sand binder & medicinal antidote.

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MOLECULAR STUDY OF BOVINE GROWTH HORMONE GENE IN NILI-RAVI BUFFALOES OF PAKISTAN

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Bovine growth hormone (*bGH*) is a polypeptide hormone. It has variety of functions in the animal body includes, different biological and metabolic processes such as lactation process, mammary development, carbohydrate, lipid, and protein metabolisms etc. Different mutations within the bovine growth hormone (bGH) gene produce genetic variants. The frequency of these variants is different according to the breeds. Various findings emerging from the study of genetic polymorphism of bGH gene may have some practical implementation in livestock sectors. The present study was conducted to investigate the existence of polymorphism at bGH locus in Nili-Ravi buffaloes of Pakistan through PCR-RFLP methodology.

Upon digestion of the 211bp DNA fragment from exon V using *Alu*I restriction enzyme, a homozygotic pattern was observed. All the amplified products from buffalo animals which indicate the presence of only Leucine-Leucine (LL) genotype thus, all the buffalo animals were found to be homozygous for LL genotype which was according to our hypothesis that all the buffalo animals used for dairy purposes is homozygous for LL genotype (since we have collected blood samples from dairy animals) that is mainly associated with high blood plasma growth hormone concentration. LL genotype has only one restriction site for *Alu*I restriction enzyme thus results in to two fragments.

The homozygosity in buffalo animals could be due to the loss of genetic variability among the studied population. Thus, the PCR-RFLP analysis is easy, cost effective method, which permits the easy characterization of bGH gene. The present study is the first report of Pakistani buffaloes, therefore it has been suggested the all the animals used for the breeding purposes should screened by molecular methods.

MISTAKE OF COMBINED NUTRIMENTS FABRICATION DESTINED FOR POULTRY FEEDING

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Because of some fabrication errors, in the combined nutriments (for the reproduction hens' heavy breeds) an amount of Koccisan (drug for the meat chickens, used for their protection, on growing period, against parasites.) has been introduced. After this fabrication mistake an effective and egg loss estimated at 16 987 EURO has been recorded. The fabrication of combined nutriments necessary for hens feeding intended for meat and egg production was performed in the frame of unit of production " A", unit specialized in combined nutriments production.

The farm of hens heavy breeds reproduction "B" was the only beneficiary of this producer, contracting an annual necessary about 18 000 tons, integral assuring the raw materials of fodder, inclusive the vitamin-minerals premixes and drugs, has establish the manufacture receptions and the amount of fodder input necessary. Unit of production,, A" , has received the manufacture receptions and the raw materials necessary for fabrication, achieving on their on responsibility the fodder quantity, programmed at quality parameters suiting the solicited prescription. On the nutriments fabrication route, the delegates from the farm of reproduction hens heavy breeds "B" haven give the dispositions to the execution personal of the production unit "A" and don't substituted him.

The technologic process and the responsibility of combined nutriments fabrication has been taken only by the production unit "A", which detain the installation and the qualified personal in this domain of activity. Because of some fabrication errors, in the feeds prescriptions intended to the reproduction hens heavy breeds effectives, it has been introduced a quantity of coccidiostatic (Kokcisan), drug intended to the meat chickens, for their protection on the growing period, against parazitoses (coccidiosis).

The coccidiostatics substances administrated to the youth fowl until the age of sixteen weeks, after that they are interdicted totally, the limit of toxic sill being very diminished and having repercussions on fowls live an also in the reproduction activity. In the case of Kokcisan premix (which contain as active substance *salmomicina*) the restriction is totally regarding the reproduction youth and the adult fowls, being recommended only meat chickens. From the Table I analysis can be observed that between the commanded quantity by the farm of reproduction hens heavy breeds "B'' and those delivery by production unit "A" are some differences which dignify certain problems in the technologic flow of fabrication.

TABLE I. THE REALIZATION OF FABRICATION PROGRAM

Prescription	Destination	Quantity (kg)		% realization
		Commanded\	Delivered\	
21-2	Chicken meat (growth phase)	40000	39800	99,5
21-3R	Youth breeding breeds heavy	16000	15500	96,8
21-4	Youth replacement egg consumption	10000	9950	99,5
21-5	Hen egg consumption	20000	19150	97,5
21-7C	Cock heavy breeds	2000	2200	110
21-7F	Heavy breed chickens	30000	31750	105,8
TOTAL		118000	118350	100,3

In Table II we present the order of combined nutriments fabrication realized by production unit "A''

TABEL II. THE ORDER OF COMBINED NUTRIMENTS FABRICATION

Nr. crt.	Prescription	Comamnded quantity (kg) Ferm "B"	Realized quantity (kg) Unit "A"	Bunker Depozitation	Bunker destination
1	Premix Kokcisan	-	1 000	4	Finished
2	Premix hill	-	2 000	9	Finished
3		2.000	2 200	8	Finished
4		10 000	9 950	9	Finished
5		30 000	31 750	7	Finished
6		20 000	19 150	1	Finished
7		16 000	15 500	2	Finished
8		40 000	39 800	5	Finished
TOTAL		118 000	121 350	-	-

After introducing in the poultry nutriments, the fabricated fodder from production unit "B" in the framework farm of reproduction hens' heavy breeds "B" has been take place intoxication with salinomycin to the effectives of hen and coconut (Table III).

TABLE III. THE RESULT OF ANALYSIS ON THE COMBINED NUTRIMENTS SAMPLES EXAMINED

Sample	Laboratory analysis	Results obtained (g) salinomycin / kg feed	Comments
21-7C	Laboratory 1	*0,128*	Salinomycin not be present
	Laboratory 2	*0,040*	
	Laboratory 3	*0,138*	
	Laboratory 4	*0,223*	
21-7F	Laboratory 1	*0,564*	Salinomycin not be present
	Laboratory 2	*0,088*	
	Laboratory 3	*0,561*	
	Laboratory 4	*0,518*	
Premixture Kokcisan	Laboratory 1	-	Level of salinomycin 6g/kg
	Laboratory 2	*0,818*	
	Laboratory 3	*1,200*	
	Laboratory 4	*1,138*	
Premix Kokcisan	Laboratory 1	-	Level of salinomycin 120g/kg
	Laboratory 2	*80,620*	
	Laboratory 3	*120,930*	
	Laboratory 4	*120,300*	

In the combined nutriments components, beside the basic raw materials (cereals, coarse-ground grist, protean flour) that are included fodder additives (methionine, lysine, hill, etc.), vitamin-minerals concentrates, prepared medication, enzymes, bio stimulant. From the category of prepared medicated make part the antibiotics, vitamins, coccidiostatic substances. These products are presented in the concentrated shape or on the support, which can assure the possibility to be integrated in the final product.

In the case of the kokcisan premix, utilized to prevent the meat chicken coccidiosis, the product contain salinomycin as active substance, being utilized by the producer firm already premixed in an granulated form of 12 % concentration, recommended to be technological processed only by the FNC units as an ingredient in the preparation of the combined nutriments. For the augmentation of the secure degree in the homogenization process of the nutriments component, S.C. Combivra S.A. Focşani has effectuated an premixed from this product with the incorporated rate of 1% from the

final combined nutriment. The premixing was effectuated in the case of chloride hill and lysine. Any of the fodder additives can generate serious intoxications. In case that the incorporating module is not respected. Pursuant to the result presented in tab.3 can be observed the incorporating extreme high of salinomycin in the 21-7C şi 21-7F prescription and inconclusive in the kokcisan premix.

As a result of the intoxication with salinomicină in the framework of the reproduction heavy breeds farm, many losses of effectives has been produced, of eggs for incubation, perturbation the activity due to losses of coconut effectives.

CONCLUSIONS

1. The fabrication of the Kokcisan premix on the technological fabrication line of combined nutriments and his storage in the bunker of final products was a very serious mistake.

2. The fabrication of the 21-7C prescriptions for coconut immediately after the premix fabrication resulting their contamination with salinomycin.

3. The quantity of 200 kg combined nutriments 21-7C, which result in plus following the fabrication compared with the effectuated command, demonstrated that she is emanated from the previous premixed.

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UTILIZATION OF ZINC METHIONINE SUPPLEMENTATION IN FRIESIAN COWS: SOMATIC CELL COUNT IN MILK AND MASTITIS

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Two hundreds and forty lactating Friesian cows on the 1st to 8th of lactation and different stages of lactation were used to study some factors affecting on somatic cell count and its effects on milk yield and composition. Also, 12 normal cows, 15 subclinical and 15 clinical mastitis cows were used to study the effect of zinc methionine supplementation on somatic cell count and mastitis. Cows were divided into three similar groups, the first groups was unsupplemented, while the second and third groups were supplemented with 5 and 10 gm zinc methionine / head / day, respectively. Subclinical and clinical mastitis cows were intramammary injected by antibiotic Gentamast (Gentamicin 100 mg) till complete recovery.

The obtained results showed that winter season showed significantly ($P < 0.05$) the highest somatic cell count followed by summer season, while the lowest value was in autumn season. Somatic cell count tended to decrease with the progress of lactation up to the peak period and increased significantly ($P < 0.05$) thereafter and also with the progress number of lactation.

The percentages of normal, subclinical and clinical mastitis cows were 77.71, 15.82 and 6.46%, respectively. Milk yield and composition and its output decreased significantly ($P < 0.05$) with increasing somatic cell count. Zinc methionine supplementation resulted in significant ($P < 0.05$) decrease in somatic cell count in milk. Zinc methionine supplementation for subclinical and clinical mastitis cows led to significant decrease ($P < 0.05$) on somatic cell count, electrical conductivity, recovery time and the cost of therapy compared with unsupplemented group.

It could be concluded that increasing somatic cell count decreased milk yield and composition. Zinc methionine supplementation at the level of 5 g per head daily to lactating Friesian cows reduced somatic cell count in milk, recovery time and therapy cost of mastitis.

USING BACTERIAL INOCULANTS TO CONTROL THE GROWTH OF *E. COLI* O157:H7 IN MAIZE SILAGES UNDER ANAEROBIC AND AEROBIC CONDITIONS

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The aim was to determine if bacterial inoculants could eliminate *E. coli* O157:H7 (ECOL) in contaminated corn silages and if inoculants transferred antibacterial activity to silages. Chopped corn forage was ensiled in triplicate after treatment with:1) distilled water (control); 2) 5 x 10^5 cfu/g of ECOL (EC); 3) EC and 1 x 10^6 cfu/g of *Pediococcus pentosaceus* and *Propionibacterium freudenreichii* (EC+BII); 4) EC and 1 x 10^6 cfu/g of *Lactobacillus buchneri* (LB; EC+LB); 5) EC and 1 x 10^6 cfu/g of LB and *P. pentosaceus* (EC+B500). Silos were opened after 3, 7, 31, and 82 d and analyzed for pH and ECOL counts as well as VFA, lactate, and aerobic stability on d 82. By d 3, all silages had pH was <4 (SE=0.33; p=1) and pH did not increase subsequently; therefore ECOL *w*as not detected in any silage. The Kirby-Bauer disc diffusion test showed that all pure cultures of inoculants had pH-independent antibacterial activity against ECOL but inoculated silages did not, suggesting that ECOL elimination was mediated by pH reduction. Inoculation with LB resulted in less lactate (SE=0.31; P < 0.05), more acetate (SE=0.35; P < 0.05), and greater aerobic stability (SE=7.1; P < 0.05) versus control. Day-82 silages were reinoculated with EC at silo opening (immediate) or after 144 h of exposure (delay) and ECOL were enumerated 24 h later. All immediately reinoculated silages had low pH values (<4) and no ECOL 24 h later. Control, EC, and EC+BII silages reinoculated after the delay had relatively high pH values (4.71, 5.67, and 6.03) (SE=0.74; P < 0.05) and ECOL counts (2.87, 6.73, and 6.87 log cfu/g) (SE=1.4; P < 0.05), whereas those treated with LB had low pH values (<4) and undetectable (EC+B500) or low ECOL counts (1.96, cfu/g; EC+LB). Inoculants did not enhance elimination of ECOL during ensiling, but *L. buchneri inoculants* increased stability and eliminated or inhibited ECOL in aerobically exposed silages.

EFFECT OF DIETARY PROTEIN SOURCES OF ON BLOOD OR MILK UREA NITROGEN OF NATIVE COWS

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When feed protein metabolism in ruminants produces urea in the liver and recycles or blood urea (BUN) filters into milk urea nitrogen (MUN), an indicator of protein status in diets or feeding urea as one of the non-protein nitrogen sources for ruminants is scientifically acceptable throughout the world; a section of environmentalists, policy makers or even professionals often raise question of residual effects in milk and/or meat of fattening and/or dairy cattle fed with diets containing urea. Keeping their views in consideration, a feeding trial on 30 Pabna milking cows of 2 to 4 parities dividing equally into 5 groups was arranged to determine the effect of feeding of different sources of protein on BUN and MUN, and milk yield or protein content.

To achieve the objectives, a group of cows was fed a diet of rice straw and concentrate as the control (T_0), two out of the rests was fed either with urea-molasses straw (UMS) (T_1) or Matikalai (*Vigna mungo*) hay (T_2) as sources of basal roughage. The rest two groups of cows were fed the control diet replacing % of feed protein by the amount of urea and molasses fed to UMS group. The amount of urea and molasses was fed daily either in two meals (T_3) or fed to cows mixing with other concentrate feed (T_4).

In addition, a concentrate mixture containing 45 % wheat bran, 24% Khesari bran, 12% Til oil cake, 12% soybean meal, 4% fishmeal, 2.0% oyster-shell, 0.5% DCP and 0.5% common salt, was supplied twice daily. Having adjusted the cows with the diets for 20 d, a 20 d feeding trial was conducted, when feed intake and samples of blood and milk were collected.

Milk samples were collected from individual cow after feeding the experimental diets in the morning and evening milking. Samples were collected from milk bucket after complete milking and mixing thoroughly. Samples were analyzed for milk urea content (MUN) using a Colorimetric p-dimethylaminobenzaldehyde (DMAB) method as described by Bector et al., [1]. Concentration of MUN in milk was calculated from the standard curve shown in Fig. 1.

Blood samples were collected from jugular vein of a cow after feeding the diets using heparinised tubes. Immediately after collection, samples were placed on ice and refrigerated for 1.0 h. followed by centrifugation. Plasma was removed and serum samples were analyzed for urea content (BSU) using a colorimetric method described by Patton and Crouch [2].

Daily feed intake was measured by deducting the amount of feed remained in the manger in each day from the total feed supplied. The feed samples were collected daily, composited and analyzed for nutrient composition using methods described by AOAC [3].

Data were analyzed by using General Linear Model Procedures of SPSS in the computer to determine treatment effects. Duncan multiple range test was used to test significant differences in treatments.

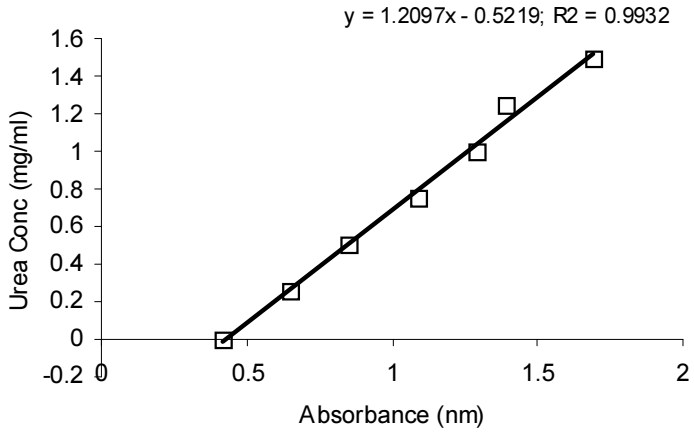

FIG. 1. Standard Curve

TABLE I. MILK UREA NITROGEN (MUN), BLOOD SERUM UREA (BSU) AND NUTRIENT INTAKE BY COWS UNDER DIFFERENT TREATMENT GROUPS

Parameters	T_0 (Control)	T_1 (UMS)	T_2 (Matikalai hay)	T_3 (Meal)	T_4 (Mix.)	Sig.
Blood serum urea (mg/dl)	45.70c	45.10c	37.32ab	35.70a	35.04a	*
MUN in Morning (mg/dl)	65.00d	61.00c	69.00b	55.00a	54.00a	*
MUN in evening (mg/dl)	79.00d	73.00a	72.00a	69.00b	65.00 c	*
Milk fat (%)	4.23d	4.01c	4.72a	3.78b	4.67a	*
Milk protein (%)	3.65	3.62	3.65	3.64	3.69	NS
Milk production (kg/day)	3.5	3.6	3.6	3.1	3.2	NS
DM intake (kg/day/ Cow)	7.24a	7.76b	7.06c	7.12c	7.45a	*
OM intake (kg /day/Cow)	6.33a	6.87b	6.20c	6.22c	6.53a	*
CP intake (g/day/Cow)	490d	770a	760 a	630b	580c	*
ME intake (MJ/day) (Calculated)	62.00a	65.31b	60.74a	61.20a	63.47ab	*

Dl= Deci litre; NS= Non-significant * = Significant ($P < 0.05$)

Feeding a basal diet of UMS, DS or leguminous hay did not affect milk protein (%) and daily milk production (Table I). Feeding urea and molasses in meals or mix (T_3 and T_4) did not affect significantly ($P > 0.05$) BSU and MUN contents. It indicates that feeding urea and molasses in two meals a day either as a single mix of the two or as a mix of the two with concentrates significantly ($P > 0.05$) reduced the concentration of BSU or MUN without having any change in milk protein (%) of the cows. Dry matter (DM) intake was significantly ($P < 0.05$) higher in T_1 treatment group followed by T_4, T_0, T_3 and T_2, respectively. Similarly, CP intake was significantly ($P < 0.05$) higher in T_1 and T_2 treatment groups followed by T_3 and T_4 treatment groups. The values of CP intake were 490, 770, 760, 630 and 580 g/day for treatment groups T0, T_1, T_2, T_3, and T4, respectively. Feedings urea and molasses as meals ($T_{3)}$ significantly ($P < 0.05$) reduced the fat content in milk compared to other treatment groups. Similar to T_3, UMS feeding also significantly ($P < 0.05$) reduced fat content in milk compared to Matikalai hay and T4 treatment groups. Milk urea nitrogen (MUN) content in morning milk was lower compared to evening milk. This similar findings also reported by Broderick and Clayton, (1997), they reported that MUN concentrations was generally lower for samples collected at morning milking. Others explained that the differences of MUN in morning or evening milk might be influenced by the differences in feeding to milking intervals [4].

These data show that feeding urea or protein of organic sources had effect on BSU and MUN contents in the morning milk but had no significant effect on evening milk. The lower BSU or MUN content in milk of the cows fed urea and molasses either in daily meals or as mix with concentrates may be due mainly to a lower CP intake compared to UMS and Matikalai.

The results and discussion evinced no significant effect of feeding urea or organic protein on milk protein per cent (%.). Determination of dietary protein concentration or sources on different components of milk protein is important to identify to determine feeding effect of urea on the quality of milk. Further detail study, may be worth a try to answer these questions.

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GUT MICROBIAL DIVERSITY STUDIES ON ETHIOPIAN BLACKHEAD OGADEN SHEEP WITH PARTICULAR EMPHASIS ON RUMEN METHANOGENIC ARCHAEA AND TOTAL BACTERIA

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This study was conducted in Somali (Jijiga zone) and Oromia (Borena zone) regional state of Ethiopia, which was designed to determine the rumen microbial composition of black head Ogaden sheep in comparison with highland sheep. The areas experience shorter rainy seasons (October/November and March/April) and the prolonged dry season. During the rainy season animals have access to fresh grass and water but during the dry periods animals hardly get grass pasture as the severe weather dries out the grass and what remains in the grazing field is a highly fibrous and lignified material. During the dry periods animals are usually compelled to survive on the scarcely available thorny trees and shrubs among which Acacias and Cactus are the most prominent.

A total of 44 rumen fluid samples were collected both in dry and rainy seasons and kept at -80^{o}C until processed and finally DNA was extracted. Also rumen samples were collected from five highland sheep (from sheep of the institute) for comparison. The randomly selected four samples (two collected in dry season, one collected in rainy season and one highland sheep samples) were tested with real time PCR against total bacteria, protozoa, anaerobic fungi, F. *succinogenes*, R. *flavefaciens* and methanogens.

The study indicated that the population of methanogenes of black head Ogaden sheep showed that 12.6 to 23.58 fold lower and also R. *flavifaciens* 6.4 to 10.12 fold lower than highland sheep. The population of rumen microbes of Black head Ogaden sheep samples collected during rainy season were generally much lower than highland sheep (61.3 to 95.8% less), while anaerobi fungi of Blackhead Ogaden sheep samples collected during dry season was exceptionally 117 to 311 % higher than highland sheep.

Generally this study indicated that Blackhead Ogaden sheep has low rumen microbial population including methanogens.

TABLE I. REAL TIME PCR ROW DATA FOR THE RELATIVE QUANTIFICATION OF RUMEN MICROBIAL DIVERSITY OF BLACKHEAD OGADEN SHEEP IN COMPARISON WITH HIGHLAND SHEEP

	CT	ΔCT	2^-△△Ct	% difference
23 Highland sheep				
G.bacteria	11.77	0	1.00000	
Methanogen	21.88	10.11	0.00090	
Protozoa	16.3	4.53	0.04328	
Fungi	21.74	9.97	0.00100	
F.succi	18.2	6.43	0.01160	
R.flavifaciens	19.57	7.8	0.00449	
12 Blackhead (rainy season)				
G.bacteria	12.68	0	1.00000	0.0
Methanogen	27.35	14.67	0.00004	-95.8
Protozoa	20.82	8.14	0.00354	-91.8
Fungi	27.03	14.35	0.00005	-95.2
F.succi	20.48	7.8	0.00449	-61.3
R.flavifaciens	24.25	11.57	0.00033	-92.7
5 Blackhead (dry season)				
G.bacteria	12.15	0	1.00000	0.0
Methanogen	25.92	13.77	0.00007	-92.1
Protozoa	17.49	5.34	0.02469	-43.0
Fungi	21	8.85	0.00217	117.3
F.succi	18.53	6.38	0.01201	3.5
R.flavifaciens	23.29	11.14	0.00044	-90.1
27 Blackhead (rainy season, Borena)				
G.bacteria	12.99	0	1.00000	0.0
Methanogen	27.33	14.34	0.00005	-94.7
Protozoa	19.02	6.03	0.01530	-64.6
Fungi	20.92	7.93	0.00410	311.2
F.succi	20.26	7.27	0.00648	-44.1
R.flavifaciens	23.47	10.48	0.00070	-84.4

PHENOTYPIC CHARACTERIZATION OF THE BAROTSE AND TONGA CATTLE OF ZAMBIA

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The Barotse and Tonga are among the known indigenous cattle of Zambia they belong to the Sanga cattle. Barotse cattle are long horned found predominantly in Western Province while Tonga cattle are small framed, medium to short horns with a rudimentary hump found in Southern Province of Zambia.

A total of 271 mature Barotse cattle and 268 mature Tonga cattle were included in the morphological characterization study. The aim of the study was to create an understanding of the physical characteristics of the two types of cattle.

The comparisons of least – square means on the dimensional measurements between the male and female mature Barotse cattle revealed that males are bigger than females. There were very high significant differences ($P < 0.001$) in favour of males for withers height, body length, heart girth, head length, head width and horn circumference.

In Tonga cattle wither height was highly correlated to rump height, body length, heart girth, and barrel size. Body length was highly correlated to heart girth and barrel size. Barrel size was also very highly correlated (0.834) to the heart girth.

The phenotypic characterization of the two cattle groups shows variations in measurements. Preliminary findings on genetic characterization using RAPD markers showed remarkable differences in the two breeds of cattle. A more comprehensive study including the production parameters and genetic variation is ongoing.

SESSION 3: TRANSBOUNDARY, EMERGING AND ZOONOTIC DISEASES

THE IMPORTANCE OF EMERGING AND RE-EMERGING ZOONOTIC DISEASES: RECOGNITION, MONITORING AND CONTROL

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Whilst communicable diseases mainly affect the developing world, new and emerging diseases have re-awakened the developed countries to the importance of these infections. Zoonoses are of main concern. In these diseases, the animal reservoir is of prime importance for its control, and elimination becomes a task always believed to be far from affordable. Therefore, the greatest efforts are nowadays concentrated on human non-zoonotic diseases. Among zoonoses, there are several usually neglected parasitic diseases, which need to be added to the priority list. These are diseases for which it is very difficult to get funds for research, despite being of high human impact globally, regionally or locally. Most of them are globally or regionally/locally emerging or re-emerging at present, including both vector-borne and non-vector borne diseases.

Molecular tools are recently showing that these diseases are more complex than previously believed, explaining the reduced success of control initiatives despite the great efforts carried out. Molecular marker combinations, from high resolution DNA sequencing up to less detailed techniques, are very useful tools for zoonoses. In epidemiology they enable the distinguishing between different strains of the causal agent and their relationships with higher/lower prevalence and intensities in humans and animals, concrete animal species which constitute the reservoirs and infection sources for humans, concrete vector species, climatic factors and environmental characteristics, geographical distribution and spreading capacities. In clinics and pathology, they enable the distinguishing of strain relationships with more or less pathogenicity and immunogenicity. In diagnosis, they are useful for the development of highly sensitive and specific diagnostic techniques to be applied in humans, reservoir animals and vectors. In treatment, they are useful in the characterisation of resistant and susceptible strains, as well as in post treatment assessment. In control and surveillance, they furnish tools for the development of vaccines and the follow up of post treatment re-infections. Avian flu is a very recent, still ongoing excellent example of application of molecular tools.

Examples of zoonoses in which molecular tools have decisively helped in clarifying disease aspects are numerous. In cryptosporidiasis, molecular tools have proved that the causal coccidians include a number of human-infecting species and reservoir hosts markedly higher than initially believed. In hidatidosis, different *Echinococcus granulosus* strains with different host ranges and geographical distributions can at present be differentiated. Trichinellosis was believed to be caused by *Trichinella spiralis*, whereas there are in fact different *Trichinella* species with different cycles and distributions involved.

In Chagas disease, molecular results have shown that the causal agent *Trypanosoma cruzi* is very heterogeneous throughout Latin America, including two main phylogenetic groups I and II each comprising different lineages. This, together with triatomine vectors also showing to be more complex than previously believed, is indicating that known transmission patterns, clinico-pathological pictures, diagnostic kits and traditional control strategies need to be reassessed.

Molecular results are also giving rise to a revolution in fascioliasis. In this disease, the impact is higher because of its worldwide distribution, the present emerging situation everywhere, its great pathogenicity in humans and livestock, it is under developing impact on human communities, and the 17 million people affected, mainly children and females. The large intraspecific variability of liver flukes and their lymnaeid snail vectors is in the background of the capacity of this disease to spread and emerge in very different areas, environments and human behaviours.

The complexity of zoonoses offers, moreover, several problems. Knowledge on local epidemiology and transmission characteristics is still lacking or insufficient in many cases. Multidisciplinary approaches and Tran professional team networks are needed. Efforts will be needed to convince different ministries and health responsible to co-work. Funding agencies shall be convinced about the need for increasing efforts at animal level. Studies on geographical distribution and epidemiology of zoonoses by using modern tools are crucial to ascertain the appropriate control measures. The need for "old-fashioned" disciplines as Medical Malacology and Entomology shall be emphasized, molecular techniques offering an excellent way for the re-launching of research in these fields. Fieldwork shall again be encouraged. Today, one of the greatest problems is that the epidemiological situations are unknown in many areas of the developing world. Moreover, control needs sustainability and sustainability needs specifically trained scientists in endemic countries and areas. Consequently, we need to include training and technology transfer high in the agendas of research projects on zoonotic diseases.

Interestingly, when today performing fieldwork and surveys, the results usually suggest that many of these diseases are emerging or re-emerging. Whether this is related to the higher performance of today diagnostic methods when compared to old ones or not, one conclusion is evident: zoonotic diseases are still there and continue to be as prevalent as always!

EARLY, COMPLEX AND RAPID DIAGNOSTIC TECHNOLOGIES: A VIEW FROM TWO COLLABORATING CENTRES OF THE WORLD ORGANIZATION FOR ANIMAL HEALTH (OIE)

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Transboundary animal diseases (TAD), such as highly pathogenic avian influenza, Newcastle disease, foot-and-mouth-disease, classical swine fever, African swine fever, bluetongue, etc are highly pathogenic infectious maladies that migrate across boundaries between regions or countries, causing very high economic and socioeconomic losses worldwide. These diseases are fought at the international level by international organisations such as the World Organisation for Animal Health (OIE). The new generation molecular diagnostic technologies, such as high-throughput, robust real-time PCR assays, solid- and liquid-phase microarrays (Luminex), padlock probes, proximity ligation, full-genome sequencing and phylogeny, together with other novel methods of direct and/or indirect virus detection offer hitherto unparalleled methodologies in the biotechnology-based diagnosis of infectious diseases, including TAD. These novel technologies are vital for the positive detection and identification of pathogenic agents as well as the effects of the pathogens on the production of antibodies.

The development phase of the novel technologies entails a thorough understanding of accurate diagnosis and discrimination of present and emerging diseases. These diagnostic improvements will allow early warning of potential disease spread and the safeguarding of human and animal health. The development of novel technologies can only be successful if they are transferred, and used, in the field with a sustainable quality assured application to allow for the optimal detection and effective control of diseases. The aim of these new tools is to detect the presence of a pathogen agent before the onset of disease. This presentation is focusing mainly on the experiences of two Collaborating Centres of the World Organisation for Animal Health (OIE) in context to molecular diagnosis and molecular epidemiology of transboundary and endemic animal diseases of viral origin, food safety and zoonoses. By applying these novel technologies to address the listed wide range of problems, there is a hope that the “One World One Health” principle will be followed and the quality of human and animal life will be improved. The novel assays facilitate both the laboratory-based and the “on site” detection of the targeted pathogens and support the improved control of the devastating infectious diseases.

CLIMATIC CHANGES, SEASONALITY AND THE DYNAMICS OF INFECTIOUS DISEASES IN ANIMALS

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In the last few years the potential impact of climate change on infectious and parasitic diseases has drawn an increasing attention although the issue is still quite controversial. Many infectious diseases, especially of wildlife, have a remarkable meteo-climatic footprint. In some cases, outbreaks are clearly synchronized with seasonal fluctuations in temperature, humidity and rainfall patterns. Seasonal changes are ubiquitous in ecology and affect the timing of both outbreak and wildlife demography on a yearly basis but more subtly, can contribute to generate more complex, inter-annual dynamics on a longer time scale. Meteo-climatic fluctuations can affect the infective agent directly by modifying the life expectancy of the free-living stages or, indirectly, through changes in immune response, behaviour, demography (timing of reproduction, mortality, etc.), abundance (birth pulses, resources availability) of the host and vectors. This may result in turn in a change of probability of transmission between susceptible and infected animals or between susceptible hosts and infective stages/propagules with remarkable effects on the epidemiological patterns at the population or community level. Changes in the statistical properties of climate, especially in the combination of temperature and rainfall patterns, can thus ultimately affect the geographical distribution and the dynamics of pathogens and vectors.

In the present work, I briefly illustrate two specific examples of how seasonality in meteo-climatic variables can affect the dynamics of infectious diseases caused by micro and macroparasites. In the first case, I investigate how seasonal fluctuations in demography of the host affect the dynamics of rabies epidemics and show how short-living, fast-reproducing host species may respond to seasonality differently than long-living, slowly reproducing ones. The second example is about the effect of seasonality in the development of hypobiosis (arrested stage of development of parasite larvae in the gut mucosa of the definitive host), a strategy carried out by a number of nematodes species to overcome harsh environmental conditions - such as extremely drought summers or very cold winters - during which survival of free-livings stages (and, thus, the probability of infection) is low or negligible. In both cases, modifications in the seasonal forcing due to anthropogenic climate change have the potential to alter the parasite burden, the prevalence of infective individuals and the abundance of the host. In the temperate areas of Europe, for instance, winter seasons have been progressively milder in the last 30 years and possible effects both on host demography and on the transmission of infectious diseases have begun to be observed. Alteration of rainfalls patterns with dry spells between intense precipitation periods may affects diseases in tropical regions such as Rift Valley fever.

Of course, the ultimate impact of climate change will depend not only on the interaction between a pathogen and its host but also on the fragility of ecosystems and the intensity of the changes and on a country's ability to prepare and adapt to such changes. In fact, the effect of anthropogenic climate change on infectious diseases can be substantially altered by several cultural and socio-economic factors, including sanitation and vaccination programs, development of drug resistance, intensive management of livestock, land use changes, etc. An additional problem is that even basic information on epidemiological parameters and on host demography in the wild is often scanty and anecdotic (probably with the exception of rabies, Lime and few others diseases on human concern). Even when this information is available, our prediction power of future outbreaks under changes of climatic conditions can be disconcertedly poor: in fact, microclimatic conditions usually have tremendous impacts on the survival and persistence of free-living stages, propagules and vectors but fine down-scaling of global climate forecasts is still affected by a dramatic levels of uncertainty. As a consequence, definitive conclusions about the influence of climate on infectious diseases at the local level should be taken cautiously. In many cases, a huge effort is still needed to achieve a better understanding of the epidemiology of infectious diseases and their relationship with climate.

OVERVIEW OF EMPRES

J. Lubroth
FAO

OIE ACTIVITIES FOR THE GLOBAL IMPROVEMENT OF ANIMAL DISEASE DETECTION AND CONTROL

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The OIE, the World Organisation for Animal Health, which was created in 1924 to prevent animal diseases from spreading around the world has, since then, enlarged its mandate to the improvement of animal health worldwide. The OIE is an intergovernmental organisation with a total of 172 Member Countries and Territories.

It is recognised by the sanitary and phytosanitary agreement (SPS agreement) of the World Trade Organization (WTO) as the international reference organisation for international standards, guidelines and recommendations related to global animal health with the main purpose of facilitating international trade in animals and animal products.

The OIE develops and publishes two types of international health standards for animals and animal products – trade standards and biological standards. These standards are developed through the elected Specialist Commissions and are adopted democratically by OIE Members during the annual OIE General Assembly. They are developed for use not only by the veterinary services of Members, but also by the private sector. For strengthening surveillance of disease, public–private sector partnership is essential and should be based on the collaboration between official veterinarians, private veterinarians, farmers and other stakeholders.

The most effective way of detecting, diagnosing, controlling and responding to animal disease and zoonotic incursions, is to ensure good veterinary governance in Member Countries. Integral to good governance is the ability and capacity of all Member Countries to comply with the guidelines, recommendations and international standards of the OIE and to establish efficient chains of command.

The OIE has therefore embarked on a unique strategic initiative to develop an assessment and evaluation system to assist countries to identify weaknesses in their system that makes it difficult for them to comply with the minimum standards, guidelines and recommendations of the OIE. The evaluation system based on the performance, strategy and vision of a country to move towards compliance (commonly referred to as the PVS-strategy) is applied successfully in a number of developing and in-transition countries. The assessment system considers critical aspects of veterinary service delivery such as technical capability, human and financial capital, interaction with the private sector and other needs. The OIE has, by linking this initiative to its overall aim to establish awareness and acceptance of the delivery of veterinary services as an international public good, elicited major financial support for this project from the World Bank and other donors, including some Member Countries of the OIE. Integral to the assessment process is also the identification of the need to establish scientific and technical expertise within these countries to enable them to become self-sufficient to early detect and diagnose diseases.

Scientific and technical expertise for OIE is mainly provided by the OIE Reference Laboratories and Collaborating Centres. They play an essential role in enabling the OIE to carry out its principal functions, but also in supporting Member Countries and Territories in their capacity of disease detection.

As the large majority of OIE Reference Laboratories and Collaborating Centres and expertise is still situated in the most industrialised countries, it is of critical importance to establish an even geographical spread of available expertise within developing and in-transition countries. Taking that into consideration the OIE has developed a twinning programme that could imply a transfer of knowledge, training and expertise from the ‘North’ to the ‘South’ or from an existing OIE Reference

Laboratory or Collaborating Centre of the South to another less advanced laboratory applying for such assistance.

The main objective of twinning is therefore to assist laboratories in developing or in-transition countries to build their capacity and scientific expertise with the eventual aim that some of them could become OIE Reference Laboratories in their own right. To practically apply this concept, a link between an existing OIE Reference Laboratory or Collaborating Centre with another laboratory in a developing or in- transition country must be established for exchange of scientific expertise and capacity building.

Through twinning OIE aims to extend its network of capacity and expertise to provide a more even global geographical distribution, so that more countries will have access to high quality diagnostic testing and expertise. This is essential for effective detection, prevention and control of important animal diseases and zoonoses as well as for giving OIE members more ready access to scientific expertise for applying OIE standards and debating the setting of OIE standards.

ORAL PRESENTATIONS

EPIDEMIOLOGY, PATHOLOGY AND SENSITIVE TOOLS FOR THE DIAGNOSIS OF PPR IN BANGLADESH

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Peste des petits ruminants (PPR) are an acute viral disease of small ruminants caused by *Morbillivirus.* In Bangladesh, outbreaks of PPR have been occurring in goats since 1993 and cause severe losses to small ruminant production and considering top most threats to about 22 million small ruminant population of the country. Although PPR has been prevalent in Bangladesh for more than a decade, the prevalence and epidemiology of the disease is not know and sensitive diagnostic method is not available. Therefore, the present study has been proposed with the following objectives: to study the descriptive epidemiology and sero-prevalence of PPR in Bangladesh, to adopt RT-PCR for quick detection of PPR virus and study the efficacy of locally produced PPR vaccine. Data were collected from the outbreaks in prescribed questionnaire for epidemiological investigation, 600 sera were collected from six different geographical locations of the country, 100 goats were vaccinated, sera were collected pre, 21st day post and 6 month post vaccination, and was analyzed by cELISA. Gross lesions were recorded at necropsy from confirmed cases and formalin fixed tissues were processed for histopathological study. Field samples (soaked with filter paper and extracted RNA) were subjected to RT-PCR for the amplification of the F gene. Mortality was higher in the young goat (<1 year). The overall morbidity and mortality was 51.35% and 13.51%, respectively. Weather changes like sudden high temperature, movement of animals and introduction of newly purchased animals from the market played an important rule in transmission and maintenance of the virus. Congested and consolidated pneumonic lungs, enlarged edematous lymph nodes, brush paint haemorrhages in the heart, intestine and atrophied congested spleen were main post mortem changes. Loss of tracheal lining with infiltration of mononuclear cells, lost of pneumocyte I and proliferation of pneumocyte II with infiltration of large mononuclear cell were the major histological findings.

An overall 21% sero-prevalence has been found in the country. The sero-prevalence varied greatly in different geographical locations/districts (Table I). Results of vaccine efficacy are in progress. A filter paper method of PPRV RT – PCR was developed using blood, nasal swabs and tissue suspensions. A 448 bp fragment of F gene from the locally produced vaccine virus was successfully amplified by using the published method (A 448 bp fragment of F gene from the locally produced vaccine virus was successfully amplified by using the published method (Özkul et al., 2002). Later it was validated by the field samples. Using the same primer set, RT-PCR with filter paper was developed for detection of PPRV from field samples and its sensitivity was also compared with conventional RT-PCR using tissue samples. The fragment of F gene was amplified at the product 448 bp for each sample such as nasal swab, blood and tissue suspension in filter paper method. Different samples from three animals were tested for the comparison (Table II). Nasal swab was found positive in both cases. Blood was found positive in one animal. Two tissue homogenates were found strongly positive. Although nasal swabs and blood used from the same animals, only nasal swab gave consistent results. Filter paper method of RT-PCR using nasal swab samples could be sensitive tools for the diagnosis of PPR in infected herd.

TABLE I. SERO-EPIDEMIOLOGICAL STUDY OF PESTE DES PETITS RUMINANTS (PPR) IN GOAT IN THE DIFFERENT AREA OF BANGLADESH BY CELISA

SL no	District	Sampling Area	No. of Goat tested	% prevalence
1	Mymensigh (North East)	Nelokha,Boyra	200	12.5
2	Chittagong (South)	Boalkhali	80	6.25
3	Jessore (South West)	Monirampur	80	49.37
4	Rajshahi (North West)	Godaghari	80	26.71
5	Sylhet (North East)	Sadar, Jaintapur	80	20
6	Dhaka (Centre)	Sonarga	80	10.5
		Total no. of Goat	600	20.88 (overall)

TABLE II. COMPARISON BETWEEN FILTER PAPER METHOD RT-PCR AND CONVENTIONAL RT-PCR

Animal No	Dried soaked filter paper used as template RNA RT- PCR method			RNA Extracted from bronchial/mesenteric Lymph node
	Nasal swab	Blood	Tissue homogenate	
1*	+	+		
2**	+	-	+	+
3**	+	-	+	+

* live animals. ** dead animal

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RAPID DETECTION OF PESTE DES PETITS RUMINANTS VIRUS USING A REVERSE TRANCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

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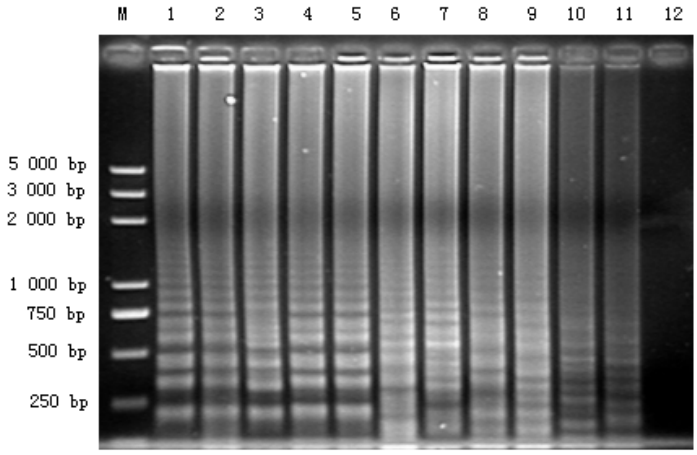
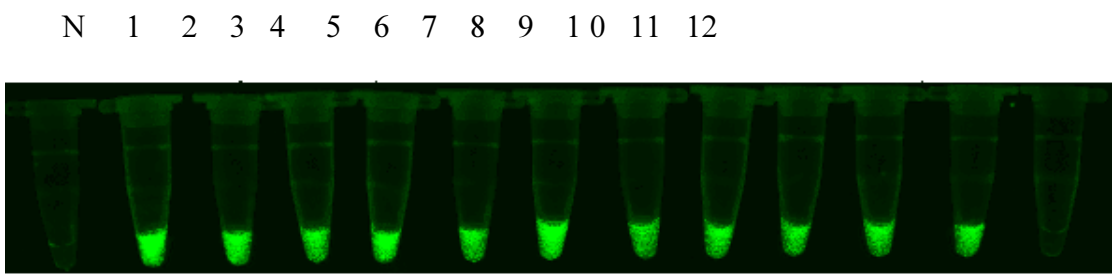
A one-step, single-tube, accelerated, quantitative reverse transcription (RT) loop-mediated isothermal amplification (RT-LAMP) assay targeting the N gene for the rapid and real-time detection of peste des petits ruminants virus (PPRV) are reported. The feasibility of PPRV RT-LAMP for laboratory diagnosis was validated with vaccine virus samples Nigeria 75/1. The comparative evaluation of the RT-LAMP assay demonstrated exceptionally higher sensitivity by conventional RT-PCR with a detection limit of 2 copies. In addition, the field applicability of the RT-LAMP assay was also demonstrated by standardizing SYBR Green I-based RT-LAMP wherein the amplification was carried out in a water bath at 63°C for 70 min, which was followed by monitoring gene amplification with the naked eye through colour changes. These findings demonstrated that the RT-LAMP assay is a valuable tool for rapid, real-time detection as well as quantification of PPRV in the samples without requiring any sophisticated equipment and has potential usefulness for clinical diagnosis and surveillance of PPRV in developing countries.

A set of four primers was designed by targeting the PPRV *N* gene. With Bst DNA polymerase large fragment, ladder like DNA fragments can be seen with agarose gel electrophoresis. The RT-LAMP reaction system was optimized; the sensitivity and the specificity were tested. The process of one step RT-LAMP assay was performed within 70 minutes and amplification results was visualized, the sensitivity of RT-LAMP assay was 1000 times of RT-PCR, ten times of nest RT-PCR. The RT-LAMP described in this study is a cheap, sensitive, specific and rapid protocol for the detection of PPRV infected cells and tissues effectively. It can be simply applied both in field condition and in laboratory operation for specific detection of PPRV.



Line M, DL 2000Plus maker; Line 1 to 12, RT-LAMP with ten time dilutions of PPRV RNA (12ng/μl ~$12X10^{-11}$ ng/μl)

FIG.1. Sensitivity of RT-LAMP



Line N, negative control; Line 1 to 12, RT-LAMP with ten time dilutions of PPRV RNA (12 ng/μl ~$12X10^{-11}$ ng/μl)

FIG. 2. Visualized result of RT-LAMP

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PPR DYNAMICS AND PROGRESSIVE CONTROL

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Peste des petits ruminants (PPR), also known as goat plague, is a highly contagious and infectious viral disease of both domestic (goats and sheep) and wild ruminants. PPR is caused by a virus belonging to the genus *Morbillivirus* (family *Paramyxoviridae*). It is clinically and pathologically similar to rinderpest in cattle and is serious viral disease of small ruminants in areas where it occurs, including its economic impact. Historically, it was first described in 1942 by Gargadennec and Lalanne when they investigated a previously unreported syndrome in sheep and goats in Côte d'Ivoire (Africa). PPR is characterized by the sudden onset of depression, high fever, serous to mucopurulent discharges from the eyes and nose, erosions of the oral cavity and lips, respiratory difficulty and cough, foul-smelling diarrhoea, dehydration and death. The mortality rate is variable but can be as high as 90%.

PPR spreading

PPR infection has long been recognized in many African countries south of Sahara and that lie between the Atlantic Ocean and the Red Sea, from Egypt to the north to PR Congo in the Equator region, from Mauritania in the West to Kenya and Tanzania in the South-East. The disease had not been recognized in most of North and southern Africa, until recent events in 2007 and 2008. The number of newly reported outbreaks of PPR in Africa had remained constant through 2005 but its distribution is changing. On average there are 400 new reported outbreaks per year. There are field findings and laboratory evidence suggesting a widespread occurrence in DR Congo, Ethiopia, Somalia, Sudan and PR Congo. The first outbreak of the ongoing epizootic in East Africa was initially suspected in Turkana District, Kenya, in March 2006. At that time, livestock owners described a disease that was killing large numbers of small ruminants and that had not been seen before. The neighbouring countries of Uganda and Tanzania subsequently declared their first cases in July 2007 and January 2009, respectively. The most alarming event in 2008 was that of PPR recognition in Morocco. Though initially only two outbreaks were reported in mid-July 2008 in the central part of the country, the disease was identified in the following weeks to be widely spread and included most of the central and northern parts of the country. By mid-November 2008, a total of 257 outbreaks were recorded in 36 provinces. Apart from Egypt which is known to be affected since 1989, the Morocco outbreak is the first case of this disease in North Africa. This emerging situation was of great concern for Algeria and Spain which have historically maintained intense commercial interests with Morocco. Partial sequencing of the amplicons from infected tissue samples from the Moroccan epizootic confirmed that the causative agent was PPRV of lineage IV - a lineage circulating in the Middle East and Asia but not reported before from Africa. The Moroccan virus is very closely related to viruses found earlier in Saudi Arabia and in Iran.

In the 1980-1990s, the disease was described in countries in the Middle East, including Iran. The disease spread westwards to Turkey (1994) and Afghanistan (1996). The outbreak that was noted in Tajikistan in 2004 was probably the first case in Central Asia. Outbreaks are now known to be common in India, Nepal, Bangladesh, Pakistan and Afghanistan. By 2007 it had spread to China (Tibet) across the Indian border. There are serological suspicions of PPR in Uzbekistan, Mongolia and Turkmenistan but this needed further confirmation.

A FAO Strategy

A strategy needs to be formulated to: (i) define the northern and southern edges of affected areas through assessments of infected countries; (ii) sensitisation and communication avenues to governments and other stakeholders as to the importance of PPR, its impact, the risks of spread to new areas and regions; (iii) conduct epidemiological field studies, continued socio-economic impact assessments, and studies to understand production and marketing management patterns; (iv) develop a improved local and regional small ruminant husbandry systems that allow for targeted interventions; (v) prevent viral spread from infected area through strengthening of surveillance, development of emergency response, introduce inexpensive diagnostic and survey tools to relevant laboratories; and, (vi) combine with other campaign to improve small ruminant flock health and disease prevention and thus maximise available resources.

Conclusion:

There are many challenges in the prevention and disease management options to control of PPR in the currently affected areas that have contributed to the rapid spread of the disease. Knowing them would contribute for improved control, detection and elimination of PPR and safeguarding areas where PPR is known not to occur. With coordinated control measures there are good reasons to believe that the eradication of PPR is an achievable goal like rinderpest.

FIELD SURVEILLANCE AND LABORATORY DIAGNOSES OF AFRICAN SWINE FEVER IN NIGERIA

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African swine fever (ASF) is a highly lethal haemorrhagic disease of domestic pigs that may results in up to 100% mortality [1]. Although, the disease was originally described in East Africa by Montgomery in 1921, it has subsequently been reported at epizootic scale in Central, Southern and more recently West Africa [2, 3, 4]. Following an initial sporadic infection reported in 1973 which was subsequently eradicated, the recent waves of epizootics was first noticed in Nigeria around September 1997 but was confirmed in November 1997. The local government areas from Ogun and Lagos States closely bordering the previously infected Benin Republic confirmed index cases. Despite the early warning systems, it seemed the country was not well prepared for the infection and the disease reporting structure appeared deficient, hence the virus spread rapidly and the quick containment of the virus was unrealistic (see Figure).

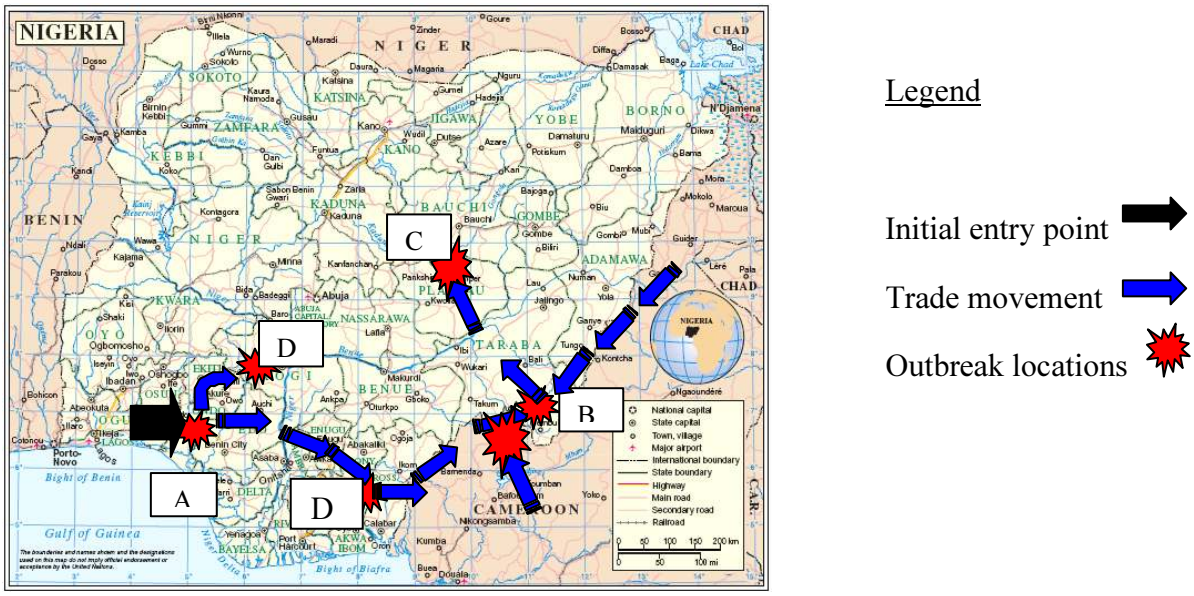

FIG. 1. Probable spread of ASF within Nigeria, 1997-1998 and 1999 to date.

In the first year of infection, a total of 125,000 pigs were lost at an estimated cost of $3.5million. Since the time of these first outbreaks, the virus has continued to infect the country with waves of epizootics and as at the time of this report, it has been confirmed that 18 of the 36 states of the country has recorded infection [5] with loss of an estimated one million pigs. Since a good surveillance system and rapid diagnosis of transboundary animal diseases (TAD) like ASF is key to the control and effective eradication of the virus, we carried out a nationwide epidemiological surveillance (serological and virological) in the country to determine the sero-prevalence of ASF in Nigeria, the strains of the virus currently circulating in Nigeria, and plan an effective strategies for the control and eradication of the virus through the understanding of the means and routes of spread of ASF in Nigeria.

Specifically, collaborations were set-up with CISA/INIA, Spain and ILRI; the country was mapped, stratified sampling with cluster sampling within each stratum was used in farm site or slaughter slab/abattoir selection. A key factor in the selection of sites includes the main pig producing,

marketing and consuming areas of the country. It must be understood that religious factors prevent close association with pigs in certain regions of Nigeria and as such, it was considered a futile exercise to expend or concentrate effort in such area in a bid to carry out surveillance for African swine fever. Sera and tissues (spleen, liver, kidney, lung and lymph nodes) were collected. Laboratory tests including i-ELISA and PCR were done. The results are tabulated below:

TABLE I. ANALYSES OF SERA FROM DIFFERENT REGIONS OF NIGERIA TESTED BY I-ELISA

Region (States)	Number of serum	Number Positive	Prevalence (%)	PP (35-50%)	PP (51-75%)	PP (≤76%)
South-West (Lagos, Oyo and Ogun)	101	26	26	17	3	6
South-East (Enugu, Imo and Abia)	68	4	6	2	1	1
South-South (Cross-River, Akwa-Ibom, Edo and Delta)	195	30	15	5	7	18
North-West (Kaduna and Kebbi)	74	5	7	3	0	2
North-East (Taraba and Adamawa)	128	45	35	19	9	17
North-Central (Plateau and Benue)	526	123	23	49	40	40
Total	1092	239	22	95	60	84

PP= Percentage positive; *= Samples collected during the 2007 sporadic outbreaks of ASF in Delta state; ** Samples collected during the October 2006 widespread ASF outbreaks in Plateau state.

Although work is still on going on the tissue samples, approximately 51% (70/137) of the tissue tested so far are positive. These results are similar to previous reports given in regional studies carried out in Nigeria. They indicated that since 1997, the ASF virus is still in circulation in Nigeria. It has been emphasised that no effective control of ASF exist without stamping out of all infected and in-contact animals. Movement of pigs within the country added to the re-circulation of the virus.

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SURVEILLANCE OF RIFT VALLEY FEVER IN CATTLE, GOATS AND SHEEP IN UGANDA

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Increasing occurrence of outbreaks of Rift Valley fever (RVF) has been witnessed in southern Somalia, north-eastern Kenya and northern Tanzania during heavy and prolonged, often unseasonal, rainfall since 1997-98 [1]. More recently epizootics were reported in Kenya, Somalia, Tanzania, Sudan, Madagascar and South Africa [2, 3, 4, 5]. Because Uganda is located in the endemic zone of RVF, surveillance was conducted to enable early detection of outbreaks. Testing of frozen and fresh bovine sera retrospectively from 1997 to 2008 using IgM ELISA revealed a seroprevalence ranging from 0 to 2.5%, while VNT revealed 9.65% prevalence.

Testing of goat and sheep samples collected from 2005 to 2008 through a cross-sectional study targeting commercial farms and free-range flocks in flood-prone zones in Uganda revealed a sero-prevalence ranging from 5.3-27.9% and 0-0.9% by IgG ELISA and IgM ELISA, respectively. Virus neutralization test revealed 32.3% prevalence among goat flocks from these same places.

Although indigenous breeds of cattle, goats and sheep, known to be less susceptible to RVF than exotic breeds (Anon., 2005), are predominant in areas surveyed, it is likely that the RVF virus is circulating in livestock in some of these locations. Outbreaks are likely to occur once favourable conditions are met. Studies to establish the distribution of RVF vector mosquitoes and the proportion of susceptible hosts in areas with high sero-prevalence are required to further elucidate the status of RVF in Uganda.

TABLE I. RETROSPECTIVE TESTING OF BOVINE SAMPLES FOR RIFT VALLEY FEVER IN UGANDA

Year	Sample size	Assay type	Prevalence (%)
1997	160	IgM ELISA	1.25
1998	80	IgM ELISA	0.00
2000	80	IgM ELISA	1.30
2001	80	IgM ELISA	2.50
2005	80	IgM ELISA	0.00
2007	683	VNT	9.65
2008	232	IgM ELISA	2.60

TABLE II. CROSS-SECTIONAL TESTING OF CAPRINE AND OVINE SAMPLES FOR RIFT VALLEY FEVER IN UGANDA 2005 TO 2008

Place	Stock type	Sample size	Assay type	Prevalence (%)
Ssembabule	Goats	240	IgG ELISA	7.10
Mpigi	Goats	240	IgG ELISA	27.90
Mpigi	Goats	697	VNT	32.30
Masaka	Goats	600	IgG ELISA	5.30
Mubende	Goats	360	IgG ELISA	8.30
Soroti	Goats	112	IgM ELISA	0.90
Soroti	Sheep	16	IgM ELISA	0.00

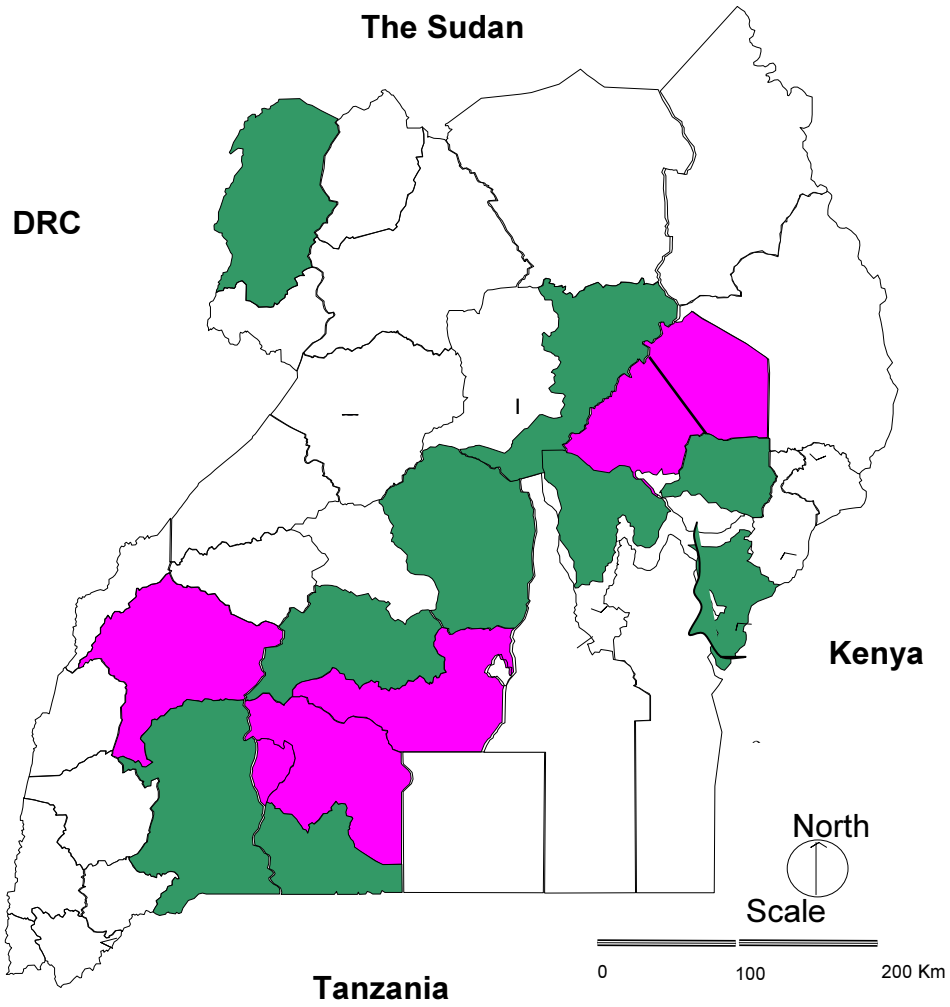

FIG 1. Map of Uganda showing places where cattle (green), goats and sheep (Indigo) were sampled for Rift Valley fever, 1997-2008.

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SERODIAGNOSIS OF RIFT VALLEY FEVER IN AFRICAN WILDLIFE USING A RECOMBINANT NUCLEOCAPSID-BASED INDIRECT ELISA

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Antibodies to Rift Valley fever virus (RVFV) have been found in many wildlife species [1, 2] but their importance in the epidemiology of the disease during the inter-epidemic and epidemic periods, and including their possible specific role in the cryptic maintenance of the virus is not elucidated. A recently developed indirect ELISA (I-ELISA) based on recombinant nucleocapsid protein (rNp) of RVF virus was reported to have high analytical accuracy for the detection of IgG antibody in African buffalo sera [3].

An indirect ELISA (I-ELISA) based on the recombinant nucleocapsid protein (rNp) of Rift Valley fever virus (RVFV) was evaluated for the detection of specific serum IgG antibody in African wildlife. Data sets derived from field-collected sera (n = 877) in Africa (antelopes = 529, black rhinoceros = 43, common zebra = 24, elephant = 73, giraffe = 81, grevy zebra = 78, warthog = 49) were categorized according to the results of a virus neutralization test. Dose response curves using different dilutions of sera known to be positive or negative in the virus neutralisation test had the expected analytical slope and the I-ELISA clearly differentiated between different levels of specific IgG antibody against RVFV in African wildlife (Figure 1).

At cut-offs optimised by the two-graph receiver operating characteristics analysis, the diagnostic sensitivity of the I-ELISA was 100% and diagnostic specificity ranged from 99.8% to 100% while estimates for the Youden's index (J) and efficiency (Ef) ranged from 0.99 to 1 and from 99.7% to 100%, respectively. The rNp-based I-ELISA is highly accurate, safe, and offers a single assay format for rapid detection of IgG antibody to RVFV in sera of different wildlife species.

This study confirm previous findings that the rNp-based I-ELISA accurately identifies sera with different concentrations of specific IgG antibodies to RVF virus, and compared to virus neutralization test it has very high diagnostic performance in various wildlife animal species. As a single and safe test format for mass and rapid detection of IgG antibody to RVF virus, it provides a useful tool for sero epidemiological studies of RVFV infections in African wildlife species. Such investigations might help to elucidate their specific role in the epidemiology of the disease during the inter-epidemic and epidemic periods, and including enigmatic mechanisms of the virus cryptic maintenance within the host-vector natural cycle.

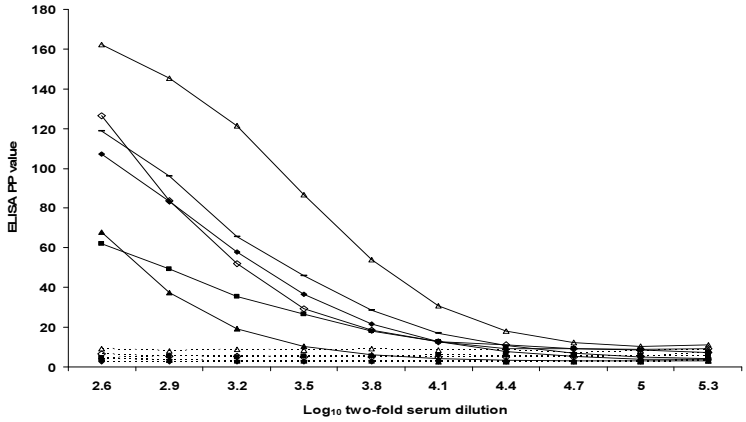

FIG. 1. Dose response curves of wildlife sera in IgG I-ELISA tested positive (□) or negative (---) in a virus neutralization test (VNT): Black rhinoceros (♦), eland (▲), gerenuk (■), kudu (◊), impala (Δ), Thomson gazelle (▬). VNT titers in positive sera ranging from $log_{10}10^{1.9}$ (■) to $log_{10}10^{3.1}$ (Δ).

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MOLECULAR DETECTION TECHNOLOGIES FOR ARBOVIRUSES INCLUDING BLUETONGUE AND RIFT VALLEY FEVER VIRUSES

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Arthropod-borne animal viruses (arboviruses) cause significant livestock and economic losses to world agriculture. This paper discusses the current and potential impact of these viruses, as well as the current and developing molecular diagnostic tools for these emerging and re-emerging insect transmitted viruses affecting livestock and wildlife. The emphasis will be on those viruses which there have been significant recent outbreaks in livestock including bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV), vesicular stomatitis virus (VSV), and Rift Valley fever virus (RVFV). The current readiness for rapid detection of arboviruses is fairly high, but there is a need for global harmonization and continued evaluation due to the genetic variation of these unique pathogens. The tool chest for molecular detection contains a range of assays from low technology to high-throughput sophisticated devices.

Biting midges in the genus *Culicoides* transmit arboviruses affecting livestock, including BTV and EHDV. These viruses cause sub-acute to lethal disease cattle, sheep, goats and/or wild ungulates resulting in worldwide losses attributed to BTV alone estimated at $3 billion annually. There was a fairly good understanding of the epidemiology of BTV until recent introduction of BTV into Europe. Of particular concern is the economic and unique disease impact BTV-8 has had on Europe and the fact that there have been multiple isolations of exotic BTV serotypes in the U.S. over the past 3 years. In Europe, killed BTV-8 vaccines are being utilized to control and potential eradicate the disease. In the U.S., there is only one commercial vaccine available nation-wide, and it is specific to BTV type 10. There is limited or no cross protection between serotypes thus complicates the control of the disease. The related orbivirus, EHDV, is of considerable interest to the captive cervid industry, and EHDV serotype 7 has been associated with clinical disease in Israeli cattle. A number of assays are available for detection of viral RNA using reverse transcriptase-polymerase chain reaction (RT-PCR) genome amplification for BTV and EHDV.[2] Additionally, real-time RT-PCR assays are available to detect all BTV EHDV serotypes.[3,5] A multiplex assay has also been developed to detect BTV and EHDV and distinguish between the two viruses in a single closed tube [3].

There are periodic outbreaks of VSV in the U.S. presumably being brought in on or in insect vectors from Central and South America where the disease is enzootic. *Culicoides*, black flies and sand flies transmit VSV to cattle and horses. Insects are believed to play an essential role in transmission of the virus from natural reservoirs to domestic livestock. Once initial infection has occurred, direct contact transmission is believed to be the major route of transmission. Humans associated with infected livestock can become infected resulting in mild to severe febrile illness. The clinical severity of VSV, and its similarity to foot-and-mouth clinical disease, results in quarantines, sale barn closures, and restrictions on the movement of livestock and animal products. As with BTV there are a number of standard diagnostic tools available for detection of VSV, including a recently developed real-time RT-PCR for detection and distinguishing VSV Indiana and VSV New Jersey. [1,4]

Recent outbreaks of Rift Valley fever (RVF) in East Africa have raised worldwide concerns of the potential impact of this virus. This virus causes a high mortality and abortion rate in small and large

ruminants. This is a zoonotic virus resulting in mild to lethal disease in humans. Retinal degeneration has been reported in humans as high as ten percent of those infected. There are a very limited number of veterinary diagnosticians that are immunized with the expensive investigational vaccine to allow them to work safely with this virus. Therefore a rapid diagnostic tool that quickly inactivates the virus during the process such as a molecular amplification technology is ideal. In addition, diagnostic assays are being developed to differentiate infected from vaccinated animals (DIVA). A number of international collaborations are being developed utilizing a variety of formal agreements to evaluate new diagnostic tests needed for an effective RVF countermeasures program.

Standard reverse transcriptase polymerase chain reaction (RT-PCR) viral gene amplification assays have become routine in many diagnostic settings. These assays are prone to cross-contamination problems and are being replaced even in developing countries by real-time RT-PCR assays that can be performed in closed tube environment thus less cross-contamination. In outbreak situations the real-time RT-PCR assays can be automated to allow high throughput. Additional technologies such a Linear-After-The-Exponential (LATE)-PCR and loop-mediated isothermal amplification (LAMP) are providing both low to high technology solutions for virus genome detection. The development of validated diagnostics tools is needed for an effective control strategies and the formulation of reasonable animal regulatory statutes to reduce the economic impact of these arboviruses.

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VARIABILITY OF THE IFN-B REPRESSING ACTIVITY OF NS PROTEINS FROM 26 wt RVFV ISOLATES AND EXPRESSION OF THEIR NS IN VERO E6 CELL

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Viral factors and a high genetic variability may be involved in the pathogenesis on Rift Valley Fever disease and may explain the wide range of clinical outcome in man and cattle. The differences in virulence on RVFv strains may be due to the immune compromising effect of wtRVFV strains through a) the suppression of the IFN-response and b) the impairment of DC. In this work diversity of wt RVFV strains was assessed after cloning and sequencing the non-structural S gene (NS). Then these RVFvNS clones were monitored for their immune modulator effects by analysing the natural variability of NS activity on the IFN-β promoter. In order to do so the NS proteins from twenty-six African wild strains RVFV isolates from different sources (animals, humans, and insects) were sub cloned. Amplificates of the NS genes including a C-terminal FLAG-tag were ligated into the NheI /KpnI sites of expression plasmid pI.18. After cotransfection of the recombinant NS-carrying expression vectors together with the reporter plasmid p125-luc into Vero E6 cells, luciferase activity as a readout for the IFN-β promoter activity was monitored. In addition, expression of NS in Vero E6 cells was monitored by immunofluorescence staining using a mouse anti FLAG antibody counterstaing.

Two RVFV NS proteins (from strains R7 and R18) led to significant IFN-ß induction when compared to wt-NS whereas the others showed efficient suppression of IFN- promoter activity. Interestingly, NS-R7, which failed to inhibit IFN- induction, was not detectable in immunofluorescence tests although sequencing results revealed an intact FLAG-tag. This might be attributed to a point mutation in the NS sequence, which results in the replacement of a leucine, by proline probably leading to an unstable conformation. For NS-R18 sequencing revealed a large internal in-frame deletion, which leads to a non-functional NS-protein very, much resembling the naturally occurring mutant clone 13. Initial experiments using RVFV wt strain ZH548 and the NS-deficient mutant clone 13 for infection of human pDC indicated that the viruses were taken up but did not replicate in these cells. Interestingly neither the wt virus nor the mutant clone 13 induced IFN- expression although the mutant is a very potent IFN-inducer in epithelial cells. On the other hand, wt virus induced three times more of the proinflammatory cytokine IL-6 compared to the NS-deficient mutant clone 13 in pDC. High amounts of proinflammatory cytokines combined with the complete lack of IFN responses in both epithelial cells and pDC might be responsible for the severe outcome of RVFV wt infections. In contrast infection with the mutant clone 13, which does not cause severe disease in animals or humans, leads to an antiviral response in epithelial cells (which represent the site of primary infection) combined with a lower IL-6 expression in pDC. In further DC experiments we will include additional RVFV strains; in particular we are interested in strains encoding those NS, which failed to inhibit IFN induction in our reporter assays.

RIFT VALLEY FEVER ECOLOGY AND EARLY WARNING

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Rift Valley fever (RVF) once again dramatically affected the Horn of Africa (Kenya, Somalia, and Tanzania) in 2006-2007. This outbreak was linked to unusual rainfall associated with climatic events (El Niño), which affected the populations of the mosquitoes acting as vectors and reservoirs of the disease. The disease also reappeared in Sudan in the autumn of 2007, following excessive rainfall driven by a post-El Nino, unusually warm sea temperature in the Indian Ocean. In the same year and in 2008, the disease affected southern Africa countries (Swaziland, South Africa) and islands in the Indian Ocean (Comoros, Mayotte, Madagascar).

Based on near real-time climatic data, forecasting models and Early Warning Systems were available at the continental level and proved to be efficient in raising the alert before the onset of the epidemic, at least for the coastal countries of eastern Africa. In addition, these recent events gave an opportunity to review the natural history of RVF, especially in some places where its ecology was poorly documented. FAO and WHO officers widely use outcomes from the different models and then identified gaps or needs that could be filled in order to improve the use of these predictions. A brainstorming meeting was organized in Rome in September 2008 to discuss adjustments and complementarities of the existing models, as forecasting and early warning systems are the key points that may provide a time window for preventive measures, before the amplification of the virus is out of control.

INTEGRATING WILDLIFE ISSUES INTO THE PREVENTION, CONTROL AND RESPONSE TO TRANSBOUNDARY ANIMAL DISEASES

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The Emergency Prevention System (EMPRES) Wildlife Unit of the Food and Agriculture Organization of the United Nations (FAO) was established to investigate the role that wildlife species play in diseases that impact livestock and agriculture based livelihoods. Land-use changes and the competition for natural resources are bringing human populations, agricultural lands and livestock into closer contact with wildlife.

This increased contact creates opportunities for the transmission of endemic and newly emerging infectious diseases between livestock, wildlife and humans. It is clear that there is a need to establish long-term, sustainable wildlife disease monitoring programs globally, with a focus on understanding the ecology and epidemiology of diseases between domestic and wild animals. With the emergence of highly pathogenic avian influenza (HPAI) H5N1 it became apparent that multidisciplinary in-country and regional capacity building was necessary amongst, biologists, veterinarians, ornithologist and others.

To date, the Wildlife Unit that has coordinated, facilitated, or implemented training of more than 1,000 in-country nationals from over 100 countries worldwide on wildlife disease surveillance. The EMPRES Wildlife Unit is leading and facilitating a range of collaborative activities to study the epidemiology and ecology of HPAI H5N1 in wild birds, migratory routes, habitat use and the role wild birds may play in virus introduction and movement. FAO has deployed almost 400 transmitters in 9 countries and is monitoring global migratory bird movements across more than 40 countries to determine whether wild bird movements are temporally or spatially associated with HPAI H5N1 outbreaks. An overview of the Wildlife unit activities will be presented, along with insights on the role of wild birds in the transmission and spread of HPAI H5N1- an area that has been intensively studied over the past several years.

FMD PROGRESSIVE CONTROL PATHWAY

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ON-SITE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS USING A PORTABLE, AUTOMATED SAMPLE PREPARATION AND PCR SYSTEM

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Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease of farm livestock. The etiological agent, FMD virus (FMDV), is a single-stranded, positive-sense RNA virus belonging to the genus *Aphthovirus* within the family *Picornaviridae*. Rapid and accurate confirmation of the presence of FMDV is needed for effective control and eradication of the disease. An on-site detection test would be highly advantageous as the time taken to transport suspect clinical material to a central laboratory can often be lengthy, thus delaying a definitive diagnosis in the event of an outbreak. This study describes the development of a molecular assay for the detection of all seven serotypes of FMDV using novel technology, namely: Linear-After-The-Exponential (LATE)-PCR [1], for transfer onto a portable, easy-to-use, fully automated sample preparation and RT-PCR instrument.

Primers and a mismatch tolerant probe were designed from consensus sequences in the FMDV 3D (RNA polymerase) gene to detect the target and its variants at low temperature. An internal control (IC) was included to validate negative results. After demonstrating that the LATE RT-PCR signal at end-point was proportional to number of target molecules over the range 10 to 1 million copies, the assay was compared with a one-step real-time RT-PCR (rRT-PCR) assay (also targeting the 3D) used routinely by reference laboratories [2].

The LATE RT-PCR assay amplified RNA extracted from multiple strains of all FMDV serotypes. Of the 121 FMDV-positive samples tested, 119 were positive by both rRT-PCR and LATE RT-PCR tests while 118 had tested positive by virus isolation at the time of receipt. Twenty-eight FMDV-negative samples failed to react in all 3 tests. There were no false positive signals with RNA from other vesicular disease-causing viruses. Each FMDV-negative sample generated a signal from the IC, ruling out amplification failures. A dilution series of an FMDV reference strain demonstrated that the analytical sensitivity of the assays was similar.

The LATE RT-PCR assay is at least as informative as the rRT-PCR assay currently used by reference laboratories. All the necessary reagents have been converted into a ready-to-use dry format inside an FMDV assay reagent cartridge. This is inserted into a sample preparation unit for use in conjunction with the BioSeeq™-Vet Portable Diagnostic Laboratory [3]: a fully integrated, easy-to-use system, which is currently undergoing field evaluation.

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GLEWS

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REAL TIME PCR METHODS FOR SIMULTANEOUS DETECTION, QUANTIFICATION AND GENOTYPING OF CAPRIPOX VIRUSES

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The Genus *Capripoxvirus* (CaPV) of the *Poxviridae* family comprises three members, namely, *sheep poxvirus* (SPPV), *goat poxvirus* (GTPV) and *lumpy skin disease virus* (LSDV) affecting sheep, goats and cattle respectively. The classification of CaPV is based largely on the host species from which the virus has been isolated and they are considered to be host specific because they seem to cause disease only in the preferred host. However, in the case of GTPV and SPPV there are conflicting reports on their pathogenicity: if most strains produce severe clinical disease in only one host, there are some cases where both sheep and goats are severely affected during the same outbreak. It is evident therefore that the classification based on the host origin of the virus isolation is unreliable and therefore requires replacing with a system that is not based simply on host pathogenicity criteria. Methods relying on molecular characterization offer a means for comparing strains genetically, independently of simple biological classification. Recently, the G-protein-coupled chemokine receptor (GPCR) gene of CaPV has been shown to be suitable for discriminating between virus isolates irrespective of their animal origin.

Based on these findings, we have designed two real time PCR for differentiating CaPV based on the GPCR genes. The first assay is based on the Fluorescence Resonance Energy Transfer (FRET) chemistry, while the second exploits the ability of a 3'black hole quencher (BHQ) labelled linear probe to quench the signal of the fluorophore in the 5' of the FRET acceptor probe and thereby induce a decrease in fluorescence during the course of the PCR. Both these methods were found to be quantitative, sensitive and able to discriminate between CaPV strains on the basis of the differences in their melting peaks. In addition, the second method, that uses quenching of the FRET acceptor can be performed without a need for a special FRET channel in the PCR machine and is therefore likely to be usable in all real time platforms.

These findings will make a significant contribution to the better understanding of the epidemiology of CaPV by enabling rapid genotyping and unequivocal identification of viral isolates. In addition, the new FRET method based on 3' BHQ labelling of the FRET donor will improve the possibilities for genotyping by real time PCR.

MYCOPLASMA DIAGNOSIS BY PCR FROM BEDDING OF MYCOPLASMAL DAIRY HERDS AND ASSOCIATION WITH DISEASE IN DAIRY ANIMALS

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Infection with *Mycoplasma* spp, typically *M. bovis*, is an important disease complex of dairy cattle. *Mycoplasma* spp can cause mastitis, arthritis, metrititis, pneumonia, septicemia, and death of cattle [1]. Standard microbial cultures of milk samples do not isolate *Mycoplasma* spp; special methods are necessary [2]. Mycoplasma infections have been reported as contagious in nature, primarily by milking machines and respiratory spread [3]. Bulk tank milk samples (n = 5 samples per tank) were collected from all bulk tanks on most dairy farms in Utah, USA (n = 222 farms, 292 tanks) at 3-4 day intervals [4], resulting in a sensitivity of 97% for *Mycoplasma* spp. Mycoplasma was detected on 16/222 dairy farms in Utah (7%), a relatively high prevalence compared to the rest of the USA.

After initial surveillance, follow up was conducted on positive farms. One farm milking approximately 4500 Holstein cows in dry lot and free stall housing experienced an outbreak of clinical mastitis (CM) caused by *Mycoplasma* spp., affecting 35 cows per month vs. the endemic rate of approximately 3 CM cases per month (aseptic milk samples from all CM cases were cultured from this herd). Bedding sand was used following a recycling and manure separation process on the farm; sand samples were cultured for mycoplasmas and other bacteria during the outbreak. *Acholeplasma laidlawii* was found in one sample, 2 samples were positive for *M. bovis* by PCR, and one month later 14/20 cow pens' sand bedding samples tested Modified Hayflick medium culture-positive for *Mycoplasma* spp. (testing by 3 different laboratories). During the same month, one recycled bedding sand sample and one cow pen sand sample tested PCR-positive at the Utah Veterinary Diagnostic Laboratory; amplicon sequencing of both isolates showed 99% homology with *M. bovis*.

Positive bedding sand (18,000 kg) was transported from the farm to Utah State University and stored in a pile outdoors. As the weather progressed from late winter (March) to summer (May), the colony forming units/gram (cfu/g) of *Mycoplasma* spp. decreased. From 770,000 and 720,000 cfu/g on the surface and 2,130,000 and 990,000 cfu/g deep in the pile during the first 2 weeks, surface counts all were negative and the deep counts decreased steadily, below 150,000/g during April and to 2,200 -7,800 cfu/g during May and June, then negative through July. Controlled incubation temperatures for 24 -72 hr showed the following: - 20^{o} C – negative (n = 4); 4^{o} C - 64,000, 59,400, 6,000 cfu/g and negative; 37^{o} C - 25,400 cfu/g in wet slurry, all 4 other (dry) samples negative; 45^{0} C – negative (n = 1); 60^{0} C – negative (n = 2). Together with the weather temperature data (not shown), results suggested that frozen or warm conditions did not support mycoplasmal growth in sand as well as cool temperatures, approximately 4^{o} C. Mycoplasma also grew readily deep within the sand pile, but not as much on the surface.

During July, 3 recycled sand bedding piles tested on the initial farm and one each from 2 other project follow up farms with mycoplasma-positive cows were culture-positive for *Mycoplasma* spp. Chemical disinfection experiments were unsuccessful except for use of 10% bleach (0.5% sodium hypochlorite) solution in a pan of sand for 10 minutes at room temperature. A mycoplasma-negative closed herd was identified and tested until 32/32 calves were negative for mycoplasmas on nasal and ear swabs, resulting in probability > 99% that the herd was negative. Bull calves (n = 12) were then obtained from the negative herd and 6 were exposed to *Mycoplasma* spp.-positive sand bedding for 28-34 d, until the sand became culture negative as freezing temperatures developed. Four calves were exposed from 24-30 d old to 52-64 d old, and 2 calves were exposed from a few days old to 15 -21 d old. The remaining 6 calves were controls, bedded with mycoplasma-negative sand.

Preliminary results are: 86 nasal and ear swabs collected every 7 d and 17 tracheal swabs collected at 35 d (n = 9) and 70 d (n = 8) after exposure to either mycoplasmal or controls and have all been negative for mycoplasmas.

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EPIDEMIOLOGY OF CAMEL TRYPANOSOMOSIS DUE TO *TRYPANOSOMA EVANSI* IN MAURITANIA AND ITS CONTROL STRATEGIES FOR SUSTAINABLE LIVESTOCK PRODUCTION

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Camel trypanosomiasis due to *Trypanosoma evansi* is mechanically transmitted by hematophagious diptera such as Tabanidae, Stomoxyinae, and Hippoboscidae. In its acute form, the disease results in a generalized weakness. The animal lies down as of the least effort; milk production falls with the abortion of females. At times, the animal dies after a prolonged decubitus. However, in 80% cases, the disease is observed in its chronic form, which is characterized by considerable economic losses resulting from abortions, reduction of milk production, loss weight, and cachexia.

Due to its extremely dry conditions Mauritania remains a favourable environment for camels as preferred livestock species of considerable economic importance. The animals are kept by shepherds who are very mobile in the field in search of good pastures and water points. Unfortunately, this pastoral system is reported to expose dromedaries to numerous pathologic conditions, especially camel trypanosomiasis due to *T. evansi*. Our investigations from 1993 to 1997 showed that *T. evansi* is present in the dromedaries in Mauritania. According the haematocrit centrifuge technique the parasite prevalence rate ranged from 1.1 to 13.6 % while seroprevalence varied from 13% to 36.7% according to the CATT test. In the Trarza region, as consequence of a good rainy season we more recently observed an abundance of tabanids and stomoxes hence a favourable ecology of *T. evansi* vectors, and subsequently an outbreak of camel trypanosomosis. Prevalence rate was 17.6% using buffy coat examination and 58.8% with the CATT. In many herds, numerous abortions were recorded and all breeders registered very important milk production losses.

In order to limit the infections due to *T. evansi*, two control strategies for camel husbandry could be practiced in the Trarza region. The first is "northern strategy" with the potential of lowering pastures availability and usage. However, it has the advantage of avoiding the direct and indirect action of the pest insects. The second consists of the capture of mechanical vectors of *T. evansi* using traps during the cold dry season and the systematic treatment of infected animals by Cymélarsan® drug. The combination of the two methods can help in establishing an effective control strategy for *T. evansi*.

With the climatic changes, the ecology of *T. evansi* vectors and the transhumance causing mixing and concentration of various livestock species on pastures and water points, it is important to review all risk factors that are necessary to integrate in an epidemiologic approach of camel trypanosomosis.

SITUATION OF ANIMAL HEALTH IN ALBANIA

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The animal healthy service policy in Albania represents an integral component of overall government, social & economic policy in the field of agricultural & rural development, public health, food processing & import/export of animal products. In order to obtain the necessary political, economical & public support, animal health service attempt to contribute effectively to the overall development of the country aiming at improving standards of living of its inhabitants.

One of this approaches is through a practical way of reducing food loses due to animal morbidity & mortality, increasing productivity in animal population, protecting human health against zoonotic diseases & ensuring human treatment of animals. An animal health strategy contributes to the creation of conditions necessary for an uninterrupted animal disease surveillance & control of the country.

The vision of National Animal Health Program (NAHP) is to improve the health and welfare of animals, which meets the needs of stakeholders, enables safe production of food, improves health of the public, sustains the rural society, and support the rural economy.

The concept and requirements for this vision

- The current veterinary services section in the country, including the veterinary diagnostic institution, is very weak. The few resources in place are fragmented and reflect a historic paradigm of previous regimen with incomplete transition to market economy. The veterinary services on the 12 districts/regions appear to have been connected from administrable aspect but their field operations are not connected with the national interest and the above mission.
- There is a need to link food safety and zoonotic aspect to public health sectors so that public interest and funding resources can be increased.
- The livestock sector is undeveloped and consumers do not have much influence or organization. The veterinary service should take this opportunity to present a comprehensive plan of national animal health with the benefits for both consumers through safety food and livestock sector through better production and trade.
- Budgets need to be in place to meet all need with coordination with many of the international organization to secure funding for specific activities within the comprehensive plan for NAHP.
- The main core for reliable NAHP is a scientifically based surveillance system in which contingency planning is incorporated for specific health events.
- Through a reliable NAHP, there is a challenge to sustain livestock production, including social needs, in the agricultural community, with modern economic approaches. The NAHP can be the core for this type of sustainability due to the trust of the agricultural community in the veterinary input. However the NAHP should not use police authority to implement its action. There is no need for this type of authority if the producers are aware of the importance of health animals for their production and family.

AN EFFICIENT STAKEHOLDER DRIVEN APPROACH TO DISEASE CONTROL

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The European Technology for Global Animal Health (ETPGAH) was established in December, 2004 with the objective of identifying the most critical issues that need to be addressed in order to control diseases in animals. The ETPGAH was funded by the European Commission and as required by the Commission, was led by industry. European stakeholders and International organisations participated in the work of the Platform and developed a Vision, Strategic Research Agenda (SRA) and an Action Plan.

The Vision developed is as follows "To facilitate and accelerate the development and distribution of the most effective tools for controlling animal diseases of major importance to Europe and the rest of the world, thereby improving human and animal health, food safety and quality, animal welfare, and market access, contributing to achieving the Millennium Development Goals".

The Vision foresees the speedier development of tools for disease control by focusing our research effort on the most important gaps in the most important diseases. These may be emerging, established or zoonotic diseases. By delivering better disease control, animal health and welfare is protected, human health benefits in terms of zoonotic disease control but human health also benefits from food security, safety and quality. Indeed, poverty and famine may be averted. The stakeholders to the ETPGAH recognise that diseases do not respect borders and took a global perspective in the knowledge that a global reduction in disease is to the benefit of everybody.

In developing the SRA, the stakeholders identified 6 major themes:

1. Prioritisation of Animal Diseases
2. Gap Analysis
3. Fundamental Research
4. Enabling Factors
5. Regulatory Issues
6. Global Perspective

It was recognised that we need to prioritise our effort and focus on finding new disease control tools by collaborative research on a limited number of critical targets. By this mechanism, we can attempt to make greatest progress in the least amount of time. Critical to this concept is the need to identify and prioritise the most important gaps in our ability to control the critical diseases.

Having identified our targets, it is then vital that we have the fundamental research capacity – infrastructure and people – to carry out the necessary research. Establishing our research capability necessitates the creation of a database with the relevant information with gaps then being addressed. Along with filling gaps, efficiency can also be improved by avoiding unnecessary capacity development.

Enabling factors such as quality assurance, intellectual property rights and facilitation of technology transfer are critical components in moving from basic research to the development of a tool that can be used to fight a disease. Financial support at critical points in the development chain is also vital. Too often, projects are dropped because intellectual property rights have not been secured and nobody is willing to invest perhaps €100 million in taking the project from the laboratory bench through development and into the market.

The correct regulatory environment is vital to stimulate innovation. A balance needs to be reached between protecting human and animal health from the risks associated with a product versus the

wish to eliminate all hazard. In addition, regulation needs to be focused on the needs of the veterinary sector, which may be quite different to those of the human sector.

From a global perspective, it is in the interests of everybody to reduce the global burden of disease. It may be much more beneficial to tackle disease at its source. This approach facilitates cooperation across the globe including capacity building.

Having explored the broad areas that need attention in the SRA, the Action Plan was then developed and published in July, 2007 identifying the actions – research or information gathering – that need to be carried out in order to deliver the SRA. The Action Plan follows the themes identified in the SRA with 30 activities outlined. For each activity, the objectives deliverables and tasks are stated.

The purpose of the ETPGAH is to now oversee the delivery of the Action Plan. Each activity needs to be progressed and funding is an important factor. Funding from the European Commission is important as it stimulates collaborative research. However, nation states are the main source of research funding with more than 90% of funding coming from this source. As such, the ETPGAH has stimulated the creation of "Mirror Groups" and seven have been formed to date. The purpose of a Mirror Group is to communicate the content of the Action Plan to a national level and also to encourage the use of national funding to deliver some of the activities from the Action Plan best suited to that country.

Progress has been encouraging to date with many activities being funded by the European Commission and with the European Research Area Network (ERA-Net) progressing many of the information gathering exercises.

DEVELOPMENT, VALIDATION AND IMPLEMENTATION OF ANIMAL HEALTH INFORMATION SYSTEMS IN AN ENVIRONMENT WITHOUT UNIQUELY IDENTIFIED ANIMALS IN TRANSITIONAL COUNTRIES

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The veterinary activities for control of animal diseases are based on the Low for Veterinary Health [2], the Programme for control and eradication of especially dangerous diseases in animals [3] and the special programmes designed for specific diseases, which are in accordance with the EU legislative. This legislative is defining a division of the country into 30 Epidemiological Areas (EAs) and 123 Epidemiological Units (EUs). Each village belongs to a defined EU, which further belongs to a defined EA.

Two animal health information systems were developed in Macedonia, the National Epidemiological Information System (NEIS) and the Laboratory Information System (LABIS). Both systems were aimed on collection/interpretation of animal disease data, in a country where animals are not uniquely identified. The development of NEIS was based on the existing legislation of compulsatory notification of infectious diseases. Field records are collected via the designated veterinary practices (DVP) and entered into the NEIS via the veterinary inspectors (VIs), (employees of the MAFWE). Sources of data for NEIS are obligatory disease control programs (Annual order), Endemic diseases, Outbreaks, Slaughterhouses and Laboratory results of annual surveys. LABIS is a separate database for managing laboratory results. It collects data from samples submitted by DVPs. The samples can be then analyzed in different laboratories, using different methods and given a “final status” by authorized person. The final status is linked to the previously performed tests and entered into the NEIS.

By this concept, the Veterinary department is capable to trace back the background for each individual sample, by reviewing the analyses performed on it. Both systems are designed as a referential integrity databases, where the field result is linked to the animal, owner, village (n = 1803), epidemiological units (n = 123) and epidemiological areas (n = 30) in the country. NEIS can also present the same data in geographical maps, showing the infected village as the smallest unit of observation. Both systems have also different levels of authorization access, allowing precise tracing of entered data.

TABLE I. SOURCES OF ENTRY DATA FOR THE NEIS

Type of informations collected in the NEIS	Responsible / way of collection
Obligatory disease control programs (vaccinations, dehelmintizations, and field diagnostics /TBC/ e.t.c.)	VI/Manually, in the local NEIS database
Endemic diseases (Anthrax, Clostridia e.t.c.)	VI/Manually, after laboratory confirmation, in the local NEIS database
Outbreaks (once an outbreak occurs /FMD/ he must not test every single animal in the lab, but count the number of animals showing clinical signs in the village	VI/Manually, in the local NEIS database
Slaughterhouses (findings during slaughtering, for example Echinococcus, Trichinella e.t.c.)	VI/Manually, in the local NEIS database
Laboratory results of annual surveys	LABIS at FVMS/Automatically

The analysis of the structure of the national flock is of great importance from many organizational reasons. Firstly, the distribution of animals inside the units of observation (EAs, EUs and villages) is important for organization of human resources for the planned activities and secondary for implementation of consequent measures (easy to vaccinate or control a flock of 10.000 sheep, difficult to do the same in 2000 flocks, each of 5 sheep!).

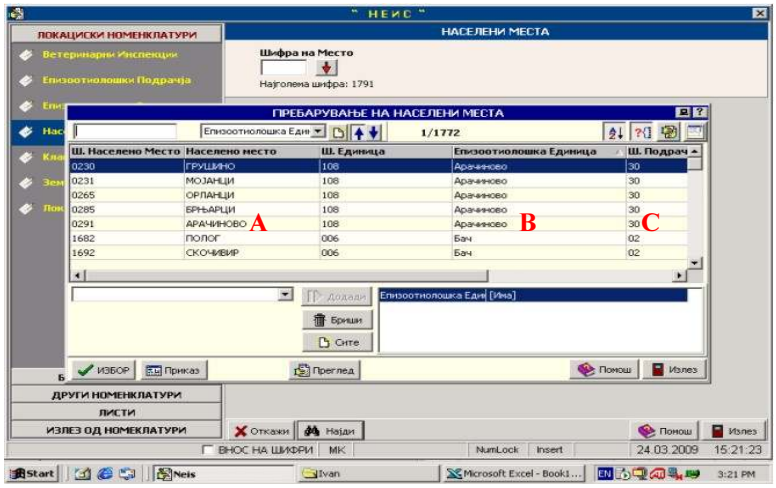

FIG 1. Nomenclatures: linking villages (column A) to appropriate EU (column B) and EA (column C).

The structure of the national flock in the country is as follows:

The total area of the F.Y.R. of Macedonia is 25713 km^2; Divided in 30 EAs, within which 123 EUs are defined. There are 1803 administrative villages, each of which belongs to a defined EU. Approximately 87% of the cattle population is bred in farms (holdings) smaller, then 30 animals. Sheep and goats are mainly kept in flocks of 50-100 animals/flock. Pigs and poultry are bred mainly in smaller (backyard) farms. The rest of the animals (approx. 13% of cattle, 20-30% of sheep and goats, 27% of pigs and 50% of poultry) are bred in professional farming facilities.

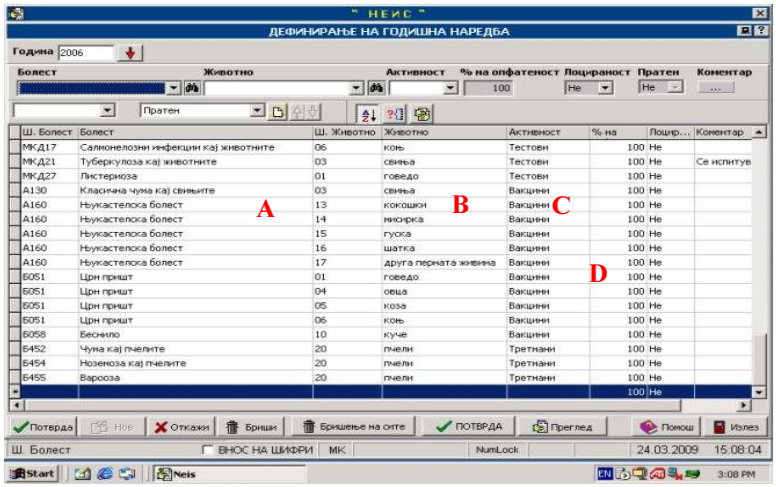

***FIG 2.** Planning of the annual order by disease (column A), animal species (column B), type of activity (column C) and coverage (column D).*

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POSTER PRESENTATIONS

CURRENT SITUATION OF PESTE DES PETITS RUMINANTS (PPR) IN SUDAN

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The current situation of Peste des petits ruminants (PPR) in Sudan was investigated. A total of 1198 serum samples were collected from camels (392), sheep (500) and goats (306) at different areas in Sudan (Khartoum, Gezira, River Nile, Kordofan, White Nile, Gedarif, Kassala, Blue Nile, Tambool, Port Sudan, Halfa ElGadida). A total of 61 tissue samples were also collected from various PPR suspected outbreaks in sheep and goats in 2008. Collected sera were examined for PPR antibodies using cELISA. Collected tissue samples were tested for PPR antigen using IcELISA. Both tests were done according to the product manual of the kits manufacturer (CIRAD EMVT, Montpellier, France 2005), distributed by BDSL.

A total of 336 sheep, 170 goat samples were found to be positive (Table I). Only one camel serum was positive. The PPR antigen was detected in 26 out of 61 samples (Table II). The PPR antibody and antigen detection results are summarized on Figure 1.

TABLE I. DETECTION OF PPR ANTIBODIES IN SHEEP AND GOAT SERA IN SUDAN USING CELISA

State	Ovine				Caprine				Total	
	Total tested	No. +ve	No. -ve	% +ve	Total tested	No. +ve	No. -ve	% +ve	Total tested	% +ve
Khartoum	16	15	1	93.8	160	90	70	56.3	176	59.7
Gezira	53	39	14	73.6	62	37	25	59.7	115	66.1
River Nile	60	32	28	53.3	12	5	7	41.7	72	51.4
Kordofan	251	153	98	61	22	14	8	63.6	273	61.2
White Nile	47	32	15	68.1	43	24	19	55.8	90	62.2
Gedarif	35	31	4	88.6	7	0	7	0	42	73.8
Kassala	20	19	1	95	0	0	0	0	20	95
Blue Nile	18	15	3	83.3	0	0	0	0	18	83.3
Total	500	336	164	67.2	306	170	136	55.6	806	62.8

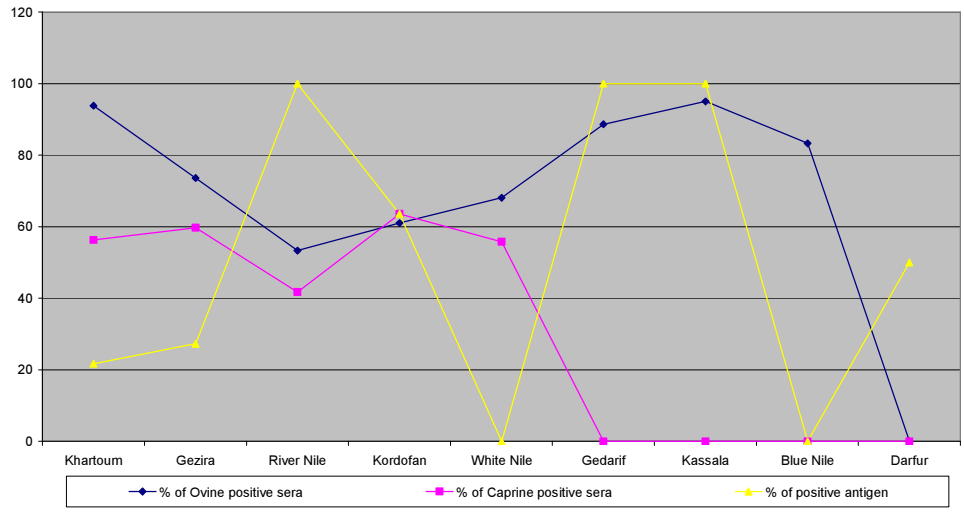
TABLE II. DETECTION OF PPR ANTIGEN IN TISSUE SAMPLES IN SUDAN DURING JANUARY-JUNE 2008 USING ICELISA

State	Total tested	No. +ve	No. -ve	% +ve
Khartoum	23	5	18	21.7
Gezira	11	3	8	27.3
River Nile	1	1	0	100
Kordofan	22	14	8	63.6
Darfur	2	1	1	50
Gedarif	1	1	0	100
Kassala	1	1	0	100
Total	61	26	35	42.6

The results showed that PPR exists in Sudan in different localities. The overall detected seroprevalence of PPR was 62.8%, which is higher than that previously reported (50%) in Sudan [1, 2]. Sheep were noticed to have higher percentage of seropositivity than goats (67.7 vs. 55.6%, respectively) in agreement with the results of Intisar et al. [1]. The highest seroprevalence of PPR (95%) was noticed in Kassala samples from Eastern Sudan followed by 83.3% in Blue Nile region (Eastern to South Sudan) and Gedarif (73.8%). PPR antibodies were found to be prevalent in sheep samples in Khartoum State (93.8%), then Gedarif (88.6%), Blue Nile (83.6%) and Gezira (73.6%). PPR antigen detection was carried out using IcELISA; the overall detected samples were 42.6% of 61 tested samples. Highest prevalence of the disease was noticed in River Nile, Gedarif and Kassala (100%) however only one sample was tested from each locality; Samples collected from Kordofan area showed 63.6% positivity while those of Gezira State were 27%.

The number of collected samples for antigen detection was low compared to collected sera, which could be attributed to the fact that usually most of outbreaks are not reported to the veterinary sector.

Fig 1: The positive percentages of PPR antigen and antibodies detected in ovine and caprine tissues and sera collected from different areas of Sudan during the first semister of year 2008.



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GENERAL INFORMATION ABOUT VETERINARY AND LIVESTOCK IN AFGHANISTAN

S. Rafa

Afghanistan

THE IMPACT OF AHIP AWARENESS CAMPAIGNS ON THE PUBLIC KNOWLEDGE OF THE DISEASE IN ZANZIBAR

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A household survey was conducted in Zanzibar between May and June 2008 to investigate the impact of the ongoing nationwide Avian and Human Influenza Pandemic (AHIP) awareness campaigns on people's understanding of the pandemic. A total of 500 families in 239 villages; all 10 districts of Zanzibar were interviewed. Factors: administrative district, age, sex, number of family members, level of education, occupational groups (farmer vs. non farmer) and access to AHIP trainings and other media tools were investigated in different models.

Preliminary results showed that over 70 percent of respondents know the disease but less than 40 percent out of them understands the cause and ways to prevent the spread of infection in Zanzibar. The district of residence featured prominently ($P < 0.001$) as an important factor of people knowing the pandemic, the causative agent, transmission of the pandemic, self-protection, protection of the country and adequacy of the campaign. The level of education significantly influenced people knowing the pandemic ($P < 0.05$), the causative agent ($P < 0.001$), transmission of the pandemic ($P < 0.001$), self protection ($P < 0.001$), country protection ($P < 0.001$) and adequacy of the campaign ($P < 0.05$). The ongoing trainings on AHIP offered significant understanding of the pandemic, the cause, transmission, self-protection and protection of the country ($P < 0.001$). The family size appeared as a significant ($P < 0.05$) factor of the family knowing the cause of the pandemic. Finally, the habit of reading news article among the respondents increased the exposure to the knowledge of self ($P < 0.01$) and country ($P < 0.001$) protection against the pandemic.

It was concluded that the future AHIP campaigns emphasis on particular disadvantaged districts and widening the use of alternative media of information.

PARASITES OF CATTLE IN THE TRANS-BOUNDARY AREAS OF OGUN STATE, NIGERIA

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An assessment of the parasites of cattle along a major trans-boundary route was carried out in Yewa division of Ogun State, Nigeria between February 2006 and July 2007. The location of study is bound to the west by the Republic of Benin, with which it shares 155 kilometres of International boundary, within latitude 6^0 15^1 N and 7^0 58^1 N in a deciduous/derived Savannah zone of Nigeria.

A total of 62 herds of between 56 and 98 heads of cattle entering Nigeria from neighbouring West African Countries by hoof along the Iwoye – Imeko – Olodo – Abeokuta route were observed for various haemo- and ecto-parasites. About 5ml of blood was collected from the jugular vein of each of 245 randomly selected animals into sterile bijou bottles containing 250µl of 200 mM disodium ethylene diamine tetra acetic acid (Na_2EDTA) as anticoagulant. Animals infested with ectoparasites were noted and the parasites on each of the animals were collected into universal bottles containing 1% formaldehyde. The pus expressed from skin nodules on 18 animals and skin scabs from 15 animals were also collected for Parasitology and Microbiology respectively. All the samples were analysed at the College of veterinary medicine, University of Agriculture, Abeokuta. The blood samples were screened for the presence of trypanosomes using the buffy coat centrifugation method and for other blood parasites by examination of Giemsa-stained thin blood smear.

Of the 245 blood samples analysed, 123 (50.20%) were positive for blood protozoans. Of these, *Babesia spp.* infection accounted for 50 (40.65%); *Trypanosoma spp.* accounted for 29 (23.58%); *Anaplasma spp.* 14 (11.38%); mixed *Anaplasma spp.* and *Babesia spp.* 9 (7.32%) and mixed *Babesia spp.* and *Trypanosoma spp.* 21 (17.07%). All the animals sampled had varying degrees of tick infestation ranging from a total count of 26 to 280 ticks per cattle. Of the 3756 ticks collected, 1080 (28.75%) were *Rhipicephalus appendiculatus*, 917 (24.41%) were *Amblyomma variegatum*, 739 (19.68%) were *Boophilus decoloratus*, 487 (12.97%) were *Boophilus microplus*, 473 (12.59%) were *Amblyomma hebraeum*, 41 (1.09%) were *Hyalomma spp*. and 19 (0.51%) were *Amblyomma gemma*.

Haematopinus was the only louse specie identified in this study. They were found on the upper eyelid, inner commissure of the pinna and tip of the tail in 16 animals. *Demodex bovis* was seen in all the 18 skin nodules, while *Dermatophilus congolensis* was isolated from all 15-skin scabs collected.

The very high parasitic load observed in the animal population in this study is an indication that many parasites enter the country on daily basis via animate vectors. These animals may be carriers of a wide range of economic important diseases, the control of which may be very expensive. This may also result in additional cost of production to the owners of in-contact animals. Since the potential for increasing livestock production in Nigeria can only be fully realized if the animals are adequately protected against major diseases, the animals in border areas should be regularly monitored and screened for diseases of economic and/ or epizootic importance to minimize spread of trans-boundary diseases to the national herd.

CLONING AND VACCINATION PROPERTIES OF THE SPOROZOITE ANTIGEN *EIMERIA STIEDAE* (RABBIT COCCIDIOSIS)

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Coccidiosis, caused by the protozoan parasite of the genus *Eimeria*, is a common disease in rabbits and chicken and is of the major economic importance to poultry industry and commercial rabbit's production around the world. Ten known *Eimeria* species infects the rabbits. Nine of them infect only the intestinal tract while one (*Eimeria stiedae*) infects the intrahepathic epithelial bile ducts.

At present, coccidiosis is controlled by chemotherapy. However, the emergence of drug resistant parasites coupled with the higher cost for developing new coccidiostats has drawn the attention to alternative means of control such as immunoprophylaxis including the development of genetically engineered vaccines.

As an initial step in the development of a genetically engineered vaccine against coccidiosis, a library of EcoRI-digested genomic DNA from *E. stiedae* has been constructed in *E.coli* using the expression vector λgt11. Screening of the library with an antiserum raised in rabbits against purified *E. stiedae* sporozoites resulted in the identification of immunoreactive clones, one of these clones Est.R1, was chosen for further characterisation. This Est.R1 genomic clone had an EcoR1 insert of 1200 bp and produced ß-galactosidase fusion protein.

DNA sequence analysis of Est.R1 revealed an open reading frame of 741 bp corresponding to a polypeptide of 247 amino acids corresponding to the C-terminal end of a 40 kd protein detected in Western-Blots of sporulated and non-sporulated *E. stiedae* oocysts incubated with an antiserum against the recombinant ß- gal/fusion protein (SPF α Est.R1). An analysis of the incomplete sequence with the Swiss-prot, PC/gene and GCG Software data bank did not reveal any homology with a known sequence; this protein was classified as a transmembrane segment.

The result of Southern-blot showed that one fragment with BamH1 restriction digest of genomic DNA was detected; it is probable that this antigen is present as a single copy gene in the genome of the parasite. In this study, PFGE was also used to separate the genomic DNA of *E.stiedae*. We were able to determine that this species had at least 6 chromosomes ranging in size from 1.1 to 5.7 megabases. However, relative staining intensities indicates that there may be co migrating chromosomes. In addition, we demonstrated that the antigen of approximately 40 Kd is conserved in *E. magna*a and *E.intestinalis* while the reacting protein in *E. tenella* (the chicken coccidia) showed a higher molecular weight of 45 Kd.

Fluorescence staining was seen in the membrane of the parasite of *E. stiedae* while with *E. magna* and *E. intestinalis* it was localised in the refractile body. No staining observed in live sporozoites *E. stiedae* suggesting that this antigen is not exposed on the surface of the parasite but localised in the inner membrane.

The antiserum SPF α Est.R1 was tested for its ability to inhibit invasion of cell culture (MDBK) by sporozoites in vitro. After 6h incubation, the treatment with antiserum caused a reduction of cell invasion by sporozoites (approximately 43%) suggesting that this antiserum appeared to inhibit sporozoite invasion.

A Pseudomonas outer membrane lipoprotein (opr I) has been used as a carrier to produce a fusion protein for presentation of epitopes at the surface of *E. coli* cells. The purpose was to evaluate administration of live E. coli transformants containing a recombinant protein for delivering antigen

to the rabbit immune system. The Est.R1 fragment was subcloned from λgtll into pVUB1 vector and after induction at 37° with IPTG to 1mM final concentration a fusion protein of about 30 kda was detected in the outer membrane fraction together with some degradation products. The adjuvant property of lipoprotein I was also demonstrated since antibodies α LppI/Est.R1 could be obtained without adjuvant.

The immunofluorescence experiments also show the antibodies raised against Lpp/R1 fusion protein (with and without adjuvant) recognize the protein native on the parasite fixed while the preimmune and ß -gal/Est.R1 (Without adjuvant) did not. The same results were also observed in immunoblot analysis. FACS (Fluorescence-Activated-Cell-Sorter) showed that the antigen L//Est.R1 was taken up by lymphoma cells.

The antigen was tested for its ability to protect in-vivo. Hence, two experiments were done; first, SPF New Zealand white rabbits were immunized subcutaneously with purified fusion protein ß -galactosidase/Est.R1 or ß -galactosidase alone and were challenged with live sporozoites (*Emagna*, *E.intestinalis*).In the second experiment, the rabbits were vaccinated orally with live *E. coli* cells producing the α LppI-fusion protein before challenge with *E.magna* and *E.intestinalis*. No protection was observed in the animals from the first experiment, while some oocyst reduction was confirmed from the second experiment (62% for *E.intestinalis*, 17% for *E.magna*).

Further work will be done to optimise the immunising capacity of this antigen. Cloning of a gDNA or cDNA containing the entire gene might improve the ability to induce protective immunity.

AN OUTBREAK OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP) AND POST OUTBREAK SERO-SURVEILLANCE IN ERITREA

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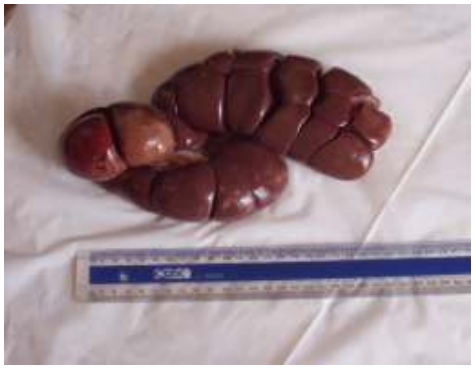
At the end of 2002 contagious bovine pleuropneumonia (CBPP) was never reported in Eritrea for the last 20 to 30 years. A major incursion of CBPP was reported from illegally smuggled Raya Azebo type cattle from Ethiopia, introduced through the Southern Red Sea administrative region of Eritrea (the Afar area bordering Ethiopia). The first case was detected in the Asmara abattoir during post mortem inspection. Characteristic CBPP clinical signs and post-mortem lesions were observed in affected herd during the outbreak of the disease. Significant number of in-contact indigenous cattle was infected by the disease. Samples were first sent to OIE reference laboratory CIRAD-EMVT in France for confirmation. The disease was confirmed by PCR on 15 January 2003.Samples of Pleural fluids and lung tissues were collected from CBPP infected animals and tested using the polymerase chain reaction (PCR) technique at the National Veterinary Laboratory. The affected cattle herds were held in an isolation pen and eventually destroyed following confirmation of the disease.

Following this incident, surveillance of CBPP was stepped-up and a total of 4,695 serum samples were collected from 313 sampling units or villages and submitted to the National Veterinary Laboratory for testing in 2004 one year after the outbreak of the disease as part of the epidemio-surveillance. Serum samples collected for sero-surveillance were tested for CBPP antibodies by the use of the monoclonal antibody specific for *Mycoplasma mycoides* subspecies *mycoides* sc as described in the competitive ELISA kit manual from CIRAD-EMVT, France. During this post outbreak sero-surveillance sampling season, all age group animals were eligible for sampling. Out of the total sampled animals 67 (1.43%) were positive for *Mycoplasma mycoides* subsp. *mycoides* sc antibodies using the cELISA test. This result suggested that very low lateral infectious agent transmission could have taken place. Vaccination as a control measure had never been applied in cattle throughout the country prior or after the outbreak.

The internal quality control (**IQC)** results were used to monitor the performance of the control sera and reagents supplied in the kit. Control charts to monitor the performance of cELISA assays used to estimate antibodies in serum samples were used for the serological tests conducted at the National Veterinary Laboratory. Some of the important clinical cases and post-mortem lesions:



Kidney infarction in CBPP infected cattle slaughtered in 2003 (photograph courtesy of T. Sebhatu)

Characteristic CBPP lesions in cattle Asmara Slaughter house, 2003 (photograph courtesy of T. Sebhatu)

Clinical cases of CBPP in Raya Azebo type (Ethiopian cattle) cattle, 2003 (photograph courtesy of T. Sebhatu)

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STUDY ON THE PREVALENCE AND THE AGE AT INITIAL COLONIZATION OF CAMPYLOBACTER IN BROILER FLOCKS IN CENTRAL PROVINCE OF SRI LANKA

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Campylobacter is considered one of the main causes of bacterial enteritis worldwide and poultry meat appears to act as a common source of infection (Vandeplas et al., 2008). In Sri Lanka, the available data to show the importance of campylobacter as a cause of diarrhoeal diseases is sparse and the involvement of poultry in relation to Campylobacter has not been identified. Therefore this study was carried out to investigate the association of campylobacter with broiler chicken.

The Campylobacter isolation and identification procedure based on ISO standard was established and poultry meat samples collected from retail markets were tested. A considerable number of meat samples were contaminated with thermotolerant Campylobacter.

As Campylobacter is a commensal in the chicken gut, though it is a pathogen to human, the prevalence of Campylobacter in broiler flocks was studied. Cloacal swabs collected at farms and caeca collected at slaughter in processing plants were analysed. Out of 59 samples collected from the Central Province 42 became positive for Campylobacter. The prevalence of Campylobacter in broiler flocks is 71%.

High prevalence indicated the importance of controlling the pathogen but there was no data available on the ecology of the pathogen in Sri Lanka. Previous studies elsewhere have shown that factors like geographical location, seasonality, farming system, bio-security measures as the key factors influencing the host pathogen relationship. However, most data have been generated in non-tropical countries with clear summer and winter seasons (Newell and Fearnley, 2003). The relative importance of these factors in Sri Lanka was questionable as it is a tropical country with no clear seasonality.

To investigate the colonization of Campylobacter in broiler flocks twenty broiler flocks reared in two farms where different management practices in use, in the Central Province were monitored during one year. From each flock, cloacae swabs were collected from randomly selected ten birds every other day until the flock became positive for Campylobacter. According to the findings, all flocks became positive for Campylobcater as shown in Table I and colonization was first seen at the age of 14-26 d. Irrespective of the absence of seasonality and low levels of bio security the age of colonization was in accordance with the literature. In this study there was no difference at the age of colonization in the farm A that practices all in all-out system and the farm B that practices multiple age production system.

Quantitative analysis of Campylobacter in the chicken gut and effect of feed modifications on the level of the pathogen load in the gut is under investigation.

TABLE I: AGE AT INITIAL COLONIZATION OF BROILERS IN FARM A AND B.

Farm	Flock	Age at initial colonization (Days)
Farm A that practice all-in all-out system	1A	20
	2A	20
	3A	26
	4A	26
Farm B that practice multiple age production system (4 groups of broilers of different ages in adjacent pens at a given time)	1B	20
	2B	20
	3B	20
	4B	20
	5B	19
	6B	14
	7B	22
	8B	22
	9B	19
	10B	26
	11B	23
	12B	21
	13B	18
	14B	18
	15B	23
	16B	23

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PREVALENCE AND CONTROL OF BOVINE DERMATOPHILOSIS IN ANGOLA

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The bovine dermatofilosis is an excudative and enzootic dermatite found in many livestock production area of in Angola. There is not limit of altitude and longitude to the presence and extend of the disease. A study carried out during the last five years with support of the Veterinary Services demonstrated the presence of the diseases on Northern, Central and Southern provinces of Angola with altitudes from 200 m to 2,000 m. Annual rainfall has real influence on the evolution of the disease.

FIG 1. Disseminated lesions

FIG 2. Pododermatite

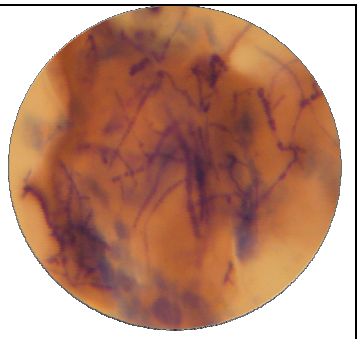

FIG 3. Micro photografia of dermatophlus congolensis.

DISEASE INCIDENCE

Among other animal diseases the dermatofilosis has an economical importance in the government restocking programme dues to its losses. Affected animals generally are in bad conditions and it is registered a decrease of productivity in term of meat, milk and drought powder. Sometimes the infection is associated to other skin diseases such as mange (*Demodex spp*). In all cases the incidence of the disease is more than 60 % in reference to mange, lumpy skin and mycosis.

ROUTINE DIAGNOSIS

The routine diagnosis is made by the Veterinary Services Field Personnel and confirmation is made in the four Laboratories of the Veterinary Research Institute (IIV), located in Cabinda, Luanda, Wako Kungo and Lubango. The clinical lesions are typical and assistance is provided to Field teams to recognize them during their activities (cattle dipping, treatment and vaccination campaign). OIE recommended techniques are used in IIV Laboratories (Fig. 3)

TREATMENT AND CONTROL

Animal dipping is practiced but not always efficient and experiment treatments are made in some commercial farms. The inexistence of curative treatment for most of the small holders is a big constraint to livestock development and rural families income. To alleviate this situation, the Veterinary Services have undertaken control regulations in term of quarantine and restrictions of animal movements in accordance to OIE rules. More engagement of this is necessary to reduce the incidence of the disease.

DISEASE PREVALENCE

Most of the cases of the disease are found in small holders, a few in commercial farms. The prevalence is between 10 to 50 % in the herds. Without treatment this prevalence always increases with pick in rain season. No significance difference was registered in reference to breed susceptibility (local or imported) and animal category. The disease lesions progress quickly and prognostic is always fatal (Vide Fig 1 and 2). The disease is as a matter of concern.

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MTADM: THE NEW JOINT MASTER PROGRAMME IN TRANSBOUNDARY ANIMAL DISEASE MANAGEMENT FOR EASTERN AFRICA

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Livestock issues are vehemently put back on Africa's agenda by the African Union (AU) and AU's New Partnership for Africa's Development (NEPAD) flagship Comprehensive Africa Agriculture Development Programme (CAADP).

Focus is on livestock for trade and export. Better policies, institutions, regulatory framework and technologies are sought for livestock production and management and delivery of veterinary services and disease control. The disease status of African countries places the pivotal constraints on trade possibilities. Animal health standards imposed by importing countries for international, regional or bi-lateral trade, and through the World Trade Organisation's (WTO) Sanitary and Phytosanitary (SPS) agreement must be met. 12 of the 15 most important transboundary animal diseases persist in Africa.

Disease control under SPS, entailing new standards, regulations and technologies, can and is not be covered by conventional veterinary training. This specialist area of its own has to be addressed in a specialised postgraduate course for young personnel already involved and responsible for public, private and hybrid animal disease control services. Ambitious visions of a new African livestock sector with changed focus on production, disease, trade, marketing, organisation, delivery and internationality are only realistic with newly trained animal disease control personnel.

To target these issues at the academic level the Addis Ababa University / Ethiopia with universities of 3 regional partner countries (Kenya, Uganda, Sudan) and the Freie Universität Berlin, Germany, successfully applied for a grant to establish a Joint Master Course in Transboundary Animal Disease Management (MTADM) for Africa. The 3-year project is funded under the EU - EDULINK Programme of the 9th European Development Funds (EDF) as from 2008 to 2010. Currently, preparatory work is ongoing on the final technical details of the MTADM Course.

The *overall* objective of the programme is to strengthen the capacity of national veterinary services in Africa to control and manage trans-boundary and epidemic diseases more effectively in a regional concerted action so as to (a) contributing towards developing or expanding exports markets and trade for animals and animal products and, (b) improve in the longer run the livelihood of livestock keepers as well as consumers demands on quality and safety of animal products.

The *specific* objectives are to build human resource capacity by producing an effective cadre of professionals in regional / trans-boundary animal disease control and management and to strengthen the regional network of the veterinary faculties of the participating African countries.

This is to be achieved through:

- the development of an innovative and state-of-the-art curriculum for the Joint MTAD Programme,
- a first MTADM course executed at Addis Ababa University/Ethiopia, Freie Universität Berlin, and the African home country of the participant,
- a second MTADM Course prepared and ready to be launched at a second Regional Partner University, and
- a first group of at least 15 graduates being highly qualified and ready to take up meaningful employment in the animal disease control sectors.

The MTADM Master Course is delivered as cross-border "sandwich" programme, in modular form (EU system of ECTS credits; 120 ECTS total) with the Master Thesis carried out as a field study project under guidance of the partner universities in the African region.

The MTADM Master Programme is directed towards young early-career professionals already involved in animal disease and sanitary control activities but who identified academic master-level training needs for innovative tools and methods in transboundary animal disease management to advance their career.

The Course Announcement will be circulated early 2009 and applications are welcome before October 2009; qualified candidates may apply for scholarships provided by German, regional and international organisations.

PRELIMINARY RESULTS OF THE c-ELISA FOR THE SURVEILLANCE OF ANTIBODIES TO BLUETONGUE VIRUS IN LIVESTOCK IN MONGOLIA

C. Tungalag

Mongolia

PCR BASED DETECTION AND EPIDEMIOLOGY OF FMDV AND PPRV IN FIELD SAMPLES FROM PUNJAB, PAKISTAN

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Foot-and-mouth disease is a highly contagious, viral infection of cloven-footed animals. It is a major threat to world's livestock and of considerable economic importance to both developed and developing countries. Peste des petits ruminants (PPR) is another acute, contagious, viral disease of small ruminants, which is of great economic importance in many parts of world including Pakistan. Sero-surveillance of PPR has recently been conducted in the Punjab province of Pakistan [1]. The emergence and re-emergence of trans-boundary animal diseases (TADs), e.g., Foot and Mouth Disease (FMD) and Peste des petits ruminants (PPR) strongly indicate the need for the development of powerful and sensitive diagnostic methods. Molecular biological techniques have been successfully exploited to improve both the speed and accuracy of disease diagnosis. Molecular epidemiology and viral phylogeny provide new possibilities to combat and eradicate infectious diseases. The polymerase chain reaction (PCR) has proved to be very powerful tool for disease diagnosis and molecular epidemiology

PCR based detection was optimized and then successfully applied to the suspected field samples of FMD and PPR collected from different districts of Punjab, Pakistan. For this purpose RNA was extracted using TriReagent^{TM}, which was then reverse transcriptised using MuLV reverse transcriptase and random hexamer primer. The resulting cDNA was then amplified using specific primers designed for the amplification of VP1 (in case of FMDV) and F or P gene (in case of PPRV) using Taq Polymerase. The PCR products showing the positive results were then cloned in pTZ57R vector and then transformed in Top 10α competent cells (a strain of *E. coli)*. The transformed colonies were picked and restriction digestion was performed using ECOR1 and Pst1 enzymes to isolate the plasmid. The digested products were then sequenced using M13 forward and reverse primers. The sequences were then aligned in Clastal W software and pylogenetic tree was developed.

On the basis of sequence analysis, it was confirmed that the FMDV isolated from different regions of Punjab was strain O with more than 90% homology with the neighbouring countries (India, Afghanistan and Iran). While, the sequences were very distinct from Middle East and European stains of FMDV O [2]. Furthermore, all the sequences fall in Pan-Asia 2 lineage of a broad group Pan-Asia and Middle East south Asia topotype. Three representative sequences of different areas of same outbreak season were submitted to gene data bank and their accession no. are AM942747, AM942748 and AM942749. The PPRV sequences of F gene were aligned with the available sequences in data bank it was confirmed that all sequences fall in lineage 4 [3]. The findings of these studies will help the authorities in Pakistan to formulate the vaccines for the effective control of these economically very important diseases of animals and also provide the recent picture of circulating FMDV and PPRV in the areas under investigation.

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AN OVERVIEW OF GOAT FARMING IN THE HAMBANTOTA DISTRICT OF SRI LANKA, WITH SPECIAL REFERENCE TO HEALTH ASPECTS

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A survey was carried out with the intention of gathering knowledge of local farmers (n=108) on goat husbandry practices and their health status in the Hambantota district in the Southern province of Sri Lanka. Information was collected using a pre-tested questionnaire by interviewing the farmers. Apart from general farm and herd data, also questions on mortality, medicinal treatments and feeding were included.

Dairy goats in the district accounted for 62% of total goats, while kids and billy goats accounted for 30 and 8%, respectively. The overall mortality in the goatherds was found to be 20% per year. Kid mortality has become a serious threat to the goat farmers in the Hambantota district where 68% out of total deaths in goats were kids, while the proportions of billy goats and dairy goats were lower with 8 and 24%, respectively (Figure 1). Death of kids mainly happens because of the prevailing dry and harsh environment in this area. Hence, kids undergo a lot of environmental stress and therefore only the fittest survive. In addition, clean water and fresh feed often was not available due to prevailing high temperatures. Because of clean water scarcity dairy goats also do not produce enough milk to nurse their kids. Travelling long distances with the herd to find feed has also been identified as major cause of kid mortality. In addition, poor sanitation practices have facilitated the prevalence of several contagious diseases such as foot rot and pustular dermatitis (CPD). Physiological disorders like bloating of the goats are common in both dry and wet seasons as well.

According to the questionnaires' results, commercial medicine to treat their animals was used by 83% of the farmers while only 3% of the farmers applied indigenous medicine. This can be explained by the well-established veterinary service network in the Hambantota district to which almost all the farmers have access. Only few farmers are not satisfied with the veterinary services and rather use indigenous medicine. Another 14% of the farmers did not use any kind of medicinal treatment on their goats, ignoring their health status. Out of the farmers that used commercial medicine, 25% used anti-parasitic treatments, vaccination, antibiotics, or minerals and vitamins as health treatments (41%, 25%, 9%, or 8%, respectively) (Figure 2). However, there is an increased risk of an epidemic outbreak in this district among goats due to their generally low health status.

Employing bio-diversity-based concepts in goat feeding was more prominent among rural than peri-urban farmers in the Hambantota district. Shrub and tree species used for feeding are *Dichrostachys cinerea* (Andara), *Flueggea leucopyrus* (Katupila), *Azadirachta indica* (Neem), *Tamarindus indica* (Tamarind) and *Leucaena leucocephala* (Ipil ipil). Feeding supplements such as rice bran, coconut oil cake and salt was practiced by 44% of the farmers and thereof by the ones living in peri-urban areas where access to supplementary feeds was easier. Besides that kitchen waste and refused coconut scrapings were also used by farmers to feed their goats during dry season in order to overcome feed scarcity. Free-living goats in the urban area tended to consume market garbage, paper posters on walls, pieces of clothes, and papers to satisfy their appetite.

Selling goats for breeding purposes or meat production is very popular among goat farmers in the Hambantota district. Billy goats (47%) and dairy goats (46%) were the most preferred categories to be sold for an increase in the farmers' monetary income. By contrast, selling goat kids seems uncommon as it accounted only for 7% of the total sales, and the kids sold were mainly of poor condition as rearing them would mean monetary loss to the farmer.

The goat management system most prominent in the Hambantota district is of extensive nature. Around 97% of the farmers keep the animals on the farm free living during daytime and collect them in barns during night-time to protect them from thefts and unfavourable environmental conditions. The remaining 3% of the farmers are situated in urban and peri-urban areas and keep goats in barns permanently.

With the present survey it could be determined that the health status of the goats in Hambantota district is not meeting desirable standards. This is mainly due to the poor knowledge and, sometimes, ignorance of the farmers. Recommendations to overcome this situation would be to educate farmers, to prevent and control diseases, and to improve condition by more strategic feeding especially during the dry season.

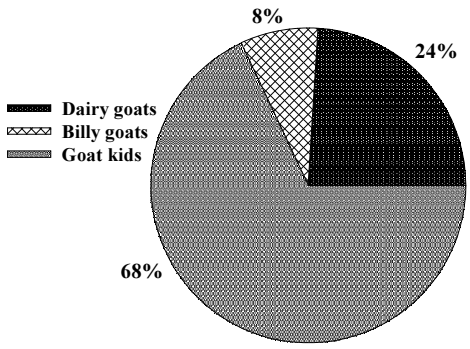

FIG. 1. Goat mortality in Hambantota district

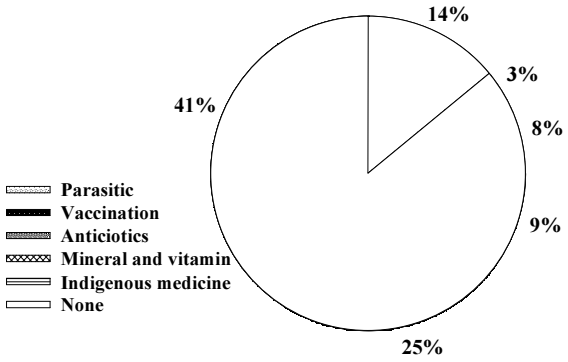

FIG. 2. Medical treatments of goats in Hambantota district

ANTHELMINTIC ACTIVITY OF MEDICINAL PLANTS EXTRACT AGAINST GASTROINTESTINAL NEMATODES IN NATURALLY INFECTED GOATS

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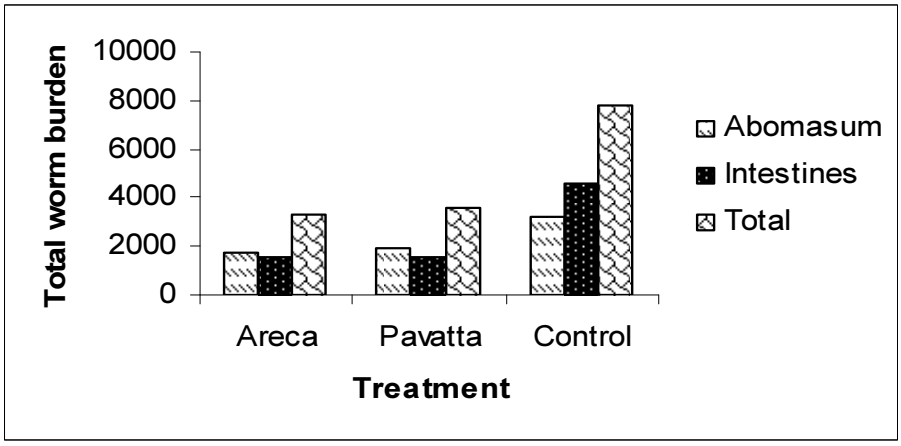
At present, goat farming, either as a full-time or part –time activity, is in increasingly being recognized as an economically beneficial livestock enterprise in tropical and sub tropical countries. Despite this popularity of goat industry, several constraints have limited the full potential of this farming activity in these countries. Among the multitude of impediments, which limit the development of the goat industry, gastrointestinal nematode infection represents a major constraint in Sri Lanka [1] like in other parts of the tropics. Recent studies in Sri Lanka have shown, gastrointestinal nematode infections decrease the productivity, particularly weight gain in meat goats in the dry zone [1]. The work done in dry zone of Sri Lanka has indicated that an overwhelming majority (99%) of goats were affected by a moderate to high gastrointestinal nematode infection. Successful control gastrointestinal nematodiasis infection is important for the sustainable productivity of the goat industry. The control of gastrointestinal nematodiasis is usually performed using synthetic anthelmintics [2]. The appearance of nematode resistance to these anthelmintics and several other factors high–light the need to developing alternatives. This stimulated the research of alternatives, such as medicinal plants to control gastrointestinal nematodiasis [3]. According to circumstances and depending on their efficacy, naturally produced plant anthelmintics offer an alternative that can overcome some of these problems and is both sustainable and environmentally acceptable.

Fifteen plant extracts were screened using an *in vitro* larval migration inhibition (LMI) assay. Out of the fifteen crude extracts *Azadiractha indica* (neem) seed (NS), *Areca catechu*(areca-nut) unripe fruit kernel(AUFK), *Adhatoda vasica* (pavatta-adathoda) leaves (PL), caused a significant($P < 0.001$)reduction in larval migration. The plant extracts with significant inhibitory activity were subjected to short-term dose titration trails and in the dose titration trial, the crude extracts of AUFK and PL significantly (<0.05) reduced the number of worm eggs in the faeces of naturally infected goats. Therefore based on these results a long-term prophylactic trial was conducted using the crude extracts of AUFK and PL for a period of six months to evaluate the effect of the extracts on gastrointestinal nematode burdens, faecal egg counts (FEC) as well as live weight gain (LWG) and packed cell volume.

Forty naturally infected goats were divided into four (A-D) treatment groups. At the commencement of the trial, each group was drenched orally with single dose of one of the following; GpA: Areca 6mg/kg BW (Body weight); Gp B: Pavatta 54mg/kg BW; Gp C: Levamisole 12 mg/kg and Gp D: remained untreated and drenching was continued at 14-day intervals. LWG of the group treated with ANFK showed a significant ($P < 0.05$) difference during the last two months of the trial and the pavatta treated group showed a significant LWG during the last month of the trail. The mean monthly FEC remained high in the control group throughout the trial, whereas in the groups treated with extracts FEC decreased significantly ($P < 0.05$) from the second month until the end of the trial. The mean packed cell volume of the treated groups increased from the second month of the trial, but level was not significant ($P > 0.05$). ANUFK and PL extracts at the dose rates used was effective in controlling the GI nematodiasis in goats. ANUFK and PL could be used in control strategies against GI nematodes in organic and conventional production systems.

Month	Treatment		
	Areca nut unripe fruit kernell	Pavatta leaves	Control
July	1.57 ± 0.20	1.17 ± 0.28	1.50 ± 0.26
August	2.14 ± 0.24	1.88 ± 0.18	1.42 ± 0.15
September	2.5 ± 0.29	2.17 ± 0.21	1.50 ± 0.13
October	2.86 ± 0.45	2.5 ± 0.22	1.92 ± 0.15
November	3.43 ± 0.32	3.08 ± 0.40	2.008 ± 0.08
December	4.29 ± 0.45	4 ± 0.50	2.58 ± 0.20

Mean live weight gain in goats of treated groups and control group



Total worm burden of control and treatment groups

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ANTHELMINTHIC ACTIVITY OF *DANIELLIA OLIVERI* AGAINST *HAEMONCHUS CONTORTUS* WORMS IN BURKINA FASO

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In the central region of the Burkina Faso, small holders like to use veterinary traditional practice to treat small ruminant gastro-intestinal parasitism. In this part of country, *Daniellia oliveri* plant is usually used against this disease. Aqueous decoction obtained from the stem bark of *Daniellia oliveri* was screened to determine his phytochemical composition and *in vitro* anthelmintic activity against *Haemonchus contortus* adult worms. Results showed that the decoction lyophilized obtained is giving to small ruminant (25 kg) at the rate of 242.5 mg per body weight. *In vitro* anthelminthic revealed that *Daniellia oliveri* extract has significant effect ($P < 0.05$) on mortality or the paralysis of the adult worms of *Haemonchus contortus* compared to the control group. In fact, use of *Daniellia oliveri* leaves by pastoralist smallholders in traditional therapy against the gastro-intestinal small ruminant parasites is justified in the central region of the Burkina Faso. Therefore, the survey suggests to achieve other studies (phytochemical and toxicity) on *Anogeissus leiocarpus* leaves studied in order to develop a further drug antiparasitic subsequently.

Photo 1: Un arbre de Daniellia oliveri intensément exploité dans la région centrale du Burkina Faso

TABLEAU 1 : RÉSULTATS DU SCREENING PHYTOCHIMIQUE DES ÉCORCES DE TIGES DE *DANIELLIA OLIVERI*

Tests	Réactions chimiques
Flavonoïdes	(+)
Tanins et polyphenols	(+)
Saponosides	(+)
Coumarines	(-)
Stéroïdes/triterpènes	(+)
Alcaloïdes	(-)

(+) : réaction positive (-) : réaction négative

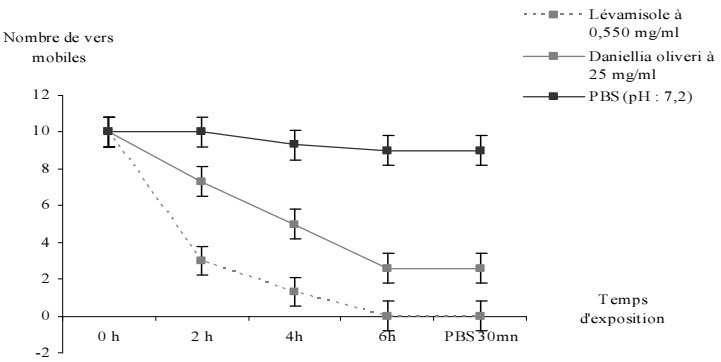

FIG 1. Effets in vitro du décocté aqueux lyophilisé de Daniellia oliveri sur les vers adultes vivants d'Haemonchus contortus d'ovins en comparaison avec les témoins positif (lévamisole) et négatif (PBS)

ANTHELMINTHIC ACTIVITY OF *ANOGEISSUS LEIOCARPUS* AGAINST *HAEMONCHUS CONTORTUS* WORMS IN BURKINA FASO

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Traditional Veterinary practice is current by pastoralist and small holders to treat small ruminant gastro-intestinal parasitism in the central region of the Burkina Faso. To treat these digestive pathologies, *Anogeissus leiocarpus* is usually used. Ethno-veterinary surveys have been achieved to understand the traditional use of *Anogeissus leiocarpus* and *in vitro* test has been carried out to value anthelminthic activity on the adult worms of *Haemonchus contortus*. Results showed that the plant part used are leaves and by oral way after decoction to obtain aqueous extract which is giving to small ruminant (25 kg) at the rate of 160 mg per body weight. *In vitro* anthelminthic activity test of aqueous extract of leaves revealed a significant effect ($P < 0,05$) on mortality or the paralysis of the adult worms of *Haemonchus contortus* compared to the control group (PBS). In fact, use of *Anogeissus leiocarpus* leaves by pastoralist and small holders in traditional therapy against the gastro-intestinal small ruminant parasites is justified in the central region of the Burkina Faso. Therefore, the survey suggests to achieve other pharmacologies and parasitologies studies on *Anogeissus leiocarpus* leaves in order to develop a further drug antiparasitic subsequently.

Photo 1 : Un arbre d'Anogeissus leiocarpus de la région centrale du Burkina Faso

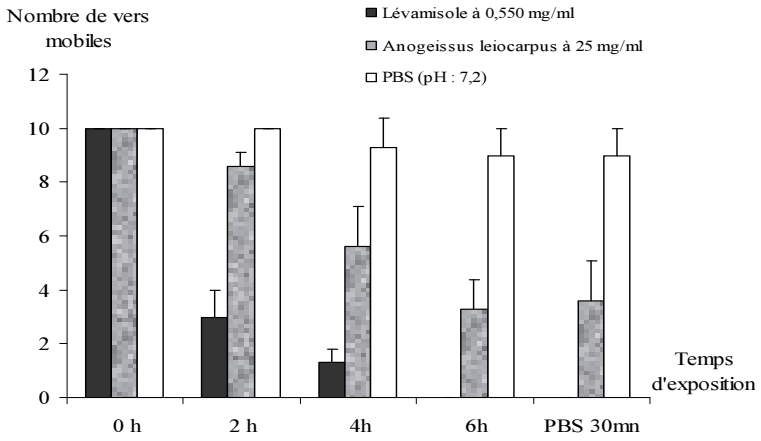

FIG 1. Effets in vitro de l'extrait aqueux de Anogeissus leiocarpus sur les vers adultes vivants de Haemonchus contortus des ovins en comparaison avec les témoins positif (lévamisole) et négatif (PBS). (: P < 0,05 par rapport au témoin PBS)*

NEWCASTLE DISEASE: MOLECULAR-BIOLOGICAL DIAGNOSIS IN THE RUSSIAN FEDERATION

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Newcastle disease is registered in the Russian Federation and for last time it is seen an upward tendency of ND outbreaks in backyards poultry due to lack of specific prophylaxis. Reports on ND outbreaks from 2004 to 2007 submitted to OIE are shown in Table I.

TABLE I. OUTBREAKS OF NEWCASTLE DISEASE IN RUSSIAN FEDERATION IN 2004-2007

Year	Registered outbreaks
2004	7
2005	25
2006	30
2007	36

The use of molecular-biological methods can reveal ND virus in the lab. ARRIAH has acquired practical experience in the diagnosis of ND virus and holds data on ND viruses identified or isolated in the course of the current diagnostic and monitoring investigations of samples from wild and domestic birds. The developed set of molecular-biological methods allows ND virus to be reliably and rapidly detected in field samples. They are also able to characterize ND viruses with F-gene sequencing that can be used for the assessment of potential virulence, their phylogenetic belonging which will aid in making decisions of how to fight the disease, to trace a possible source of infection and further spread.

The developed set of molecular-biological methods of ND diagnosis includes:

- ND virus detection with RT-PCR and F-gene sequencing, determination of potential virulence based on F_0-amino acid restriction site;
- ND virus detection with RT-PCR and ND-gene sequencing;
- ND virus detection with RT-PCR and vaccine strain La-Sota differentiation based on the F-gene;
- Genotyping of ND viruses revealed in Russia

Genetic characterization of ND viruses allows rapid and reliable determination of their group belonging and also helps make conclusions about presence or absence of any epizootic links among outbreaks, their possible source, spread routes, current and potential threat to poultry farms.

A total of 657 field samples from wild and domestic birds were PCR tested in 2007. The samples covered 20 regions of Russia and the Ukraine. ND virus was found in 8 regions of Russia. 516 samples were from wild birds with 9 positive results and 141 samples were from domestic birds with 16 positive results. PCR and sequencing identified 17 isolates of ND virus 8 isolates of which proved to be highly virulent based on F_0 amino acid restriction site. Six isolates with the following restriction site RRQKR-F were isolated from chickens. Two isolates with the following restriction site KRQKR-F were isolated from pigeons. The rest of nine isolates proved avirulent based on F_0-amino acid restriction site (GRQGR-L, GKQGR-L, ERQER-L) and were isolated from wild birds during the monitoring investigations.

EPIDEMIOLOGICAL SURVEY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP) IN MALI

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Mali is a major producer of livestock, which plays a predominant role in the economy of the country. It brings about 10% of the GDP, 15% of export incomes and constitutes the main source of incomes for the 30% of the national population. Now that rinderpest is controlled, contagious bovine pleuropneumonia (CBPP) remains the most important infectious disease of cattle not only in Mali, but in most tropical African countries causing great economic losses including mortality, loss of weight, reduced working ability, reduced fertility and indirect costs due to control programs. The disease is characterized by a severe fibrinous exudative pleuropneumonia and is caused by *Mycoplasma mycoides* subsp. *mycoides* SC (*MmmCS*). It is considered a priority disease by the Office of International Epizootie (OIE), the Pan-African Program for the control of epizootics (PACE) and the FAO-Emergency Prevention System for Transboundary Animal Diseases (FAO-EMPRES).

All these Institutions have recognized that the lack of information concerning the prevalence and the economic impact of the disease is an obstacle to implement any efficient program for its control in Africa. Therefore, research must be conducted in order to generate accurate data on the incidence and distribution of the disease before any appropriate coordinated control program can be defined for this continent.

The present epidemiological study was conducted with the aim of studying the seroprevalence of the disease among cattle in Mali, its geographical distribution and its economical impacts. Serum samples collected from 8000 cattle in different parts of the country were tested for *MmmSC* specific antibody by the competitive ELISA (c-ELISA) and the complement fixation test (CFT). In parallel, data of CBPP cases for the past 10 years and the economic impact of the disease, including production losses and control costs were evaluated. The preliminary results of this study will be presented here.

POSSIBLE IMPLICATION OF GROUND RADIATION IN THE DEVELOPMENT OF KHARI DISEASE IN LACTATING BUFFALOES IN THE FAR WESTERN NEPAL

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Khari disease, a chronic debilitating disease of lactating buffaloes that has been reported since last 2 decades from the far western Nepal [1] is characterized by hoof and skeleton deformities and appearance of chalky powder in the hooves (Figure 1). The word "Khari" has been coined locally for chalky powder and this disease is prevalent in the stall-fed animals during the winter.

Khari affected areas of Darchula and Baitadi Districts were visited by a team of a senior veterinary and radiation experts in coordination with the District Livestock Service Office. Using a portable Dosimeter (RGD 27091), ground radiation dose rates at Khari hit areas were measured. Ground radiations in these two districts were found to be higher (maximum of 15.0 μSv/hr) than other parts of the country (0.3-0.5 μSv/hr). Indoor radiation in the buffalo shed was also found to be higher than outside the shed. This finding may be suggestive of why Khari affected buffalo reportedly showed improvement after allowing her for outdoor grazing rather than keeping indoors, which might be due to lesser amount of radiation in the open grazing environment. More detailed study with respect to Khari disease and its possible linkage with ground radiation is warranted to fully elucidate the implication of radiation in the development of Khari disease in milking buffaloes and in other mammalian species including human.

Efforts were made to assess the level of Selenium in the soil, forages and water samples in Khari affected areas besides analyzing its level in sera and chalky powder from the affected animals. Due to lacks of Atomic Absorption Spectrophotometer (AAS) and good laboratory backups, no consistent level of Selenium could be measured even at a private facility during different time points. Some of the hay samples showing higher levels of selenium at a private laboratory in Kathmandu (test done in July 2007) were ground into powders to get permit from USDA, and were then analyzed at the Veterinary Toxicology Laboratory, Colorado State University. None of the samples exhibited higher levels of selenium (test done in January 2009) unlike in Nepal. Some investigators have reported on oxidative degradation of selenium from sera samples during storage [2] and our low data on subsequent analysis may be attributed to the similar reasons and that need independent verification.

Regarding Khari disease's susceptibility, apparently, only lactating buffaloes seem to be susceptible. Cases of cattle doing well, while lactating buffalo is suffering from Khari disease (Figure 2) have been recorded. Although the malnutrition and parasitic infestations are rampant throughout the hills of the country but why malnutrition of only this locality is taking heavy toll on milking buffaloes annually. This investigator feels that in above malnutrition and parasitic infestations, inciting causes like Selenium toxicity and higher ground radiation may act in a concerted manner. The first response trial with pentasulfates in three dozens of affected buffaloes is showing improvement and which also implicates the excess of selenium.

FIG. 1. Appearance of chalky white powder from the Khari disease after gentle scratch

FIG. 2. Showing susceptibility of lactating buffalo to Khari disease with normal cow

Expertise of IAEA would be of immense value especially in ruling out the involvement and sources of radiation, if any, besides assisting in laboratory strengthening for enabling us to measure selenium and other toxic and deficit minerals in the biological samples.

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DETECTION OF OVINE *THEILERIA* SPECIES IN LAHORE DISTRICT, PAKISTAN

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The prevalence of Theileria species in sheep in district Lahore during spring and summer season in year 2007 was studied by taking a total of 200 whole blood along with thin blood smears and 100 samples of ticks from 20 flocks in different locations in district Lahore with a history of tick infestation, relapse of fever and anaemia. On microscopic examination 44/200(22%) samples were positive for *theileria* while 70/200(35%)blood samples were positive theileria species by PCR out of which 79% were positive for T. ovis and 21% for *T. lestoquardi.* The clinical signs were recorded in 30% (60/200) sheep. Out of total one hundred tick samples the prevalence of *Hyalomma* was highest (45%) followed by *Rhipicephalus* (41%) and lowest for *Boophilus* (14%). The prevalence of *T. ovis* was 65.8% (27/41) as compared to 66.6% (30/45) for *T. lestoquardi* in *Rhipicephalus* and *Hyalomma* ticks, respectively.

TABLE I. *THEILERIA* SPECIFIC, *T.OVIS* SPECIFIC AND *T.LESTOQUARDI* PRIMERS USED IN THE PRESENT STUDY.

Theileria Specific: Allsopp et al. (1993): 1098 bp fragment of the small subunit ribosomal RNA (ssu rRNA) gene. 989 F;5′-AGTTTCTGACCTATCAG-3′ 990 R; 5′-TTGCCTTAAACTTCCTTG-3′
T. ovis. (Altay et al., 2005): 520 bp fragment of the small subunit ribosomal RNA (ssu rRNA) gene. TSsr 170F; 5′-TCGAGACCTTCGGGT-3′, TSsr 670R; 5′-TCCGGACATTGTAAAACAAA-3′
T. lestoquardi. Kirvar et al. (1998): 785 bp to amplify the gene coding for the 30 kDa *T. lestoquardi* merozoite surface antigene. 5′-GTGCCGCAAGTGAGTCA-3′ 5′-GGACTGATGAGAAGACGATGAG-3′

PHYLOGENETIC ANALYSIS OF THE CAPRIPOX VIRUS RPO30 GENE AND ITS USE FOR DEVELOPMENT OF A PCR FOR DIFFERENTIATING SPPV FROM GTPV

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The Genus *Capripoxvirus* (CaPV) of the *Poxviridae* family comprises *sheep poxvirus* (SPPV), *goat poxvirus* (GTPV) and *lumpy skin disease virus* (LSDV), which are responsible for economically important diseases affecting sheep, goat and cattle respectively. The nomenclature of capripoxviruses remains problematic, since there is still no molecular criterion upon which to base strain designation. Previous reports, based on partial or full genome sequences of CaPVs, indicated that SPPV, GTPV and LSDV are genetically distinct from each other and can be grouped as three different species: goat poxvirus, sheep poxvirus and lumpy skin disease virus. However, these reports were based on analysis of a very limited number of CaPVs.

In an attempt to contribute to the creation of more stringent data for CaPVs' classification as well as their genotyping, we have cloned and sequenced the homologue of the Vaccinia virus RNA polymerase subunit 30 KDa (RPO30) genes of several isolates of CaPVs of different geographical origin. The phylogenetic reconstructions based on both nucleotides and amino acid sequences show that the CaPVs can be segregated into three different lineages according to their host origins: the sheep poxvirus lineage, the goat poxvirus lineage and the lumpy skin disease lineage. This is in agreement with our previous findings with the CaPVs G-protein-coupled chemokine receptor (GPCR) gene, which was also found to be suitable for virus-animal origin discrimination. However contrasting with the results from the GPCR gene where a 21-nucleotides deletion found in SPPV strains is also present in some GTPVs strains, the alignment of the nucleotides sequences of the RPO30 gene of the CaPV strains shows a 21-nucleotides deletion in all individuals within only the SPPV group. The occurrence of this deletion was exploited to design a classical PCR method to differentiate SPPV from GTPV. This test allows a rapid differential diagnosis of diseases caused by either SPPV or GTPV strains.

SESSION 4: ONE HEALTH

ONE WORLD ONE HEALTH: WHAT CAN BE LEARNED FROM THE FARM TO THE FORK

J. Schlundt

WHO

ONE WORLD ONE HEALTH: IS THERE A NEED FOR A GLOBAL RESEARCH AGENDA?

E. Schelling

Switzerland

BENEFITS OF INTEGRATING LABORATOTY RESULTS FROM ANIMAL, FOOD AND HUMAN INVESTIGATIONS

D. Lo-Fo-Wong

WHO

ORAL PRESENTATIONS

RICKETTSIOSES IN THE CENTRAL HILLS OF SRI LANKA: PREVALENCE OF SPOTTED FEVER GROUP INFECTIONS

RPVJ Rajapakse,

Sri Lanka

USE OF ANTIBIOTICS IN ANIMAL AGRICULTURE AND THE FATE OF ANTIBIOTIC RESIDUES AND RESISTANCE GENES IN THE ENVIRONMENT AFTER LAND APPLICATION OF SWINE MANURE

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Two swine confinement facilities, designated sites A and C were the focus of study. The antibiotic regimens at both sites included chlortetracycline and tylosin. Hog manure at these sites was treated in open, unlined lagoons before being applied as fertilizer to onsite (site A) and offsite (Site C) farm fields. Sites differed in their sub-surface geology, and each site was outfitted with a network of groundwater sampling wells for the monitoring of chemical contaminants, antibiotic residues, bacterial indicators of faecal contamination, and antibiotic resistance genes. Sterile containers were used to collect water from waste lagoons and wells once in 2000, and twice in 2001 and 2002. Additionally, the presence of antibiotic resistance genes was investigated from soil samples collected from 2005 to 2007 from seven different fields that were amended with manure.

DNA was extracted from water and soil samples. Detection of antibiotic resistance genes was accomplished by PCR using primers that have been described elsewhere. These primer sets targeted three major groups of antibiotic resistance genes: 1) four classes of genes (*tet*(M), *tet*(O), *tet*(Q), *tet*(W)) conferring resistance to tetracycline by means of ribosomal protection proteins; 2) three classes of genes (*tet*(C), *tet*(H), *tet*(Z)) conferring resistance to tetracycline by means of efflux pump proteins; 3) eight RNA methylase genes (*tlr*(B), *tlr*(D), *erm*(A), *erm*(B), *erm*(C), *erm*(F), *erm*(G), *erm*(Q)) conferring resistance to macrolide antibiotics, including tylosin and erythromycin, as well as to the lincosamide antibiotics and Streptogramin-B. The RNA methylases *tlr*(B) and *tlr*(D) have been found in tylosin-producing strains of soil bacteria, while the other six *erm* genes come from a diversity of pathogenic, human commensal, and environmental bacteria. These genes were selected as targets based on preliminary surveys of lagoon and groundwater and upon the antibiotic usage of the study sites. Presence-absence data for the surveyed genes were analyzed by principal components analysis (PCA).

Manure treatment lagoons and storage pits were always found to contain every *tet* gene for which surveys were conducted (Koike et al., 2007), and, likewise, five out six *erm* genes found at these sites were detected in nearly every lagoon sample (Koike et al., *in review*). A subset of groundwater wells at site A were found to contain both *tet* and *erm* genes with much higher frequencies than other wells, and the detection frequencies of most *tet* and *erm* genes for these wells were close to 100%. These “impacted” wells (A6, A8, A9, A11, A12) were all located in close proximity to the source lagoon, and most of them were situated in a relatively porous aquifer that bisected the lagoon. Chemical indicators of lagoon leakage, such as chloride and ammonium concentrations, were previously seen to be elevated in these impacted wells (Mackie et al., 2006, Koike et al., 2007). The number of antibiotic resistance genes in other wells, including background wells, was extremely variable over time, with a tendency for the detection frequencies of many genes to be quite low. Antibiotic resistance gene pools in soils were impacted by the addition of manure. Background detection frequencies of *tet* genes in soil were close to zero, but immediately after manure injection, it was possible to detect all *tet* genes for which surveys were conducted in most soil samples. Over time, the detection frequency of some *tet* genes (*tet*(M), *tet*(O), *tet*(H), *tet*(Z)) returned to near-zero, while others (*tet*(Q), *tet*(W), *tet*(C)) persisted. The detection frequencies of *tet*(C) and *tet*(W) genes remained high five months after manure injection, and at site C the *tet*(C) gene was still detectable in many soil samples eighteen months after manure injection.

Interestingly, the temporal and spatial patterns of antibiotic resistance genes in soils and water did not seem to depend on direct selection pressure due to antibiotic persistence, as levels of antibiotic

residues were generally found to be low or below the detection limit in soil and water samples (Mackie et al., 2006). These results support previous observations that the problem of antibiotic resistant bacteria is not necessarily linked to the persistence of antibiotic residues in the environments. Different genes have differential abilities to persist in soils and waters, which suggests that a "gene ecology" perspective which includes the recognition that genes may differ in their capacity to find new hosts via horizontal gene transfer, will be important for assessing the impact of agricultural activities on antibiotic resistance. Different genes have differential abilities to persist in soils and waters, which suggests that a "gene ecology" perspective which includes the recognition that genes may differ in their capacity to find new hosts via horizontal gene transfer, will be important for assessing the impact of agricultural activities on antibiotic resistance.

THE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR RAPID SCREENING AND EARLY DETECTION OF ACUTE LEPTOSPIROSIS

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The enzyme-linked immunosorbent assay (ELISA) [1] was compared to the microscopic agglutination test (MAT) [2] in a serological survey for leptospiral infection in dogs. Results from two studies in the survey showed that the ELISA was more sensitive than the MAT: Study 1 had 81% (133/165) prevalence as detected by the ELISA whilst the MAT detected 33% (54/165) of the dogs had evidence of leptospiral infection. In another study, the ELISA detected 59% (17/29) whilst the MAT recognized only 10% (3/29). The ELISA using our own boiled antigen was found to be a useful screening technique. The antigen was easy to prepare and can be made available to any laboratory that has leptospiral cultures. The ELISA has the advantage of being a simple, rapid, sensitive and less hazardous assay compared to the established MAT. It will be a useful technique for screening serum samples for leptospiral infection. The serum samples were from stray dogs kept in a non-governmental organization. Stray dogs have been suspected of spreading leptospiral infection to other animals and humans.

In certain countries livestock have been infected with closely related leptospiral serovars and both the ELISA and MAT have difficulties to identify the causal leptospiral serovar. In New Zealand, serovars *hardjo* and *balcanica* are closely related and have been placed in the same serogroup. Both serovars infect cattle in New Zealand and it is critical to identify the true causal serovar as their maintenance host and epidemiology are entirely different. In our study, using the type-specific main (TM) antigen [3], the ELISA was made to be more specific and was able to differentiate closely related leptospiral serovars such as between serovars *hardjo* and *hebdomadis*. The TM antigen which is the Fraction V protein was extracted from a serovar *hardjo* culture. The TM-ELISA was applied to a small number of serum samples obtained from local cattle and it was seen that the ELISA was more sensitive than the MAT. TM-ELISA detected a prevalence of 93% (14/15) whilst the MAT detected only 67% (10/15). Only one serum sample was negative to both ELISA and MAT. With the other positive sera, the titres obtained by ELISA were greater than that obtained by the MAT. Four serum samples that were negative by the MAT, had ELISA titres between 1/1000 and 1/2000. In general the TM-ELISA was about 3 times more sensitive than the MAT.

Another important finding of the ELISA using serovar *hardjo* TM antigen was that the assay was able to differentiate serovar *hardjo* infection from that caused by the other leptospiral serovars tested. The *hardjo* TM-ELISA was tested on a number of known infected serum samples obtained from rabbits and it was seen that it was able to differentiate the titres due to the other serovars including closely related serovars like serovars *hardjo* and *hebdomadis*.

The ELISA is not only good as a screening assay but has the potential as an assay to detect early acute leptospirosis which is critical in the detection of clinical leptospirosis especially in humans. Early diagnosis meant that the patient would be given early treatment and perhaps save him from acute clinical leptospirosis and possibly death. A total of 832 human serum samples from 3 groups of patients were examined by the MAT and IgM ELISA to detect early acute leptospirosis. 172 (96%) serum samples from patients with clinical signs of leptospirosis and positive to MAT were IgM ELISA positive whilst 291 (58%) samples from patients with clinical signs of leptospirosis but negative to MAT were positive to IgM ELISA. and 20 (13%) random samples that were negative to MAT were positive to IgM ELISA. It was observed that the IgM ELISA was able to detect low titres which were apparently not detected by MAT and this possibly indicate early infection as evidence by presence of clinical signs but with low titres to leptospiral infection.

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MOLECULAR BIOLOGY CHARACTERIZATION, ORIGIN AND SPREAD OF HUMAN AND ANIMAL FASCIOLIASIS IN THE AMERICAS

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In Latin America, our collaboration studies have proved that numerous countries present great public health problems caused by *Fasciola hepatica*. Fascioliasis is a zoonotic disease of domestic ruminants caused by liver fluke parasites and transmitted by freshwater lymnaeid snail vectors. This disease is of well-known veterinary importance because of its great pathogenicity and impact in livestock, especially sheep, goats and cattle, but also pigs, buffaloes and donkeys, as well as horses, camelids and other domestic herbivores. Moreover, this disease is today emerging in humans in Europe, Africa, Asia and the Americas, with 51 countries presenting human infection.

A large hot spot of the disease has been detected in altitude areas of Andean countries as Argentina, Chile, Bolivia, Peru, Ecuador and Venezuela, with very high prevalence in livestock and humans in endemic areas where transmission and epidemiology follow whether altiplanic-permanent or valley-seasonal patterns related to vectors of the *Galba*/*Fossaria* group. Another hot spot includes Caribbean islands as Cuba and Central American countries as Mexico in which transmission and epidemiology are marked by lymnaeids as *Lymnaea cubensis* and *Pseudosuccinea columella*, and where animals present very high prevalence and intensities and a hypoendemic situation with periodic epidemics in humans.

Molecular studies were performed during several years to ascertain the origin and spread of fascioliasis in the New World. Specific objectives were the genetic characterisations of both liver flukes and lymnaeid vectors by combined haplotyping. Molecular markers obtained were the complete sequences of the nuclear ribosomal DNA (rDNA) internal transcribed spacers ITS-1 and ITS-2 and the mitochondrial DNA (mtDNA) genes of the cytochrome c oxidase subunit I (*cox*1) and NADH dehydrogenase subunit I (*nad*1) and respective aminoacid sequences of the proteins COX1 and NAD1. Respective sequence lengths proved to be of 432, 364, 1533 and 903 nucleotides.

Sequences were aligned using CLUSTAL-W, homologies assessed using BLAST, genetic distances measured using PAUP, and pairwise alignments made with MEGA. Genetic variation was evaluated using DnaSP and a hierarchical analysis of molecular variance (AMOVA) performed using Arlequin. Phylogenies were inferred by maximum-likelihood (ML) using PAUP and PHYML. The evolutionary model was determined using the hierarchical Lihelihood Ratio Test (hLRTs) and the Akaike Information Criterion implemented in Model test. A median-joining network analysis was performed using Network. A distance-based phylogeny using the neighbour-joining algorithm with the ML pair wise distances was obtained. Statistical support was evaluated with 1000 bootstrap replicates. A Bayesian phylogeny was applied to obtain posterior probabilities with MrBayes.

Fasciolid flukes showed a surprising homogeneity both at nuclear rDNA as in mtDNA levels. No one nucleotide difference appeared in the ITS-1 sequences of the different South American, Central American and Caribbean countries, whereas only one mutation appeared in the ITS-2 sequence. Similarly, differences detected when comparing *cox*1 and *nad*1 sequences were so few that bootstrap values obtained by using one or the other gene independently showed to be insufficient. Significant values in mtDNA were only obtained by combining both genes within the same analyses and when comparing different countries. These results contrast with those obtained in the sequencing studies of lymnaeid snails, in which several different species showing vectorial capacity appeared. Almost all vector species proved to belong to the *Galba*/*Fossaria* group of small-sized lymnaeids, and, with a few exceptions, endemic areas showed to present more than one vector species involved in disease transmission. These results clearly suggest a recent evolutionary origin

of the disease in the Americas and also, in its majority, a common geographic origin, in spite of the use of both authochthonous and introduced lymnaeid vector species.

Among the several conclusions which can be reached from this global analysis of fascioliasis in Latin American endemic countries, the following shall be emphasized because of its applied interest: (i) owing to the intraspecific genetic homogeneity of the causal fasciolids, the differences in transmission patterns and epidemiological situations may be related to the differences of lymnaeid vector species present in the endemic areas; (ii) because of the same reason, no important differences in clinics and pathogenicity are to be expected in the different endemic areas, excepting those linked to potentially different susceptibility of livestock breeds or human ethnic groups; (iii) fasciolid susceptibility to treatments and their capacity to give rise to resistance may be expected to be uniform throughout; (iv) DNA markers evolving pronouncedly faster than *cox*1 and *nad*1 are needed to analyse disease transmission; (v) lymnaeid vectors present shall be taken into account as the key issues for the control initiatives. These results open new doors for future, crucial molecular research in the way to establish the appropriate control measures for endemic areas of the different Latin American countries.

CLIMATIC CHARACTERISTICS OF AREAS WITH PRESENCE OF LYMNAEID SNAILS IN FASCIOLOSIS ENDEMIC AREAS OF MENDOZA PROVINCE, ARGENTINA

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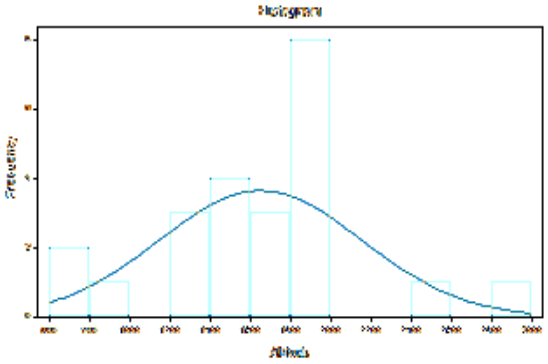
A digital climatic analysis on fasciolosis endemic areas with presence of lymnaeid snails was performed by using DIVA-GIS 5.2 software. The aim of the study is to characterize the climate of sites where the intermediate vectors of Fasciola hepatica, snails of the Gastropoda: Lymnaeidae family, are present; as well as some probable limiting climatic factors. The information generated could be of great importance while assessing for risk areas and control measures.

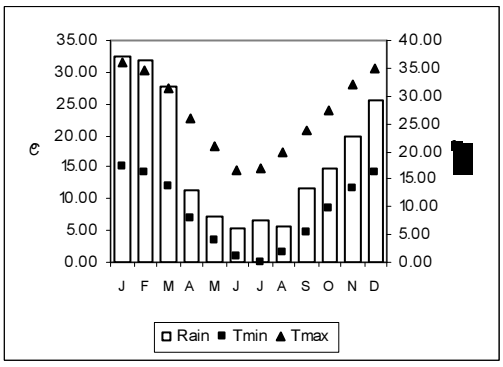
The study performed took into account 25 sampling sites, detected during the last 5 years by continuous field campaigns, covering the most important river bassins of the province, and specially aimed to the areas described in former researches as being endemic for livestock fasciolosis (Mera y Sierra et al., 2005, 2006; Gonzalez et al., 2006). Coordinates were registered with standard GPS (Garmin Vista Cx®).

The altitudes of the sampling sites were graficated in an histogram, in order to obtain a sampling distribution by altitudinal groups. By using the DIVA-GIS 5.2 software and WorldClim climate data (WorldClim 1.4, 2.5 min resolution climatic layers) (Hijmans et al., 2005), digital climatic information was obtained for every site. WorldClim provides monthly maximum temperature, monthly minimum temperature and monthly precipitation, as well as 19 derived bioclimatic variables. This information was analyzed by altitudinal groups with descriptive statistics. A combined dispersion graphic was developed for the 19 bioclimatic variables for every site.

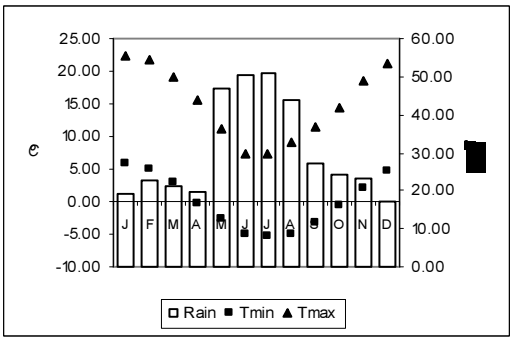


The histogram allowed to obtain 3 altitudinal groups, in order to analyze the information. Group 1: 600-1000 masl; Group 2 1200-2000 masl; Group 3 2400-3000 masl.

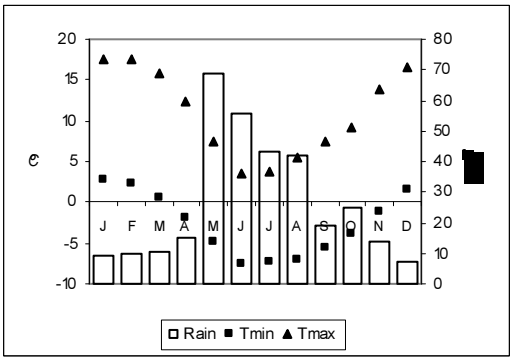
Group 1		
Bioclimatic Variable	**Mean**	**DesvEst**
Annual Mean Temperature [1]	15.60	0.721
Mean Monthly Temperature Range [2]	15.60	0.265
Isothermality (2/7) (* 100) [3]	49.50	0.755
Temperature Seasonality (STD * 100) [4]	589.30	16.076
Max Temperature of Warmest Month [5]	31.53	0.709
Min Temperature of Coldest Month [6]	0.03	0.416
Temperature Annual Range (5-6) [7]	31.50	0.436
Mean Temperature of Wettest Quarter [8]	22.07	1.361
Mean Temperature of Driest Quarter [9]	8.20	0.529
Mean Temperature of Warmest Quarter [10]	22.63	0.874
Mean Temperature of Coldest Quarter [11]	8.20	0.529
Annual Precipitation [12]	228.67	20.984
Precipitation of Wettest Month [13]	37.33	0.577
Precipitation of Driest Month [14]	5.67	1.528
Precipitation Seasonality (CV) [15]	63.10	9.968
Precipitation of Wettest Quarter [16]	105.33	4.041
Precipitation of Driest Quarter [17]	20.00	5.196
Precipitation of Warmest Quarter [18]	102.67	4.509
Precipitation of Coldest Quarter [19]	20.00	5.196

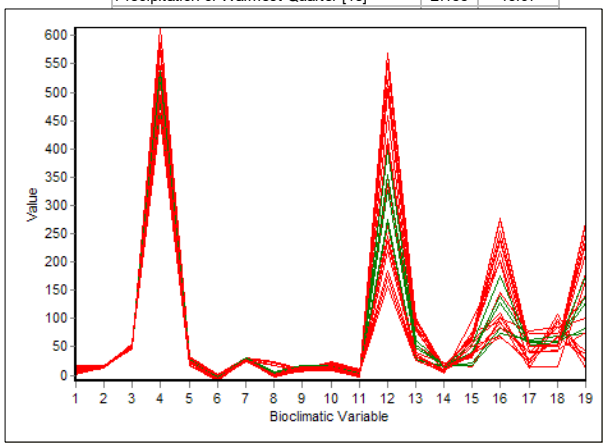


Group 2		
Bioclimatic Variable	**Mean**	**DesvEst**
Annual Mean Temperature [1]	7.41	4.417
Mean Monthly Temperature Range [2]	15.13	0.615
Isothermality (2/7) (* 100) [3]	54.45	1.504
Temperature Seasonality (STD * 100) [4]	489.27	36.016
Max Temperature of Warmest Month [5]	22.46	5.010
Min Temperature of Coldest Month [6]	-5.36	3.810
Temperature Annual Range (5-6) [7]	27.82	1.635
Mean Temperature of Wettest Quarter [8]	3.54	6.529
Mean Temperature of Driest Quarter [9]	11.03	4.575
Mean Temperature of Warmest Quarter [10]	13.49	4.803
Mean Temperature of Coldest Quarter [11]	1.44	4.100
Annual Precipitation [12]	365.95	122.392
Precipitation of Wettest Month [13]	57.40	26.428
Precipitation of Driest Month [14]	15.05	4.097
Precipitation Seasonality (CV) [15]	45.82	18.333
Precipitation of Wettest Quarter [16]	153.90	72.424
Precipitation of Driest Quarter [17]	51.95	13.256
Precipitation of Warmest Quarter [18]	58.65	10.956
Precipitation of Coldest Quarter [19]	144.85	76.024



Grupo 3		
Bioclimatic Variable	**Mean**	**DesvEst**
Annual Mean Temperature [1]	4.15	0.78
Mean Monthly Temperature Range [2]	13.50	0.57
Isothermality (2/7) (* 100) [3]	53.80	0.57
Temperature Seasonality (STD * 100) [4]	461.25	11.38
Max Temperature of Warmest Month [5]	17.70	1.13
Min Temperature of Coldest Month [6]	-7.45	0.35
Temperature Annual Range (5-6) [7]	25.15	0.78
Mean Temperature of Wettest Quarter [8]	-0.80	0.57
Mean Temperature of Driest Quarter [9]	9.70	0.99
Mean Temperature of Warmest Quarter [10]	9.70	0.99
Mean Temperature of Coldest Quarter [11]	-1.50	0.57
Annual Precipitation [12]	320.00	26.87
Precipitation of Wettest Month [13]	69.00	18.38
Precipitation of Driest Month [14]	7.50	4.95
Precipitation Seasonality (CV) [15]	76.65	27.08
Precipitation of Wettest Quarter [16]	168.00	45.25
Precipitation of Driest Quarter [17]	27.00	16.97
Precipitation of Warmest Quarter [18]	27.00	16.97





The combined dispersion graphic showed a small dispersion between temperature related variables (1-11), while a greatest divergence between precipitation related ones (12-19). This may indicate that, in general aspects, temperature has a greatest incidence in lymnaeids distribution than precipitation.

Despite the mentioned, convergence between values is specially observed in variables 6 (Min Temperature of Coldest Month) and 14 (Precipitation of Driest Month), showing that these aspects may be considered limitations to these snails survival.

All the same, it is considered that lymnaeids snails have a really great adaptability, enabling them to colonize and survive in extreme and diverse environments, such as the high altitudes of the Andes (with mean minimum temperature of coldest month of -7.45 ºC, in Group 3) or the arid plain lands of central Mendoza province (with precipitation in driest month of just 5.67 mm3, in Group 1).

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CURRENT STATUS, SURVEILLANCE AND CONTROL OF AVIAN FLU IN DOMESTIC AND WILD BIRD POPULATIONS IN BULGARIA

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Avian influenza (AI) is a highly contagious viral disease affecting several species of food producing birds (chickens, turkeys, quails, guinea fowl, etc.), as well as pet birds and wild birds. The AI viruses are divided in two groups based on their ability to cause disease (pathogenicity). Highly pathogenic avian influenza (HPAI) virus spreads rapidly, may cause serious disease and result in high mortality rates (up to 100% within 48 hours). The low pathogenic avian influenza (LPAI) can causes mild disease that may be undetected or no symptoms at all in some species of birds.

Since 1997 millions of chicken have been reported to have died due to HPAI H5N1 in countries of South-East Asia. In 2003 AI virus H7N7 affected poultry farms in Netherlands, then penetrated to Belgium and Germany. It had been considered that among human community there were circulating A viruses of 3 subtypes of H type (H1, H2 and H3) and 2 subtypes of N type (N1 and N2). However, for the recent years it has established that AI viruses as a result of mutation have changed their biological features and acquired a capacity of overcoming an interspecies barrier and affecting humans, mostly with lethal outcome. Once domestic birds are infected, avian influenza outbreaks can be difficult to control and often cause major economic impacts for poultry farmers in affected countries, since mortality rates are high and infected fowl generally must be destroyed -- the technical term is "culled" -- in order to prevent the spread of the disease.

Anatidae (ducks, geese and swans) is a group of water birds that is ecologically dependent on wetlands for at least some aspects of their annual cycle. Anatidae species use a wide range of wetlands, from the high arctic tundra, rivers and estuaries, freshwater or saline lakes, and ponds or swamps to coastal lagoons and inter-tidal coastal areas such as mud-flats, bays and the open sea. They also utilize man-made wetlands such as rice fields and other agricultural areas. Many of the Anatidae populations migrate between wetlands in the northern breeding areas and southern non-breeding areas and in doing so, regularly cross the borders of two or morecountries.

During the expansion of HPAI (H5N l) outbreaks from Asia to Europe, 2 events implicated wild birds, particularly waterbirds, as long-distance virus vectors. First, virus outbreaks in 2005 rapidly spread westward from Russia and Kazakhstan in July and August to Turkey, Romania, and Ukraine in October.There have been further reports of H5N1 avian influenza infection in birds in Russia, the Middle East, the Caspian Sea, Azerbaijan, a swan in Maribor, Slovenia, wild fowl in Krasnodar and Dagestan,, and further cases in swans in Italy. There have been reports of H5 avian influenza infection in swans in Slovenia, Austria, and Hungary. It is not yet known whether these swans were infected with the H5N1 strain.

Wild water birds were suggested as a vector because the virus spread through areas that had no record of any virus presence and coincided with the fall migration of wild water birds between these areas. Second, at the beginning of 2006, HPAIV (H5N1) was detected in many wild water birds in East Europe, often in areas where no outbreaks had been detected among intensively surveyed poultry; this event overlapped with unusual water bird movements associated with cold weather in the Black Sea area. Quantitative analysis of the global spread of HPAIV (H5N1) also supports the potential role of migratory wild birds in virus spread.

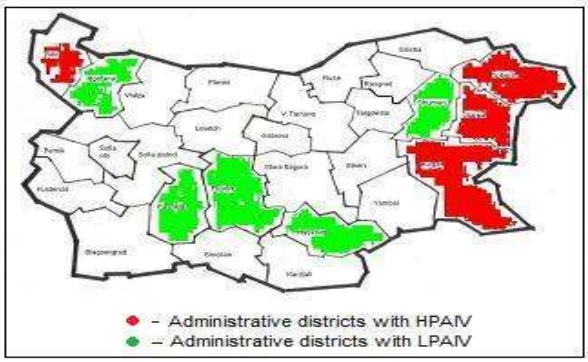

FIG 1. Areas of districts location of isolated in Bulgaria AIV during the period 2005 - 2008

The risk zones of AI penetration are connected with the wed areas and territories via the main migratory path of wild birds and the Black Sea cost, The national early warning and surveillance system also was adapted and covered these risk areas on the basement of periodic risk assessment.

TABLE I. AIV ISOLATED IN BULGARIA DURING THE PERIOD 2005 - 2008

Isolate	date	district	location	sample	Bird species	Virus isolates
A/Malard duck/05	11.11.2005	Bourgas	Poda dam	Fecal sample	Malard duck	H6N2
A/Swan/Vidin/06	31.01.2006	Vidin	Danube river	Internal organs	Swan	H5N1
A/Swan/Varna/06	06.02.2006	Varna	Tzonevo dam	Internal organs	Swan	H5N1
A/Swam/Kraimorie/06	07.02.2006	Bourgas	Kraimorie	Internal organs	Swan	H5N1
A/Swan/Dobrich/06	06.2.2006	Dobritch	Duranculak lake	Internal organs	Swan	H5N1
A/Swan/Bourgas/06	17.02.2006	Bourgas	Chengenez Skale village	Internal organs	Swan	H5N1
A/mule duck/Parvomay/06	25.04.2006	Plovdiv	Parvomay town	Cloacal swab	Mule duck	H6N5
A/mallard/Pazardjik/06	21.03.2006	Pazardjik	Kovatchevo village	Fecal samples	Malard duck	H4N6
A/mule duck/Rajevo konare//07	14.05.2007	Plovdiv	Rajevo konare village	Cloacal sample	Mule duck	H4N6
A/malard/Krepost/07	18.04.2007	Haskovo	Krepost village	Cloacal sample	Mule duck	H4N2
A/mule duck/Rajevo konare/07	22.11.2007	Plovdiv	Rajevo konare	Cloacal swab	Mule duck	H6N5
A/Malard/Chan Krum/08	31.01.2008	Shoumen	Chan Krum village	Internal organs	Malard duck	H7N7
A/malard/Montana/07	31.01.2008	Montana	Ogosta river	Fecal sample	Malard duck	H10N7

Two well equipped laboratories in Sofia and Varna covered the needs of samples investigation and research activities for AI of the whole territories of the country. The national surveillance plan

includes domestic and wild bird populations and domestic (back yards) and producing big farm livestock populations as well.

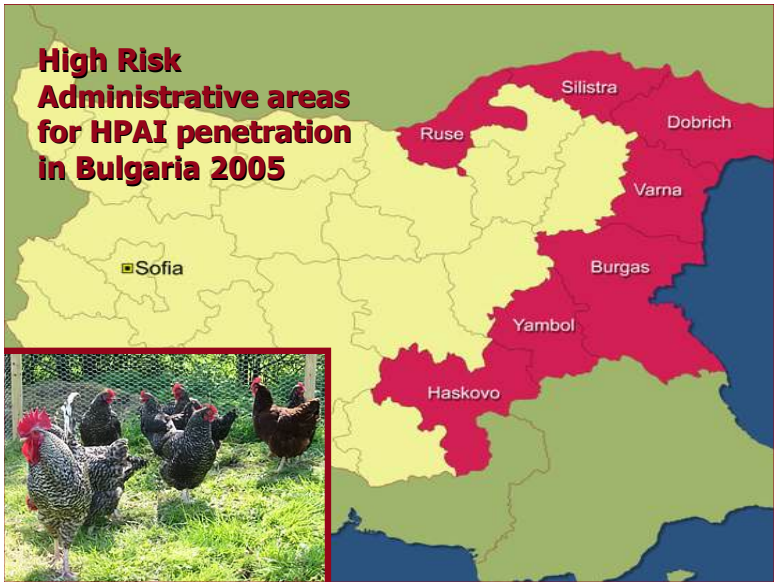

FIG 2. Areas at risk of AI penetration in Bulgaria

Based on the risk analysis (Figure 2), we think that future research studies should focus on the populations of several species wild migratory ducks, wintering at Shabla Lake (district of Dobrich), Varna- Beloslav Lake (district of Varna) and the wetlands Poda connected with Mandra Lake (district of Burgas). This surveillance will include marking of the caught birds in order to monitor their AI status in case they are caught again.

The three lakes have been selected based on the advice extended by the Bulgarian Society for the Protection of Birds (BSPB) Varna. The selection of the lakes was based on their hydrologic features so that placing of ornithological nets for catching ducks to be possible (Some lakes do not allow access by boat or freeze in the winter).

POSTER PRESENTATIONS

PREVALENCE OF RIFT VALLEY FEVER IGG ANTIBODY IN VARIOUS OCCUPATIONAL GROUPS BEFORE THE 2007 OUTBREAK IN TANZANIA

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Rift Valley fever (RVF); caused by RVF virus is a mosquito-borne viral disease that is a significant global threat to humans and livestock. Transmission in humans is via direct contact through infected animal products or contaminated foods or aborted foetuses and from the bites of infected mosquitoes, most commonly the *Aedes* species. Humans infected with RVF virus typically develop a mild self-limited febrile illness, but retinal degeneration, severe encephalitis, fatal hepatitis and hemorrhagic fever may also occur. The recent re-emergence (early 2007) of the disease among humans and livestock, covering 9 different geographical regions and the fact that RVF virus replicates in a wide range of competent mosquito vectors have raised concern that the virus might spread further into non-endemic (Tanga, Coast, Mtwara, Ruvuma, Rukwa, Kigoma, Shinyanga) regions of Tanzania. These threats emphasize the need for capable surveillance tools and a sound disease control strategy in place. Unpublished, hospital-based reports from this recent outbreak in Tanzania, indicates that RVF claimed 144 deaths and the corresponding case fatality rate of 46.6%. Much less is known of the prevalence in man and of the effect on human health in this region of the world. Such information is important to envisage when designing appropriate strategies that would help reduce its prevalence and effects.

A cross-sectional sero survey of 199(aged 14-84 years) apparently healthy persons (females, n = 67 and males, n =132) from various occupations was carried out in Tanga, Tanzania in November 2004 to investigate exposure to Rift Valley fever (RVF) virus. Occupational group categories investigated included abattoir workers (n = 41); livestock keepers (n = 67); non-livestock keepers or crop farmers (n = 38); animal health workers / meat inspectors (n =11) and others (n = 42). 'Others' category comprises people from the general community outside the traditional occupation risk groups i.e. business people, housewives, students soldiers etc. Sera were tested for the presence of antibodies to RVF virus by the inhibition enzyme linked immuno sorbent assay (ELISA) for detecting immunoglobulin G (IgG). All reactive sera were further tested by the capture enzyme-linked immunoassay test and specific RVF immunoglobulin M (IgM) assay. Eight (4.0 %; 95% Confidence interval [CI] =1.75 -7.76) tested positive for IgG while none of the samples tested positive for IgM. Among the occupational groups examined, the seroprevalence was 7.3%, 1.5% and 9.5% in the 'abattoir workers', 'livestock keepers' and 'others' categories, respectively. Seropositivity was higher in males than in females (5.3% versus 1.5%; P = 0.34) and increased markedly in males aged between 20 to 40 years, with no significant differences among the age groups and sexes (P = 0.08). The results indicate that none of the participants were aware of RVF as a zoonotic disease and a small proportion of people in Tanga municipality were exposed to RVF virus infection prior to 2007 disease outbreak in Tanzania. The low prevalence of IgG requires constant surveillance in case the prevalence rates do change. This low level of awareness is a reflection of the poor knowledge of zoonoses by livestock keepers, veterinary field staff and staff in the heath facilities which may be due to the general lack of data on RVF and inadequate communication between veterinary and human health care professionals. This shows that the emergency preparedness for RVF epidemic is low.

Education of the general public and most vulnerable occupational groups who are most at risk of contracting RVF on the transmission pathways and risk factors is required in order to lower further prevalence of human RVF in Tanga. These findings need to be taken into consideration when future disease control programs are implemented.

EFFECTS OF BETA AGONISTS IN THE DIAGNOSIS OF FASCIOLOSIS IN *BOS INDICUS* × *BOS TAURUS* IN THE STATE OF PUEBLA, MEXICO

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The Beta-agonist drugs (Beta adrenergic) international and national levels are increasing this to the improvement of performance in several species of channel domestic economic importance. It highlights the clenbuterol, zilpaterol and raptopamina, among others. These same products to be consumed by the animal increase muscle mass, but if we consider the clenbuterol, a bronchodilator, I anabolic agent and lipolytic in many species. Tend to be an agent of division of muscle mass, under which foster the production of proteins and reduces fat. Is a bit dangerous to human health and represents an act illegal and reprehensible and punishable. However, according to the literature, the active ingredient in these drugs do not represent a danger to human health because they are not mutagens or oncogenic, but if it is embryotoxic, so when given in high concentrations (doses). The problem is that it is a public health problem in terms of cardiovascular stimulation that results from eating meat from animals treated with beta-agonists and was not seen as a sign of withdrawal of four weeks. So far there are no reports of fatal cases in humans, but cases of poisoning by the use of animals and eat meat with beta adrenergic whether there are nationally and internationally.

On the other hand, induces clenbuterol in cattle at low doses as promoters of the productive performance) increased blood pressure b) transient increase in heart rate for 24 hours, c) increase in metabolic rate. But the risk is more important to the consumer for the intake of animal products contaminated with this drug. On the other hand, fasciolosis (worm is a flat, digeneo, flukes, which affects domestic and wild animals and the man accidentally) is a zoonotic disease, that is, transmitted from animal to man and vice versa; used for transmission invertebrate animals, snails Lymnaeidae of the family, whose distribution in the state is very broad, these snails act as intermediary hosts. There is currently in Mexico about 30 million cattle of which about 15000.000 million are in areas at high risk of infestation and about 5,000,000 million fascioliosis animals are with, if we estimate the economic losses per year gives us an approximate price of 4,500,000,000 pesos are lost annually, on the other hand, the lack of good pasture especially in times of drought in the state are helping farmers are feeding their animals with food additives in this case the beta-agonist (clenbuterol and the like), At present there are several municipal traces at the state level are discarding the livers of cattle producing very large economic losses.

In the state there are very few jobs on the pathophysiology of this disease, however, studies have been conducted on prevailing level of municipal trail Atlixco (zone endemic to the disease) in the year 2001-02 with a prevalence of 32-33 % While that in 2007 set a precedence of 7% over the same track municipal; this parasitic disease that affects the liver parenchyma and pipelines, affects the metabolic activity of the liver, this organ is one of the most important thing, because it participates in many metabolic pathways, as in the production and secretion of bile, regulates the metabolism of carbohydrates, lipids and proteins among others. This parasitic disease affects the production of meat and milk in ruminant animals in this very particular case Bos taurus x Bos indicus, the affected animals, these activities are depleted in previous studies have found that animals are fed with food additives such as beta -adrenergic-agonist, whose effect is to produce lipolysis, lipogenesis, are glucolíticos, and glycogen, affecting body composition, favouring neoformation tissue by the redistribution of power, also increasing the release of fatty acids into the blood, facilitating the synthesis of protein and retains the nitrogen fed into skeletal muscle, these components used as food

additives are prohibited by federal law nationals (SAGARPA NOM-015-ZOO-2002), as the consumption of meat produced visceral primarily of clinical cases as tachycardia, anxiety, trembling and symptoms like headache, weakness, facial flushing and hypertension, the group most affected are women (aged 25 to 45 years). Based on the above is intended to determine the effects of histopathology at both problems: the parasitism and doping, relating them to the metabolic profile (glucose, liver enzymes), hormonal profile (oestradiol and progesterone, prolactin), immunoglobulin (IgG1, IgM, IgG2a, IgE). The main objective of this study is to improve the quality of life of animals and products derived there from, to improve nutrition at the human level in the State of Puebla, two problems: one of natural causes and another led by the man whose target organ, the liver.

MONOCLONAL ANTIBODY SANDWICH IMMUNOASSAY DETECTION OF COPROANTIGEN TO EVALUATE THE EFFICACY OF TREATMENT IN NATURAL OVINE FASCIOLOSIS

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Considerable effort has been expended on research into a reliable method to assess the efficacy of chemotherapy in *Fasciola hepatica* infection. The most widely used method is egg counting and immunological test but none of them provide an accurate evaluation of drug's efficacy under field condition. The monoclonal antibody-based sandwich immunoassay (mAb Sandwich ELISA) was used to evaluate the effectiveness of triclabendazole treatment by the detection of coproantigens in *Fasciola hepatica* naturally infected sheep. Twelve sheep (2 to 5 years of age) were separated into two groups. The first group (three sheep) remained untreated; the other group (nine sheep) was treated with a single dose of 5% triclabendazole at 10 mg kg^{-1} of body weight. All but one of the treated groups had negative optical density values (OD_{492}) after two weeks of treatment, while seven sheep intermittently shed eggs during the course of the study. In all but one of the treated sheep, no *F. hepatica* infection; the one positive ELISA in 5th week after treatment according to the mAb Sandwich, had done fluke in the liver. The results of the parasitological examinations, as well as OD_{492} values obtained by the mAb Sandwich ELISA for the detection on coproantigens are described. The findings at necropsy, of the treated group in comparison to the untreated group are shown. The mAb Sandwich ELISA could be a useful and accurate method with which to monitor the efficacy of flukicides in *F. hepatica* natural infections.

SITUATION ON ZOONOSES IN KYRGYZ REPUBLIC

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Problems of occurrence, distribution and control of zoonotic infections as factors to risk of biological threats are priority tasks of medical and veterinary services of the Kyrgyz Republic. The necessity of solving is also a question of maintenance of biosafety and biosecurity, and all these questions are extraordinary actual today. In our republic brucellosis of cattle and small cattle animals is spread widely that causes in difficult epidemiological situation in several areas. In addition the number of people who are suffering from brucellosis is increasing every year (Figure 1). The number of ill people is increasing respectively to the number of ill animals. In the Kyrgyz Republic at the moment to eradicate brucellosis carried out research and practical work: (1) Carried out serological monitoring of all susceptible animals, (2) As a result to serological monitoring of positive animals are sent for slaughter, and (3) at this time, started small-cattle vaccination of livestock in one area (sheep and goats), REV-1 vaccine.

This experience will be spread across the country. In addition, are designing new methods for rapid - diagnosis. Serological blood researches of 974.2 thousand heads of cattle on brucellosis were carried out in 2008. Among which 8719 tests gave a positive result that makes 0,8 % from a total of the investigated tests. In 2008 have been existed not an easy epizootic situation of rabies and had been registered 196 pockets of the disease rabies, of which 157 were eliminated pockets. By type of animal's were showing: cattle - 64, sheep and goats - 11 horses - 12 dogs - 106 cats - 1, wild animals - 4. Antiepizootic and veterinary-sanitary measures were organized in the places where niduses of infection were registered. Veterinary experts carried out vaccination of 385 thousand heads of cattle, and also were provided liquidation of vagrant dogs and cats was done in order to prevent an epizootic of rabies. As shown in our study the cases of human and animal rabies are increased. For this purpose, we conducted research to determine the epizootic and epidemiological and ecological factors of the spread of rabies in the way, as reservoirs are wild animals will be vaccinated with a vaccine using baiting. Work on the study of patterns of rabies in the past 10 years.

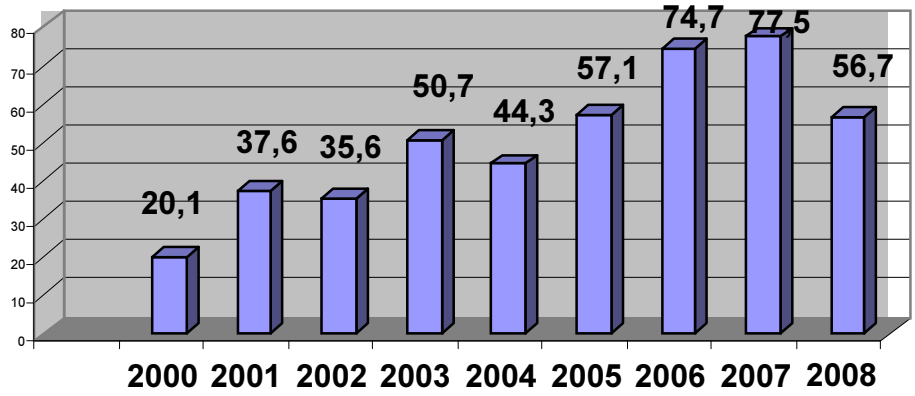

FIG 1. Dynamics of sickness rate of brucellosis among people in Kyrgyzstan 2000-2008.

REVIEW OF THE BIOSECURITY IN DIFFERENT POULTRY SECTORS TO PREVENT HIGHLY PATHOGENIC AVIAN INFLUENZA IN TANZANIA

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Distribution of the poultry operations in Tanzania in accordance to the FAO classification system of sectors 1 – 4 has been done and a review of biosecurity in each of those production systems documented. It was found out that typical sector 1 characterised by an industrial integrated system with high level of biosecurity is non-existent in the country (Table I). Sector 2 represents high levels of commercial poultry production system with moderate to high biosecurity, which involve raising parent stock (PS) and operating Hatcheries mainly but also raise commercial poultry – layers and/or broilers.19 farms were identified to fall under this category located in Regions of Pwani (6), Dar es Salaam (6), Arusha (2), Mwanza (2), Mbeya (1), Kilimanjaro (2) (Table I). Sector 3 represents farms involved in commercial poultry production of eggs and broiler production from hybrid chickens with low to minimal biosecurity and birds/products usually enter live bird markets. 25,624 small-scale commercial production farmers raising commercial layers and broilers were classified in this category. Village, peri-urban or urban backyard production with minimal biosecurity and birds/products consumed locally is classified as FAO sector 4 and included 34 million local village chickens kept by 2,992,145 smallholder households in Tanzania

The objectives of the study were to identify areas of possible biosecurity risks in the production cycle in each of the poultry production sectors, with special emphasis on sectors 3 and 4 in Tanzania so as to outline strategies to minimise the occurrence of the HPAI at all levels of poultry production, distribution, processing and marketing, thereby reducing the risk of human infection by bird flu virus. The study will contribute to drawing attention of all those handling poultry and its by-products, all along the food supply chain.

The methodology of the study was basically a desktop study that involved collection of data and documents from various sources and extensive consultations and discussions with assorted stakeholders. The HPAI biosecurity risk points in the whole production and marketing cycle (the Value Chain) of each Sector were identified. To ensure a comprehensive coverage of the entire production cycle, a review was made of the Standard Requirements (By the farm, by the law etc), Actual/Current situation in Tanzania and based on these, recommendations were made for each of the following factors: source of stock: (Imported or local); housing (design, sanitation); husbandry (raising and maintenance); feeds and feeding (quality and quantity); health management (vaccinations, isolation, treatment, disposal of dead); trade and marketing (traders, live markets slaughter facilities); animal-human HPAI transmission, farm worker biosafety, personal hygiene; consumer (meat/egg) protection, ante- and post-mortem inspection, kitchen hygiene and sanitation as well as on the ecology of wild birds (flyways, wetlands).

It was found out that the three major components of biosecurity (isolation, traffic control and sanitation) are highly adhered to in PS production and other Sector 2 commercial farms, but not in Sector 3 smallholder units and that biosecurity is highly deficient in Sector 4 which is found in extensive village chicken production systems. Sector 2 can be major sources of infectious diseases such as HPAI on account of the large numbers of birds, which are sold and distributed to many, sector 3 smallholder farmers.

The probability of infection (with HPAI) is higher in production sector 3 than in sectors 1 and 2. The risk of infection in sector 4 however is not as high despite the low biosecurity because of the small and isolated flocks. However, if the virus does enter farms in sectors 1 and 2, infection may have a greater impact due to the concentration of susceptible poultry in these farms. Risks also could be due to the distribution mechanisms of birds from breeding farms to production farms all over the country.

TABLE I. POULTRY PRODUCTION SYSTEMS (SECTORS 1 TO 4) AND THEIR DISTRIBUTION IN TANZANIA (2007)

Administrative Regions	FAO System as adapted to the Tanzania situation. Number of farms (Sector 2) and Households (Sector 3 and 4)						
	1	2	3 A	3 B	4 A	4 B	Total
Dar es Salaam		4					
Pwani		8					
Arusha		2					
Mbeya		1					
Mwanza		2					
Kilimanjaro		2					
Total	0	19	25624	0	2,992,145	0	3,120,487

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IgM CAPTURE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) USED AS DIAGNOSTIC TOOL FOR RIFT VALLEY FEVER IN SUDAN

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Rift Valley fever (RVF) is a mosquito-borne viral disease. It causes abortion in sheep, goats and cattle, and deaths of young animals. Humans can acquire infection from contact with infected tissues of livestock, or less frequently from mosquito bite. This study is aimed to use RVFV IgM ELISA as rapid serological techniques to detect IgM immunoglobulin in 323 blood samples collected from different species caprine, ovine and bovine from different region Gizera, White Nile, Sinnar and Blue Nile States where the environment and climate suited occurrence of the disease.

The study was conducted in White Nile, Gizera, Blue Nile and Sinnar states. These States are agricultural areas where we found frequent rainfall, water reserves and dams. We took animal presence in the area as essential element in disease happening.

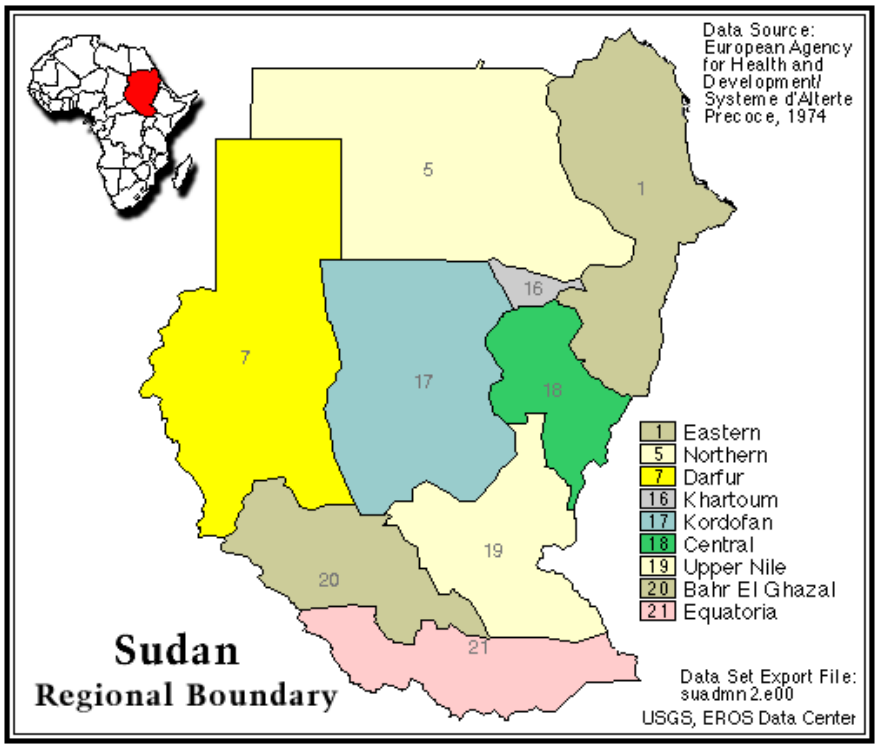

FIG 1. Illustrates study area, central Sudan whit Nile, Gizera, Blue Nile and Sinnar states. Vegetation, water reserves, dams and river in the area facilitate breeding site for mosquitoes and other biting insect.

Blood sample were collected from different animals caprine, ovine, and bovine and different ages so as to study the development of disease taking into account the affect of intrinsic factors species, breed, sex and age. Serum samples were prepared, preserved in cooled containers at 4° C and transported to laboratory.

The ELISA kits were purchased from the special pathogen unit, National institute of Virology, Johannesburg, South Africa. The kit contains the following: Rabbit anti-sheep IgM (capture antibody), freeze-dried 2 x 250µl; RVFV antigen (RVF Ag), freeze-dried, 2 x 300µl; Control antigen (control Ag), freeze-dried, 2 x 300µl; anti-RVFV serum (detection antibody), freeze-dried 2

x 100µl; Goat anti-mouse IgG horseradish peroxidase (HPRO) conjugate, 1 x 100µl; Control sera: high positive (C++) and negative control (C-), freeze-dried 1 x 200µl each; low positive (C+) control serum, freeze-dried 2 x 100µl; Phosphate-buffered saline (PBS) powder, 20 x sachets; Skim milk powder, 2 x 50g; Tween 20, 1 x100ml; Immunoplates, 25 x; ABTS-substrate, 3 x 100ml; 10% SDS stop solution ("Electran") 1 x 100ml.(J.T. Paweska, *et al.,* 2003).

The ELISA is based on a capture format in which the plates were coated with rabbit anti-sheep IgM capture antibody and then reacted with test sera. Anti sheep capture antibody can be used for detection of IgM in sheep, goats and cattle. The captured IgM antibody was reacted with RVF antigen, and the bounded antigen was then detected with mouse anti-RVF antibody anti-mouse horseradish peroxidase (HRPO) conjugate plus ABTS substrate.

TABLE I: SHOWS STATES INVOLVED IN THE STUDY, SAMPLES TESTED IN DIFFERENT SPECIES CAPRINE, OVINE AND BOVINE AND ITS PERCENTAGE OF POSITIVITY FOR IGM CAPTURE ELISA.

State	caprine			ovine			bovine			total tested
	Sample tested	+ve	pp*	Sample tested	+ve	pp*	Sample tested	+ve	pp*	
ALGAZIRA	103	63	61%	74	38	51%	43	10	23%	220
WHITE NILE	22	4	18%	12	6	50%	29	5	17%	65
SINNAR & BLUE NILE	_	_	_	7	1	14%	31	7	22%	38

*PP: positive percentage for Elisa test.

Total of 323 serum samples from different animals caprine, ovine and bovine in ALgazira, White Nile, Sinnar Blue Nile states were examined for rift valley fever virus IgM antibodies. 63(61%)caprine, 38(51%)ovine and 10 (23%) bovine were positive in Algazira state. 4(18%) caprine , 6(50%) ovine and 5(17%) bovine were positive in White Nile state .In Sinnar and Blue Nile state no caprines sample were tested , but 1(14%) ovine, 7(22%)bovine were positive.

RVF was previously reported in Sudan based on serological survey that indicated presence of antibodies to RVF virus in animals and human sera, (Findlay, 1936, Eisa et al., 1977, 1980, 1984,Watts et al., 1994, and Kambal, 1997) from all the data collected by aforementioned authors, it seems that RVF was prevalent in subclinical form, but the lack or poor of reporting system of the clinical cases did not reflect the real situation, because indigenous animals may not show clinical signs due to innate resistance (FAO, 2002).the virus activity may be revealed by random isolation from mosquitoes or by occasional human disease (FAO, 2002).

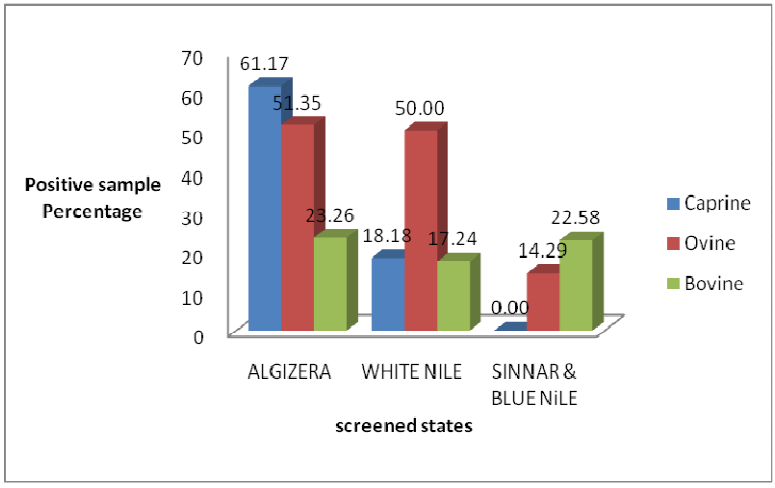

FIG 3. Explicit screened states, positive sample percentage and animal species involved in the study.

Caprine (39.5%), ovine (38.33%) and bovine (20.66%) of average percentage IgM antibodies level in study area. Gizera state showed highest morbidity rate in comparison to White Nile, Sinnar and Blue Nile states .presence of IgM antibodies is indicative of active and circulating virus in study area , but no virus isolation is done.

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EPIDEMIOLOGY OF LIVESTOCK *FASCIOLOSIS* IN MENDOZA PROVINCE, ARGENTINA

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Fasciolosis, parasitic disease caused by the trematode *Fasciola hepatica*, affects many mammals, particularly ruminants, and is now considered an important zoonotic disease. In Mendoza province, the data from the local slaughterhouses and observations made by veterinarians indicated that fasciolosis could be an important disease in cattle with 5,7% (1) liver condemnation registered which is well over the national average that oscillates yearly around 1%. Thus, even though fasciolosis seemed to be an important disease for livestock, the epidemiological information was lacking. Since the recent identification of the most efficient vector of fasciolosis in Mendoza, the introduced lymnaeid snail *Galba truncatula* (2), the epidemiological situation of fasciolosis needs to be addressed. Our objective was to gain insight in the epidemiology of fasciolosis in Mendoza province.

Mendoza province is in the west of Argentina. To the east is a plains region and at the west are the Andes Mountains. The rivers run from the mountains in the west towards the east traversed by valleys were livestock is managed extensively and trashumance is a common practice To investigate the distribution of the lymnaeid snails, samples where recovered following an altitudinal gradient, from the plains region at 600 m.a.s.l. up to above 3000 m.a.s.l. In each sampling point, by means of a GPS, altitude, longitude and latitude was registered, water pH, conductivity and hardness evaluated, and the environmental characteristics of the site recorded. To investigate the possible reservoirs fasciolosis coprological studies were performed (Lumbreras rapid sedimentation and Formo-ether sedimentation) and liver inspection at the slaughterhouse was recorded.

Lymnaeid snails where found in 27 sites, belonging to all of the river basins of Mendoza. The range of altitude was from 649 masl to 2971 masl, (μ1674). They were found in small streams with slow current in 18 sites (66,7%) small irrigation channels in 6 sites (22,2%) and ponds with still water in 3 sites (11,1%). In 24 sites (88,8%) the snails where constantly exposed to direct sunlight since there was no high vegetation or banks surrounding the water body, 23(85,2%)of the sites were in a rural setting and 4 (14,8%) of them where urban areas. In all the rural sites, livestock was to be found near the snail populations. The conductivity of the waters where the lymaneid snails were presented ranged from 121-2830 mΩ (μ 675), ph ranged from 5,95-7,4 (μ 6,91) and hardness 48-1210 ppm (μ288,7)

Cattle, sheep, goats, horses mules, donkeys and llamas where positive for fasciolosis. Out of 705 coprological studies performed, 186 (26.38%) where positive. The highest prevalence where in goats, out of 434 animals tested, 139 (32%) where positive. In equines, out of 114 tested, 29 (25%) where positive. All the positive animals where from altitudes of over 900 m.a.s.l. and no positive animals where found in the plains region. At the provincial abattoir, out of 754 cattle raised in Mendoza, 258 (34%) where positive for fasciolosis. All the positive animals came from the Andean valleys. At the local abattoir, which only butchered cattle from Tupungato region, principally Andean valleys, out of 653 animals inspected, 441 (67,5%) had fasciolosis.

In Mendoza province, the prevalence in livestock is amongst the highest in Argentina, superior to what could have been initially concluded from the national abattoir statistics. Even though livestock is found from the plains regions up to the mountain valleys, fascioliosis affects almost exclusively animals from the mountainous regions where the highest prevalence are to be found, being a very rare and almost unknown disease in the lowlands. This correlates almost perfectly with the

lymnaied distribution that we found. The altitudinal range of the snail populations, which can be found at very high altitudes, speak of the great adaptability that it has to extreme environmental conditions. It also colonized many man made irrigation channels, and even though it is usually found in soft waters with low conductivity, extreme values found again reflect its great adaptability. The fact that it is found in equines, including mules and donkeys, is unique for Argentina since there are no published reports of fasciolosis in other provinces. Fasciolosis is a disease that is clearly affecting the livestock production of Mendoza and further studies should be implemented to elaborate control measures.

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TRACKING *FASCIOLA HEPATICA* TRANSMISSION USING ND1 AND CO1 GENE POLIMORPHISMS IN ENDEMIC AREAS

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An initiative to reduce the high burden of human infection by *Fasciola hepatica* of population of endemic areas has been recently launched in Andean countries such as Peru and Bolivia as part of a collaborative effort of WHO and Health authorities in these countries. In order to reduce the risk of re-infection in areas under control programs for human infection and to diminish the negative impact on productivity on animals, it is necessary to analyze the transmission pattern in endemic areas where the parasite is infecting a variety of species such as cattle, sheep, equine, swine, lagomorphs and rodents. Genetic diversity from a *F. hepatica* population from an endemic region in Peru (La Encañada – Cajamarca) was analyzed by automated DNA sequencing of the variable fragment of ND1 gene (175 bp) and CO1 gene (216 bp). *F. hepatica* adult parasites were collected from naturally infected sheep, pig and cattle. Three variable sites for ND1 gene (1.71%) and 4 variable sites for CO1 gene (1.85%) were observed in the parasite population sample. Parasite infecting different species (sheep, pig and cattle) showed four different haplotypes for each gene. Non private specie-specific haplotypes associated to species host were observed. Preliminary results show that *Fasciola hepatica* populations in Cajamarca - Peru are distributed in three major groups that might be useful to track transmission patterns of this parasite (*Fig. 1 and 2*).

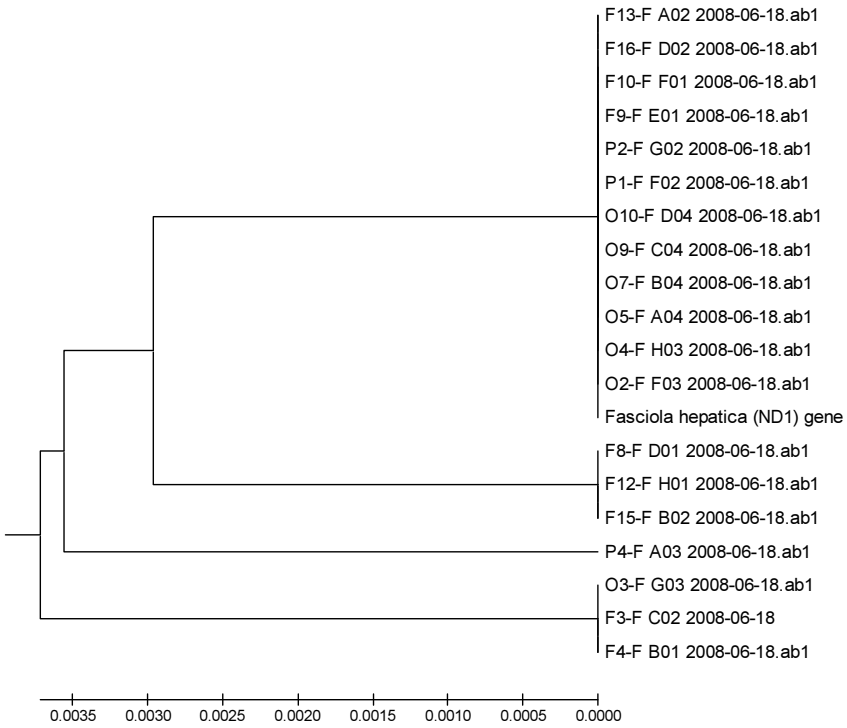

FIG. 1. UPGM dendogram of 175 bp ND1 gene from Fasciola hepatica using Mega v4.0 software. Letter F denote Fasciola hepatica from catle, letter P Fasciola hepatica from pig, letter O Fasciola hepatica from sheep and F .hepatica ND1 gene (M93388) was used as control sequence.

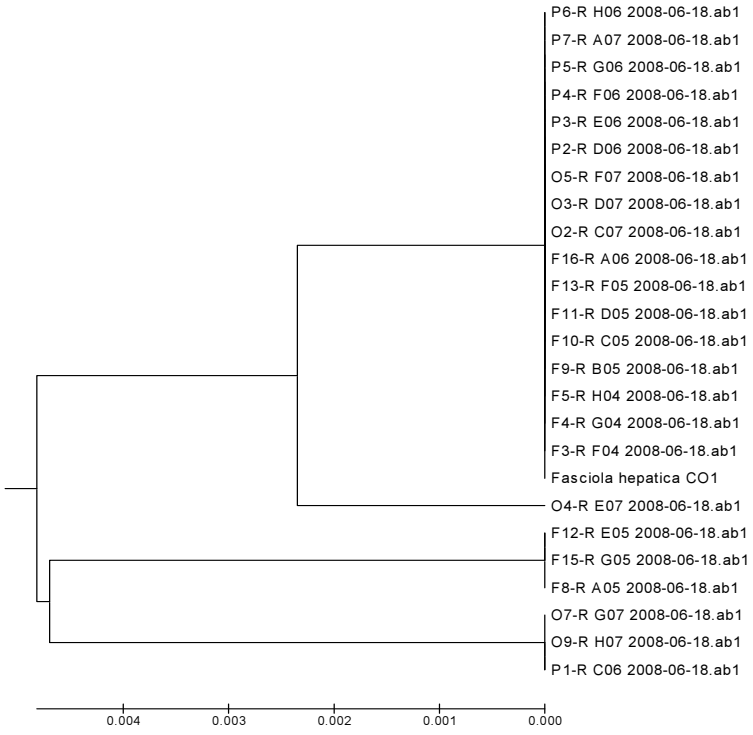

FIG. 2. UPGM dendogram of 216 bp CO1 gene from Fasciola hepatica using Mega v4.0 software. Letter F denote Fasciola hepatica from catle, letter P Fasciola hepatica from pig, letter O Fasciola hepatica from sheep and F .hepatica ND1 gene (M93388) was used as control sequence.

IMMUNODIAGNOSIS OF *FASCIOLA HEPATICA* INFECTION IN CATTLE USING FAS2-ELISA

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Performance of Fas2-ELISA, ES-ELISA and coprology for the detection of *Fasciola hepatica* infection in cattle was evaluated in serum samples from 168 cattle killed in the abattoir of Lima Peru. In this population sample, 59 cattle had liver fluke infection determined by direct visualisation of flukes in the liver and by faecal inspection. The sensitivity, specificity, positive predictive value and negative predictive value of Fas2-ELISA were 96.6%, 93.5%, 89.6% and 98% respectively. Corresponding values for sensitivity, specificity, PPV and NPV for ES-ELISA were 94.9%, 83.4%, 75.6% and 96.8% and for faecal inspection were 89.8%, 100%, 100% and 94% respectively. No cross-reaction with sera from cattle infected with *Moniezia sp. Bunostomum sp*. and *Dictyocaulus viviparus* was observed with Fas2-ELISA as ES-ELISA cross-reacted to sera from *Bunostomun sp.* infected cattle (2/17). Fas2-ELISA is more sensitive and specific for the detection of *F. hepatica* infection in cattle than ES-ELISA and faecal inspection and might be useful as a reliable tool for the screening for bovine fasciolosis.

AVIAN INFLUENZA DIAGNOSIS IN THE RUSSIAN FEDERATION: ACHIEVEMENTS AND PERSPECTIVES

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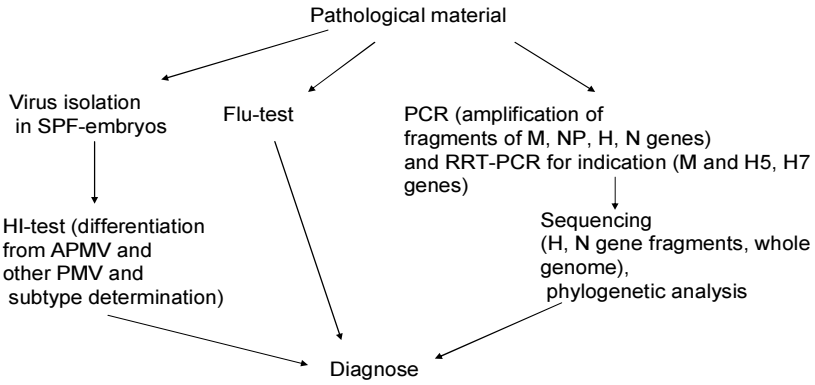
According to the Rosselkhoznadzor data, during 2005-2006, the avian influenza H5N1 outbreaks were reported in the Russian Federation in the Siberian, Ural, Central and South Federal Okrugs. In 2007, the RF officials notified the IOE about HPAI/H5N1 outbreaks in the territories of the Krasnodarsky Krai, Republic of Adygea, Moskovskaya and Kaluzhskaya Oblast. In 2008 there was one report about HPAI/H5N1 outbreak in Primorskii Krai (Far Eastern Okrug).

To detect and characterize the avian influenza virus the following diagnostic scheme was used in ARRIAH:



Approximately 4000 samples were submitted from different regions of Russia and Ukraine from AI suspected cases (poultry, wild birds) and for monitoring purposes. 392 samples were positive in PCR to avian influenza virus type A. The most part of them were HPAI H5N1.

In 2005 it was discovered 618 samples (223 – from poultry and 395 are from wild birds). Avian influenza type A virus genome was detected in 174 samples (85 – from poultry and 89 are from wild birds). 84 poultry samples and 36 wild birds samples were positive to subtype H5N1 (HPAI). 44 AI virus isolates were recovered (28 – from poultry and 16 are from wild birds).

In 2006 it was discovered 1014 samples (159 – from poultry and 855 are from wild birds). Avian influenza type A virus genome was detected in 144 samples (84 – from poultry and 60 are from wild birds). Most part of these samples were positive to subtype H5N1. 67 AI virus isolates were recovered (50 – from poultry and 17 are from wild birds).

In 2007 there were analyzed 833 samples (233 – from poultry and 600 are from wild birds). Avian influenza type A virus genome was detected in 55 poultry samples. All are positive to H5N1 subtype. Avian Influenza type A virus genome was detected in 7 samples from 1 region. Avian Influenza subtype H5N1 virus was not found**.**

In 2008 we analyzed approximately 1400 samples. Most of them are from wild birds. Only 30 samples are from poultry. Avian influenza type A virus genome was detected in 1 poultry sample

(HPAI H5N1). Avian Influenza type A virus genome was detected in 11 samples (1 – H5N1, 3 – H4, 4 – H5). Three positive samples were not sub typed. A positive H5N1 sample was not isolated. H4 positive samples are recovered.

The HA and NA segments of 136 A/H5N1 isolates were partial sequenced and phylogenetically compared with another one from Europe, Asia and Africa, which previously deposited in public databases. The whole genome of 3 A/H5N1 isolates was sequenced and phylogenetically compared too. All Russian HPAI H5N1 isolates belong to A/Goose/Guangdong/1/96 genetic line, genetic sub lineage Qinghai like isolates. A/Ck/Russia/Primorsky/0085/08 belongs to /Goose/Guangdong/1/96 genetic line, but not belongs to subgroup Qinghai like isolates. This one belongs to sub lineage FJ-like AI isolates.

Due to point mutations in the genome and reassortment of the fragments influenza viruses keep evolving rapidly now, thus, generating new variants of viruses with biological characteristics different from the characteristics studied earlier and causing great economic losses in the sphere of poultry farming throughout the world. Taking into account current situation the use of highly sensitive rapid methods including PCR and sequencing that enable early diagnosis of the disease is of high priority.

ZOONOTIC IMPLICATIONS DUE TO THE PRESENCE OF GALBA *TRUNCATULA*, VECTOR OF *FASCIOLA HEPATICA* IN MENDOZA, ARGENTINA

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Fasciolosis, the disease caused by the trematode *Fasciola hepática* and *Fasciola gigantica*, has been recognized as a very important problem affecting livestock. Since the 1990 s it started to be considered a zoonotic disease with important public health implications. Areas of human endemicity where described, principally in Andean regions of South America. The vector of the disease is different species of lymnaeid snails, but *Galba truncatula* is considered the most efficient one. In Argentina it has always been considered a disease of veterinary importance and the human cases reported have been sporadic. Traditionally, the main vector implied has always been described as *Lymnaea viatrix*, but recently *G. truncatula* has been described in Mendoza (1). This snail is of European origin and was introduced to Bolivia, where hyperendemic regions of human fasciolosis have been described in the Bolivian Altiplano (2), which highlights the vectorial capacity of *G. truncatula* in the zoonotic transmission of fasciolosis. Since the first description of *G. truncatula* in Mendoza our objective was to investigate the extent of its distribution in the province of Mendoza and evaluate the potential zoonotic risk.

The human population of Mendoza is 1, 576, 939 and is concentrated in the artificial oasis, principally of the Mendoza, Tunuyan and Atuel rivers since wine and fruit production are the main economical activities. The presence of lymnaeid snails was investigated in the basins of the rivers of Mendoza province: Mendoza, Tunuyan, Diamante Atuel and Malargue. These rivers are born in the Andes Mountains to the west, from altitudes that can exceed 6000 masl. The rivers run to the East, until they reach de plains region, which discourses under 700 masl. Above 3000msl the mean yearly temperature is below 0 C. Precipitation is scarce, mostly below 300 mm and it occurs in the form of snow in the mountains. Samples were recovered following an altitudinal gradient, from the plains region at 600 masl up to approximately 3500 masl. For identification, the snails, following an initial morphological characterization, where fixed in 70° ethanol and sent to the dpt. of Parasitology, University of Valencia. The rDNA ITS-2 sequence where obtained. A bibliographical review of the human cases was done taking into account not only published material but also communications to scientific events.

According to the revised literature, Mendoza has published 24 human cases, which would rank it third in Argentina after Cordoba and San Luis. The first human case in Mendoza was in 1955 the most recent in 2008. Five of the patients got infected in the Mendoza river basin, three in the Tunuyan river basin and 15 in the Malargue river basin. One case did not specify origin of the patient

Of 27 sites where lymnaeid snails where found 12 of them were identified as *G. truncatula*. Five of the *G. truncatula* populations are from the Mendoza river basin, two of them from the Tunuyan river basin, four from Malargue river and one from Atuel river. *G. truncatula* was found in an altitudinal range from 1387 up to 2971 m.a.s.l. The haplotype found was haplotype HC, the same for the Bolivian Altiplano from our study, it is evident that the distribution of *G. truncatula* in Mendoza province is very ample and it is present in rivers of the basins of the oasis regions here the greatest human populations are concentrated. It is also found in many recreational and touristic localities, where it is very common for people to engage in outdoor activities that can expose them to fasciolosis, such as the consumption of wild watercress or drinking of water from the mountain streams. The recent development of reservoirs such as the Potrerillos dam which collects water that subsequently provides water to the capital of Mendoza is a potential site for fasciolosis transmission

that should be monitored. Even though the published cases are scarce, we have to keep in mind that this disease does not have to be officially reported, so, there is the possibility that there could be cases not accounted for. We have found *G. truncatula* populations in all the regions where human cases have been historically reported. Thus, the distribution of G. truncatula and its proximity to important human populations imply that there can be a potential risk for human fasciolosis transmission in Mendoza province that should be urgently addressed.

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RIFT VALLEY FEVER IN CENTRAL AFRICA: SEROLOGICAL EVIDENCE AND VIRUS ANTIGEN DETECTION IN CATTLE IN THE DEMOCRATIC REPUBLIC OF CONGO

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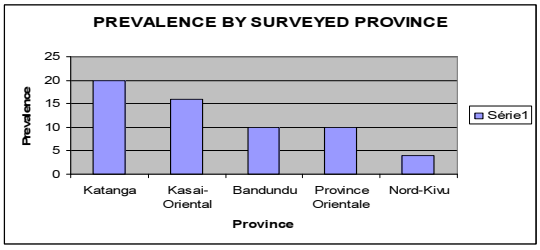
In order to assess the disease status within the cattle population in the Democratic Republic of Congo, a survey was carried out using two investigation strategies, i.e. 1) anti - RVF virus (RVFV) Ig G antibodies (Abs) detection, and 2) virus detection. Five provinces were selected due to their high bovine exploitation activity. 962 sera samples randomly collected in 26 locations from the 5 mentioned provinces were tested exploiting the recombinant N protein indirect ELISA (I - ELISA)[1] for antibodies detection and prevalence estimate and for virus detection, two diagnostic methods were exploited: a) - virus antigen (Ag) detection in tissues using the immunoperoxidase (IMP) staining with Avidin Biotin Complex (ABC)[2] technique and, b) -virus cDNA detection using RT – PCR. Only some tissues from syndromically suspected cases from one location with the highest prevalence (20%) were analysed for virus detection. The structural, nucleo – capsid (**N**) protein that the small segment (**S**) of the RVFV genome encodes with forms the key antigen targeted in all the 3 diagnostic methods. As a matter of fact, N protein is the most immunocompetent and most expressed protein of RVFV; it is also the highest conserved protein amongst members of the Bunyaviridae family.

With regard to I – ELISA, the recombinant N protein of RVFV (zim688/78 strain) was used as the diagnostic antigen and the following steps were respected: 1 - immunoplates (Maxisorb, Nunc, Denmark) precoating using 1/2000 dilution in carbonate – bicarbonate buffer pH 9.6 and incubation at 4ºC overnight on; 2 - blocking with 200 µls 10% fat free milk powder (Elite, Clover SA, Pty, Ltd.) and incubation in moist chamber at 37ºC for 1 hour; 3 – analysing of control and test sera, diluted at 1/400 in PBS containing 2% milk powder (diluting buffer) of which 100 µls each were added to plates (test sera in duplicate and control sera and conjugate in quadruplets) and incubated at 37ºC for 1 hour; 4 – 100 µls of Horseradish peroxidase conjugate (HRPO) anti – species Ig G at 1/15000 dilution was added to the plates that were incubated at 37ºC for 1 hour. *Each of the above mentioned steps were followed plates washing: 3 times with a washing buffer consisting of PBS pH 7,2 and 0.1% Tween 20*. ABTS (100 µls) was then added to each well and the reaction stopped with 100 µls of 10% SDS. The OD was determined at 405nm. Regarding IMP staining, previous works had demonstrated that the N protein of RVFV is histologically significant. Paraffin-embedded tissues from clinically suspected cases, i.e. some diseased liver specimens from abortions, stillbirth and non-viable calves were analysed.

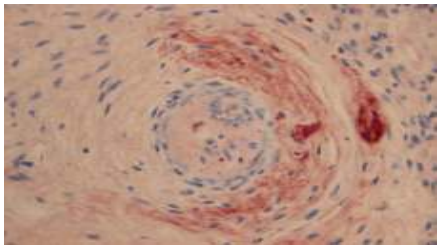
These paraffin – embedded tissues from a location with highest prevalence (20%) were dewaxed, rehydrated and pre-treated with 0.5 Trypsin and allowed to incubate for 15 min at 37°C. Antibodies non-specific bindings were blocked and endogenous Peroxydase suppressed. RVFV antigen sites were detected as immunocomplex created by the binding of Primary Ab to Secondary Ab facilitated by the ABC. The other steps such as PBS washing and incubations in humidified chambers were relevantly respected. Very Intense Purple (VIP - Vector Labs) was used as peroxidase substrate. Tissues sections were counterstained with Hematoxylin and Ag sites smartly overlooked as red purple spots on the tissue section. For RT – PCR manipulation: - RNA from suspected tissues was extracted using the QIAmp Vital RNA/DNA kit (cat. 14123), - the cDNA synthesis and PCR with

Taq DNA Polymerase High Fidelity were performed using the Invitrogen Thermoscript ™ RT – PCR System, (Catalog nos 1146 – 024 and 1146 – 040). The following primers were used, **NSa: CCTTAACCTCTAATCAAC, nts 841 – 824** and **NS2g: TGATTTGCAGAGTGGTCGTC, nts 61 – 80**. The amplification was completed within an Eppendorf Mastercycler machine using the following programme: cycle 1, 94˚C for 2 min, 95˚C for 1 min, 55˚C for 1 min, 72˚C for 1 min; cycles 2 to 45: 95˚C for 1 min, 55˚C for 1 min, 72˚C for 1 min. *PCR* products were separated and sized by *Electrophoresis* against a HyperLadder IV (Bioline), in 1 % Agarose gel and Ethidium Bromide and TAE buffer. As results: - the **Abs** prevalence at the country level was 14%, with the highest value in the Katanga (20%) province where syndromic cases are observed; - the IMP staining revealed RVFV antigen in fetus liver tissue section; - for the RT – PCR, apart from the sample used as positive control, i.e. inactivated RVF vaccine (Onderstepoort Biological Product – batch no 104), which was positive (*gel picture*), no cDNA was detected in field samples including the one positive at IMP, which is an indication of poor sampling and conservation systems. As a matter of fact: - no Guanidin isothyocyanate was used when collecting the tissues, - these tissues were conserved at - 20˚C (if not at + 4˚C) for several months before RT – PCR assay.

(1) = marker, (2) = negative control, (3) = crude vaccine, (4) = vaccine cDNA, positive: **800 bp**, (5) – (10) = field samples, all negative.



20% of virus Abs in Katanga



RVFV Ag in fetus liver section

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DETECTION AND CONTROL OF TRANS-BOUNDARY ANIMAL DISEASES, INCLUDING ZOONOSES IN MYANMAR

S.S. Kyi

Myanmar

RABIES CONTROL IN MAURITANIA

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AVIAN INFLUENZA IN CROATIA – CURRENT STATUS

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Wild birds can carry a wide range of viral and other zoonotic agents, which may be transmitted to humans. From October 2005 to March 2006 HPAI H5N1 virus was isolated from wild birds (mute swans, black-headed gulls and a mallard duck) in Croatia at five locations. After isolation of H5N1 virus at 2006 from mallard duck near City of Zagreb (capital of Croatia) Department of Poultry Diseases with Clinic at the Faculty of Veterinary Medicine, has conducted monitoring of avian viruses that could endanger human health. Samples (999 pharyngeal and cloacal swabs) from 23 wild bird species were taken. After year 2006 Croatia has regular monitoring for avian influenza in wild birds and poultry (especially in the backyard flocks). During 2007 (6,928 wild birds and 18,000 blood samples from poultry) and 2008 (2,486 wild birds; 20,000 blood samples and 1,500 cloacal swabs from poultry) were taken. Isolation was performed with classical virus detection method by inoculation of 10 day old chicken embryos, and molecular methods by conventional PCR and Real Time PCR (M gene, H5, H7 and N1 genes), and serological methods by antibody detection from blood samples (inhibition hemagglutination and ELISA). All samples were HPAI virus negative but investigators from the Poultry Centre of the Croatian Veterinary Institute isolated from wild birds LPAI viruses: H2N3, H3N8, H5N3 and H10N7. The results obtained by these investigations and monitoring revealed the need for permanent monitoring of wild bird's health status, especially the water birds species. Vaccination against AI is never practiced in Croatia. Quick and accurate detection of wild migratory birds infected with the H5N1 virus prevented the spread of the virus to the domestic poultry in Croatia which would have had enormous consequences.

MOLECULAR EPIDEMIOLOGY OF RIFT VALLEY FEVER VIRUS BASED ON GENETIC ANALYSIS OF THE VIRUS ISOLATES RECOVERED IN 1944-2008 FROM DISTINCT GEOGRAPHIC REGIONS

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Rift Valley fever (RVF) is an emerging mosquito-borne viral zoonosis caused by a RNA virus named Rift Valley fever virus (RVFV), a *Phlebovirus* member of the *Bunyaviridae* family. Historically the disease was present in Africa and Madagascar where outbreaks occur at irregular intervals when heavy rains facilitate the breeding of vector competent mosquito vectors. The occurrence of the first confirmed outbreaks of RVF in 2000-2001 among humans and livestock outside Africa, in the Arabian Peninsula, carries the implication of further spread of infection into non-endemic areas since the virus is capable of utilizing a wide range of mosquito vectors. This work undertook investigation of the molecular epidemiology of the disease (1944-2008) with special reference to South Africa where the first documented outbreak of RVF occurred in 1951 and the most recent in 2008 [1, 2].

A total of 149 isolates of RVF recovered over a period of 65 years from various hosts and during endemic and epidemic periods of disease in 15 African countries, Madagascar and Saudi Arabia were characterised by partial genomic sequencing of a 535-nucleotide segment of the G2 glycoprotein coding region of the M segment and the genetic relatedness determined using MEGA software.

Pair-wise comparison of RVF isolates revealed divergences ranging from 0-5.6% at the nucleotide level, corresponding to 0-2.8% at the amino acid level. Most isolates are compartmentalized geographically and belong to one of 16 genotypes within three main lineages. Isolates from South Africa collected over 57 years belong to one of 4 genotypes. The 2008 South African isolates were closely related to isolates from the recent east African outbreak in 2006 and a 2003 Mauritanian isolate (Fig.1)

Phylogenetic analysis indicates that circulation of RVFV is highly compartmentalized but with favourable climatic conditions a single genotype can rapidly spread from endemic areas over vast distances to cause outbreaks in susceptible human and animal populations.

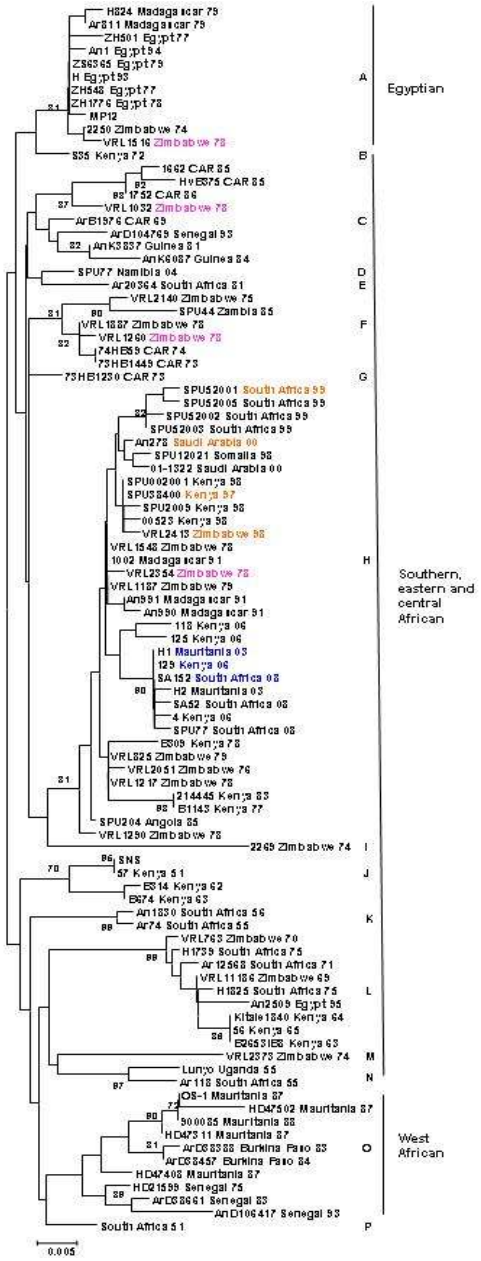

FIG.1. Phylogenetic relationship RVF virus isolates exhibiting unique genetic sequences. Values at the nodes indicate the level (%) of bootstrap support for that node from 1000 replicates. Tree topology indicates the existence of 3 lineages of genetically related isolates.

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EPIDEMIOLOGY OF RIFT VALLEY FEVER AND RISKS FOR ITS INTRODUCTION INTO EUROPE

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Rift Valley fever virus (RVFV), a member of the *Phlebovirus* genus of the *Bunyaviridae* family, is a causative agent of Rift Valley fever (RVF), a mosquito-borne viral zoonotic disease that poses a significant health threat to domestic ruminants and humans in Africa. Infection with RVFV in livestock is characterized by an acute hepatitis, abortion and high mortality rates in new borne animals. Humans infected with RVFV typically develop a mild self-limited febrile illness, but retinal degeneration, severe encephalitis, fatal hepatitis and hemorrhagic manifestations occur in small proportion of patients. Since original isolation of the virus in 1930 following an outbreak of "enzootic hepatitis" on a sheep farm near Naivasha in the Rift Valley region of Kenya, for the next four decades, epizootics were recorded only in eastern and southern Africa. However, in 1977 RVF spread to northern Africa, in 1987 to West Africa, and in 2000 to the Arabian Peninsula. The latter spread represents the first outbreaks of RVF in livestock and humans recognised outside Africa [1]. The fate of the virus during inter epizootic periods has long constituted a central enigma in the epidemiology of the disease. Cryptic maintenance and transmission cycles have been hypothesized but the exact mechanisms are not well understood. The current prevailing hypothesis is that at least in eastern and southern Africa, RVFV is maintained in the eggs of floodwater breeding aedine mosquitoes, which breed in isolated grassland depressions called dambos. The aedine mosquitoes overwinter as eggs, which can survive for long periods in dried mud. Flooding of the dambos during heavy and prolong rainfalls results in hatching of transovarially infected aedine mosquitoes followed by virus transmission to livestock. Infected livestock serve as a source of infection for culicines and anopheline mosquitoes, which act as epizootic vectors. Biting flies such as stomoxids, phlebotomids, midges, and simulids might serve as mechanical transmitters of infection.

Epizootic vectors spread the virus beyond the dambo habitat to additional livestock and humans [1]. Serological results indicate that a number of wild ruminant species are susceptible to RVFV infection but it remains to be determined whether these animals play any specific role in the virus maintenance during inter epizootic periods and virus amplification prior to outbreaks in domestic ruminants and humans [2]. The ability of RVFV to utilise a wide range of mosquito vectors and cause extensive outbreaks with severe socio-economic losses, as well as global climate change that facilitates spread of vector-borne diseases outside their traditional geographic boundaries, are of great international concern. Recent analysis of potential risks for further RVFV spread and its persistence outside traditional geographic coffins indicate that a number of mechanisms might be involved, including wind-borne movement of infected mosquito vectors, illegal importation of live animals and contaminated animal products, and rapid intercontinental air transport of infected people [3].

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PRELIMINARY EVALUATION OF A RECOMBINANT RIFT VALLEY FEVER VIRUS NUCLEOCAPSID PROTEIN AS AN IMMUNOGEN IN COMBINATION WITH DIFFERENT ADJUVANTS IN MICE AND SHEEP

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The nucleocapsid protein (NP) of Rift Valley fever virus (RVFV) is the most abundantly expressed and immunogenic viral component [1] and therefore it is an obvious choice for development of immunoassays for rapid and accurate detection of specific antigen [2] and antibody in RVF infected individuals [3]. The role of the anti-NP host immune responses in protection against infection with RVFV has not been elucidated.

Mice and sheep were immunized with recombinant RVFV NP combined with four adjuvants (ISA50, Alhydrogel, TiterMax Gold or SaponinQ) and respectively boosted with recNP/adjuvant. All vaccinated mice groups generated strong Th-2 mediated IgG1 responses (Fig. 1), whereas recNP/SaponinQ vaccinated mice generated a much stronger Th-1 mediated IgG2A response than other antigen/adjuvant combinations.

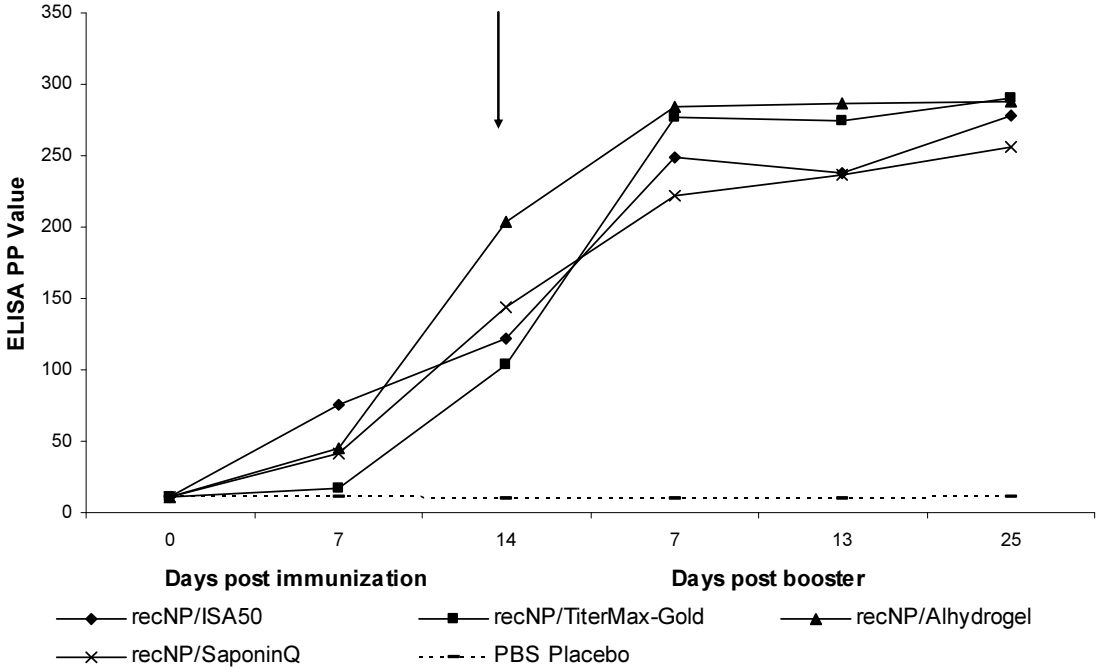


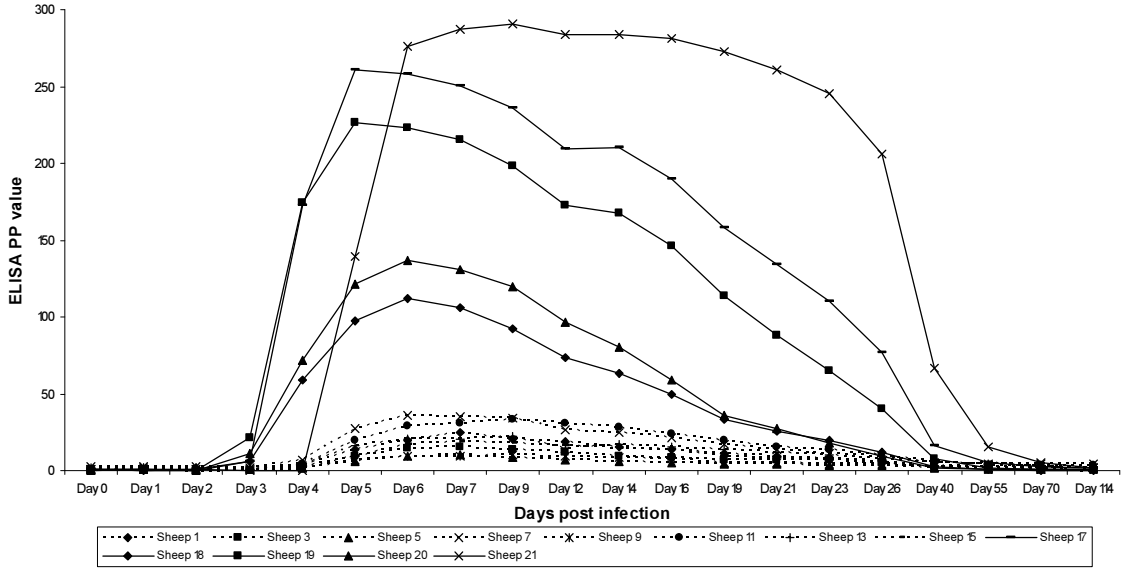
FIG. 1. Mouse IgG1 isotype immune responses after immunization and boosters on day 14 p.i. (indicated by the arrow) with different recNP/adjuvant combinations.

Among the placebo control groups, 96% of the mice showed severe clinical signs or died between day 2 and 6 after challenge whereas vaccinated animals showed 40 to 100% protection against sickness or death depending on the adjuvant used. Anti-NP humoral immunity did not prevent viral replication as demonstrated by RVFV recovery from various tissues of clinically normal mice but viral loads were lower compared to non-vaccinated groups.

The recNP was highly immunogenic in sheep. Its administration with adjuvants resulted in increased but varying kinetics of humoral immune response which after challenge with wild type virus was

lower in vaccinated than in control sheep (Fig. 2). The level of viremia post-infection varied between vaccinated sheep but was significantly lower compared to control groups. Anti-NP antibodies did not neutralize RVFV *in vitro.*

The type of adjuvant used with recNP appears to play an important role in protection against clinical disease implicating that cellular immune responses are responsible in protection and/or controlling infection with RVFV in a host.



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G. 2. IgM response in vaccinated sheep (1, 3, 5, 7, 9, 11, 13, 15) and controls (17, 18, 19, 20, 21) challenged with RVFV 37 d after the booster.

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PREVALENCE OF LIVER FLUKES *FASCIOLA HEPATICA* IN CATTLE OF PUEBLA STATE IN MEXICO

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Fasciolosis caused by *Fasciola hepatica* is not a priority for Mexican agriculture authorities, even though it has been known since XVIII century and their affectations oscillate between 5 - 80% of animals infected in several cattle zones of the country, fundamentally between the small producers. The introduction of *Bubalus bubalis* fortify the epidemiologists conditions and possible introduction of *Fasciola gigantica* in the states of Puebla and Veracruz. This study was performed in 870 animals of reproductive age in 30 farms of the state of Puebla, in order to evaluate the effectiveness of the coprological assay (Fascidig) technique (ELISA), for the determination of circulating antigens of *Fasciola* from a monoclonal Antibody. 25% of the productive animals of each farm were selected and coproparasitological technique of Lumbreras was used to have a better idea of the real prevalence of *Fasciola hepatica*. Selected areas were: zones of altitude between 1500 and 2000M above the sea level, an area of operation below the 700 msnm and an area of total stabulation. In all areas of transmission the presence of *Fossaria cubensis* and the potentiality for transmission of viable metacercarias was verified.

A higher prevalence (57,1%) to the hypothesis of study in low zones of the municipality Tlatlauquitepec, North Sierra of Puebla was obtained, the high zone of Teziuitlán showed an elevated percentage of infected animals (67%), but in this case they verified the lack of measures of sanitary regulation, when Bovine gained being transferred *Bos taurus* X *Bos indicus*, infected from white Earth and other areas of transmission well-known in Veracruz, where it is notified in sign until more of 50% of infected animals. In the case of Atlixco Puebla, a high prevalence in ten milk farms were detected, which surpass percentage notified in previous years, although the inmunoassay study reflects not only the egg shedding, but those animals infected at the moment of the study. Several field protocols were made to evaluate the effects of fasciolicides. Stabulated animals compared with grazing animals didn´t show differences in the Chipilo locality due to the high consumption of alfalfa, perhaps contaminated by the water used for irrigation of zones of high incidence in bovines. The prevalence determined in this zone is of more than 43%, which means that the zone of Atlixco could be considered a zone of transmission.

ACTIVE AVIAN INFLUENZA SURVEILLANCE IN BACKYARD POULTRY POPULATION IN FEDERATION OF BOSNIA AND HERZEGOVINA DURING 2008-2009

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Following the case of avian influenza that occurred in Bosnia and Herzegovina in February 2006, the Federation Ministry of Agriculture Water Management and Forestry and FAO in cooperation with BiH NRL for Avian Influenza and Newcastle Disease and veterinary authorities promoted, founded and implemented a Federation-wide surveillance programme. The main objectives of the surveillance effort were to identify if there are avian influenza viruses (AIV) circulating in backyard poultry flocks and to determine their actual prevalence in the same population. Over 5 months (December 2008 to April 2009), 3.556 cloacal swabs and 296 blood samples were collected from more than 100 households. Out of total number of samples only 5 were positive on ELISA test but they have not been confirmed with rT-PCR or embrionatedd SAN eggs.

RNA INTERFERENCE OF AVIAN INFLUENZA VIRUS H5N1 BY DIRECTLY INHIBITING mRNA WITH siRNA EXPRESSION PLASMIDS

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Avian influenza virus H5N1 causes widespread infection in the birds and human respiratory tract, but existing vaccines and drug therapy are of limited value. Here we show that small interfering RNA (siRNA) specific for conserved regions of the viral genome can potently inhibit influenza virus production in cell lines, embryonated chicken eggs and BALB/c mice. SiRNA expression plasmid pBabe-Super was chosen in the study, which directed the synthesis of small interfering RNA in cells. The inhibition depended on the presence of a functional antisense strand in the small interfering RNA duplex, suggesting that viral mRNA is the target of RNA interference. Among three small interfering RNA expression plasmids we designed, we found that small interfering RNA for nucleocapsid protein (NP) had a specific effect in inhibiting the accumulation of RNA in infected cells because of a critical requirement for newly synthesized nucleocapsid proteins in avian influenza viral RNA transcription and replication. The findings reveal that newly synthesized nucleocapsid, polymerase A (PA) and polymerase B1 (PB1) proteins are required for avian influenza virus transcription and replication and provide a basis for the development of small interfering RNA as prophylaxis and therapy for avian influenza infection in birds and humans.

SESSION 5: ACHIEVING FOOD SAFETY AND SECURITY IN THE 21ST CENTURY

BIOSECURITY AND TRADE IN A GLOBAL MARKET PLACE

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International travel and free trade are modern bywords and the international movement of people, animals and livestock products seen essential for the global market place to function. Yet is this compatible with a bio-secure national environment? Governments around the world seek to manage the risk posed by infectious disease on livestock, man, the environment and the related ecosystems whilst at the same time permit free trade. Ample examples exist of these competing elements as illustrated by recent outbreaks of Avian Influenza, bluetongue, SARS and most recently in Australia, equine influenza. Whilst the recognition that some 70% of new infectious diseases in man come from animals even those diseases that affect only animals, such as Foot and Mouth disease, can have devastating effects on trade and economies. The word "biosecurity" now encompasses most of these elements with processes being developed to identify these biosecurity risks, to mitigate or eliminate the risks and to ultimately prevent adverse biosecurity events. An added dimension to be recently considered is that of bioterrorism.

The OIE or World Animal Health Organisation was established in 1924 to address the risks posed by trade in animals and their products but the biosecurity issues we now face seem well beyond the initial remit of this Organisation. The World Health Organisation has for many years provided a public health framework to address zoonotic infections but again many aspects of biosecurity goes well outside this remit. The Food and Agricultural Organisation, often in partnership with OIE and WHO have sought to provide processes for identifying and managing biosecurity but often on a disease by disease basis, or in response to disease emergency. The Joint FAO/IAEA Division has contributed much through setting standards and developing a quality assurance system for laboratories, which provides critical underpinning data. But even together is this enough to manage the biosecurity risks we now face globally?

By examining in more detail some recent major disease outbreaks it is possible to indentify some underlying biosecurity principles that will help guide those now managing biosecurity at the national level. Whilst new "one health" partnerships at the national level between those managing agriculture, the environment and human health could maximise available resources or even marshal additional sources this is likely to be challenging and even agreeing priority areas difficult. Compounding and underlying much of the dilemma will be the risk of the unknown and how best to evaluate and manage this.

So is it time for a new global co-ordinated and collaborative approach to managing biosecurity with a recognition that we need to encourage not restrict, the global market place. Seeking to identify the risks not from a national but global perspective and directing national resources to mitigating these risks internationally could be an effective new paradigm. A good starting point would be the embracement of a one health approach both national and internationally.

THE FUTURE OF AQUACULTURE, AND ITS ROLE TO ENSURE FOOD SECURITY – DISEASE IMPACT AS A CHALLENGE TO SUSTAINABLE DEVELOPMENT IN PRODUCER COUNTRIES

R. Enriquez

Chile

PRODUCTION OF BIOPHARMACEUTICAL COMPOUNDS IN PLANTS: POTENTIAL AND APPLICATIONS

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Plants are gaining widespread acceptance as a suitable system for the large-scale production of recombinant proteins. As molecular farming has come of age, there have been technological developments on many levels, including transfection methods, control of gene expression, protein targetting, the use of different crops as production platforms, and modifications to alter the structural and functional properties of the recombinant product. The skepticism that received this technology when first envisaged has turned into a cautious optimism. A wide variety of proteins can be produced in plants and they are almost indistinguishable from their native counterparts. Over the last few years, there has been a continuing commercial development of novel plant-based expression platforms accompanied by success in tackling some of the limitations of plants as bioreactors, such as low yields and inconsistent product quality that have limited the approval of plant-derived pharmaceuticals. Indeed, one of the most important driving factors has been yield improvement, as product yield has a significant impact on economic feasibility.

Strategies to improve the recombinant protein yield in plants include the development of novel promoters, the improvement of protein stability and accumulation, and the improvement of downstream processing technologies. Attention is now shifting from basic research towards commercial exploitation, and molecular farming is reaching the stage at which it may challenge established production technologies based on bacteria, yeast, and cultured mammalian cells. There are already several plant-produced proteins on the market including one at a large scale. Several plant-derived recombinant pharmaceutical proteins are reaching the final stages of clinical evaluation, and more are in the development pipeline. The low cost of plant-based vaccines make them ideal for large-scale programs in poor countries. It is hoped that the issue of IP does not represent an insurmountable obstacle to this end. During the talk, the potential of plant based-vaccines for veterinary use as well as current research and experimental trials, problems associated with antigen expression and immune response and the potential risks of the technology will be reviewed. The potential impact of the technology in developing countries will also be reviewed.

ORAL PRESENTATIONS

AUSTRIAN MEAT: AUTHENTICITY CONTROL BY STABLE ISOTOPE ANALYSIS

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The EU has declared that all foodstuff must be able to be traced back from "fork to farm" to increase the safety of food and the confidence of the consumers in food quality and safety. Additionally, several incidents of "food diseases and scandals" related with meat (e.g.: BSE, foot and mouth disease, antibiotics abuse, avian flu, etc.) have demonstrated the necessity to trace back the origin of meat, to be able to locate sources of infection/mismanagement.

Besides the conventional method of the control of documentation there is the possibility of control of origin by analysing the isotopic composition of meat and herewith controlling the questioned good itself. Stable isotope ratios of the elements HCNOS are varying geographically due to different environmental conditions (e.g.: climate, geology, soil, altitude, geography, etc…) thus every region possesses an individual pattern. The isotopic pattern is imprinted on plants and animals growing in a certain region, and therefore analysis of the stable isotope pattern can allow the identification of agricultural goods from different regions.

For the control of origin of Austrian meat about 500 beef and 500 pork samples have been collected from slaughterhouses and were analysed for the isotopic composition of carbon, nitrogen, oxygen and sulphur and compared with the isotopic composition of meat samples from neighbouring countries.

As Austria, despite being a small country, is very heterogeneous in its environmental conditions, thus there are significant differences in the isotopic patterns of individual Austrian provinces. The isotopic signature of meat samples from neighbouring countries can overlap with the "Austrian isotope pattern" due to similar environmental conditions. However, a correct statistical classification has been achieved for 80% and 84% of the analysed beef and pork samples, respectively. If the declared origin of meat can be pinned down to an Austrian province, the discrimination power of the database is even significantly better.

QUALITY COMPARISON BETWEEN GAMMA-IRRADIATED OR ELECTRON BEAM IRRADIATED PORK PATTIES

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This study was conducted to evaluate the microbial safety, hardness and sensory properties of pork patties irradiated with gamma ray or electron beam at the absorbed dose from 5 to 20 kGy. Minced pork was prepared in 24 hours after butchery for manufacturing of pork patties. It was produced by methods of our previous study and then packaged to vacuum condition [1]. Gamma (430 kCi, Co-60) and electron beam (2.5 MeV, electron accelerator) were used for food irradiation, and the absorbed doses used were up to 20 kGy under room temperature. The microbiological and sitological characteristics of the samples were observed during accelerated storage at 30℃ for 10 d. The results of the total aerobic bacteria in pork patties during the accelerated storage showed that the sterilization effect of gamma irradiation was superior to that of electron beam irradiation [2]. The hardness and sensory properties such as colour, chewiness, taste, and overall acceptability of pork patties were decreased depending upon irradiation dose. Gamma irradiated samples have lower hardness and sensory scores than those of electron beam irradiated samples. In conclusion, gamma irradiation on pork patties was appeared more effective than E-beam irradiation. However, further studies to reduce the quality deterioration of gamma-irradiated pork patties should be continuously conducted.

TABLE I. EFFECT ON GROWTH OF TOTAL AEROBIC BACTERIA OF PORK PATTIES WITH VACUUM PACKAGING AND GAMMA RAY OR ELECTRON BEAM IRRADIATION DURING STORAGE AT 30^{O}C (UNIT: LOG CFU/G)

Days	Gamma ray (kGy)					Electron Beam (kGy)				
	0	5	10	15	20	0	5	10	15	20
0	3.56	ND[2)]	ND	ND	ND	3.56	3.32	ND	ND	ND
2	-[1)]	6.89	5.76	3.32	ND	-	7.91	6.83	6.61	5.51
5	-	-	6.49	5.72	3.75	-	-	-	-	-
10	-	-	-	7.26	5.46	-	-	-	-	-

[1)] Bar indicates no determination of cells because of spoilage.

[2)] Not detected within the detection limit < 1 Log CFU/g.

TABLE II. EVALUATION OF HARDNESS AND SENSORY QUALITIES OF GAMMA RAY OR ELECTRON BEAM IRRADIATED PORK PATTIES AFTER VACUUM PACKAGING

	Dose (kGy)	Hardness (g)	Colour	Chewiness	Taste	Off-flavour	Overall acceptance
Gamma ray	0	431.76 ± 45.35[a]	6.8 ± 0.8[a]	6.7 ± 0.7[a]	6.8 ± 0.6[a]	1.2 ± 0.1[c]	6.8 ± 0.8[a]
	5	395.67 ± 50.32[a]	6.1 ± 0.6[a]	6.1 ± 0.5[a]	6.2 ± 0.4[a]	2.2 ± 0.2[b]	5.7 ± 0.4[a]
	10	385.06 ± 27.59[a]	5.6 ± 0.5[ab]	5.7 ± 0.4[ab]	5.6 ± 0.6[a]	2.7 ± 0.3[ab]	5.3 ± 0.5[ab]
	15	381.43 ± 20.32[a]	5.1± 0.4[b]	5.2 ± 0.4[b]	5.3 ± 0.4[ab]	3.1 ± 0.3[a]	4.6 ± 0.2[b]
	20	375.69 ± 28.35[a]	4.7 ± 0.5[b]	4.4 ± 0.3[b]	4.1± 0.3[b]	3.3 ± 0.2[a]	4.2 ± 0.4[b]
Electron Beam	0	431.76 ± 45.35[a]	6.7 ± 0.4[a]	6.9 ± 0.6[a]	6.7 ± 0.7[a]	2.1 ± 0.2[b]	6.9 ± 0.5[a]
	5	424.38 ± 6.22[a]	5.9 ± 0.6[a]	5.8 ± 0.6[ab]	6.5 ± 0.3[a]	2.3 ± 0.2[ab]	5.6 ± 0.4[b]
	10	423.21± 61.62[a]	5.8 ± 0.5[ab]	5.5 ± 0.3[b]	5.8 ± 0.5[ab]	2.7 ± 0.1[a]	5.4 ± 0.4[bc]
	15	419.93 ± 83.64[a]	5.4 ± 0.3[b]	4.6 ± 0.4[c]	5.4 ± 0.6[b]	2.9 ± 0.2[a]	4.9 ± 0.2[c]
	20	407.34 ±6 9.88[a]	5.1 ± 0.4[b]	4.3 ± 0.3[c]	4.3 ± 0.4[c]	2.7 ± 0.2[a]	4.4 ± 0.4[c]

[a-c] Means within the same column different letters differ significantly ($P < 0.05$).

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REGISTRATION AND COMMERCIALISATION OF THE EAST COAST FEVER MUGUGA COCKTAIL VACCINE

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East Coast fever (ECF) is a parasitic disease of cattle in Eastern and Central Africa and is caused by Theileria parva, which is transmitted by the tick Rhipicephalus appendiculatus. The infection and treatment method (ITM) for immunisation against T. parva was developed in Kenya in the 1970's [1] and has since proved to be the only effective method of vaccination. T. parva is a genetically diverse parasite and there appears to be little cross protection between different stocks. The Muguga cocktail (MC) comprises three stocks, viz Muguga, Kiambu 5 and Serengeti transformed and has been claimed to be effective in preventing the disease in differing regions of East Africa including Kenya, Tanzania and Uganda.

The vaccine consists of titrated preparations of the three live parasite stocks stored in liquid nitrogen and thawed and reconstituted in buffer before use. Vaccination has to be accompanied by treatment with long acting oxytetracycline injection to prevent severe clinical symptoms including death. Cattle receiving this treatment are claimed to develop lifelong immunity to ECF.

In 1998 FAO funded production of two batches (FAO-1 and-2; approximately 1 million doses) of MC at ILRI Nairobi, which was subsequently used under approval by the Directors of Veterinary Services (DVS) in Kenya, Tanzania and Uganda, but these batches are now almost depleted. A stakeholder group assembled by AU-IBAR consisted of DVS's, academics, ILRI, NGO's and other interested parties met early in 2007. In the absence of alternative vaccines and with a clear market need, GALVmed agreed to fund further production of MC with the caveats that the product should be formally registered in user countries to help ensure consistent quality and, that it should be transferred into the private sector to ensure future sustainability of supply. Most importantly it was agreed that every effort would be made to ensure access to the product by the poorest cattle farmers in line with the global access principles espoused by GALVmed.

A master registration dossier was prepared during 2007. Individual applications for each of Kenya, Tanzania, Uganda and Malawi were then prepared drawing on the data in the master dossier as needed. The Quality section described the manufacturing process in detail and was supported by a comprehensive series of standard operating procedures (SOP's). The complete batch record of FAO-1 was included. Since specific registration clinical studies had never been carried out for MC, the available reports and publications were 'retro-fitted' to meet the normal regulatory requirements of safety and efficacy.

A benefit-risk analysis was carried out which was then presented to stakeholders to gain their acceptance of the conclusions. These were as follows:

- The ECF-ITM MC vaccine as exemplified by batches FAO-1 and -2 is safe and effective at a final dilution of 1:80, when used according to instructions supplied.
- Whilst systemic clinical reactions can occur following vaccination, these can be minimised by the concurrent use of oxytetracycline LA at a dose of 30 mg/kg.
- If oxytetracycline is given at 20 mg/kg then vaccinated animals should be monitored intensively for several days in case further anti-theilerial treatment is necessary.
- Muguga cocktail should only be used in areas where it is known that Muguga, Serengeti transformed and Kiambu 5 or related stocks are likely to be present.

There is some evidence of differential shedding and transmission from vaccinated animals by the three stocks in MC. Although evidence of clinical disease in in-contact non-vaccinates is equivocal,

every effort should be made to protect susceptible animals from close contact with newly vaccinated cattle.

The stakeholder group then delegated responsibility to a smaller task force comprising the relevant DVS's, AU-IBAR, Galvmed and ILRI to pursue the national registrations and progress the privatisation. Registration dossiers have now been submitted to the regulatory authorities in Kenya, Tanzania, Uganda and registration has been granted in Malawi. The production of one further batch of approximately 1 million doses has already been completed.

Invitations for private companies to tender for manufacture and distribution of MC were advertised in the East African press in October 2008 and meetings are planned early in 2009 to select commercial partners. Given the high costs of production and distribution of MC, a programme is ongoing to streamline the manufacturing process and to thermo-stabilise the MC stabilates. Next generation vaccines are also under consideration.

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POSTER PRESENTATIONS

TRANSFER OF HEAVY METAL FROM ANIMAL FEEDSTUFF TO ANIMAL PRODUCTS

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The study was focused on the possible transfer of heavy metals from livestock feeds to animal products and assesses health risks of human food [1, 2, 3, 4]. Samples collected (503) from conventional farms in Central Greece and analyzed to determine their heavy metal (Cu, Zn, Cd, Pb, Ni, and Cr) content. A number of 271 samples collected from cow conventional farms and they consist of 45 samples of muscle tissues, 60 of livers, 63 of kidneys, 38 of animal faeces and 65 of basic feedstuff mixtures. The rest of 232 samples were collected from sheep conventional farms consisted of 40 samples of muscle tissues, 39 of livers, 39 of kidneys, 54 of animal faeces and 60 of basic feedstuff mixtures. The sampling in both cows and sheep farms took place simultaneously during the 3 years of the experiment. The feedstuff samples were taken from different phases of the productive procedure and the farms were representative of the area. Atomic Absorption Spectrometry was used for the concentration of heavy metals. GLM Univariate analysis was performed with the use of SPSS 15.0 program. Furthermore, at the results applied post hoc range tests and multiple comparisons (least significant difference, Bonferroni, Scheffé and Tamhane's T2). Figure 1, of Cu, Zn, Pb, Cd, Ni and Cr.

The results reveal that transfer of heavy metal contaminants from feedstuff to animal products fluctuated at levels below the permissible risk values.

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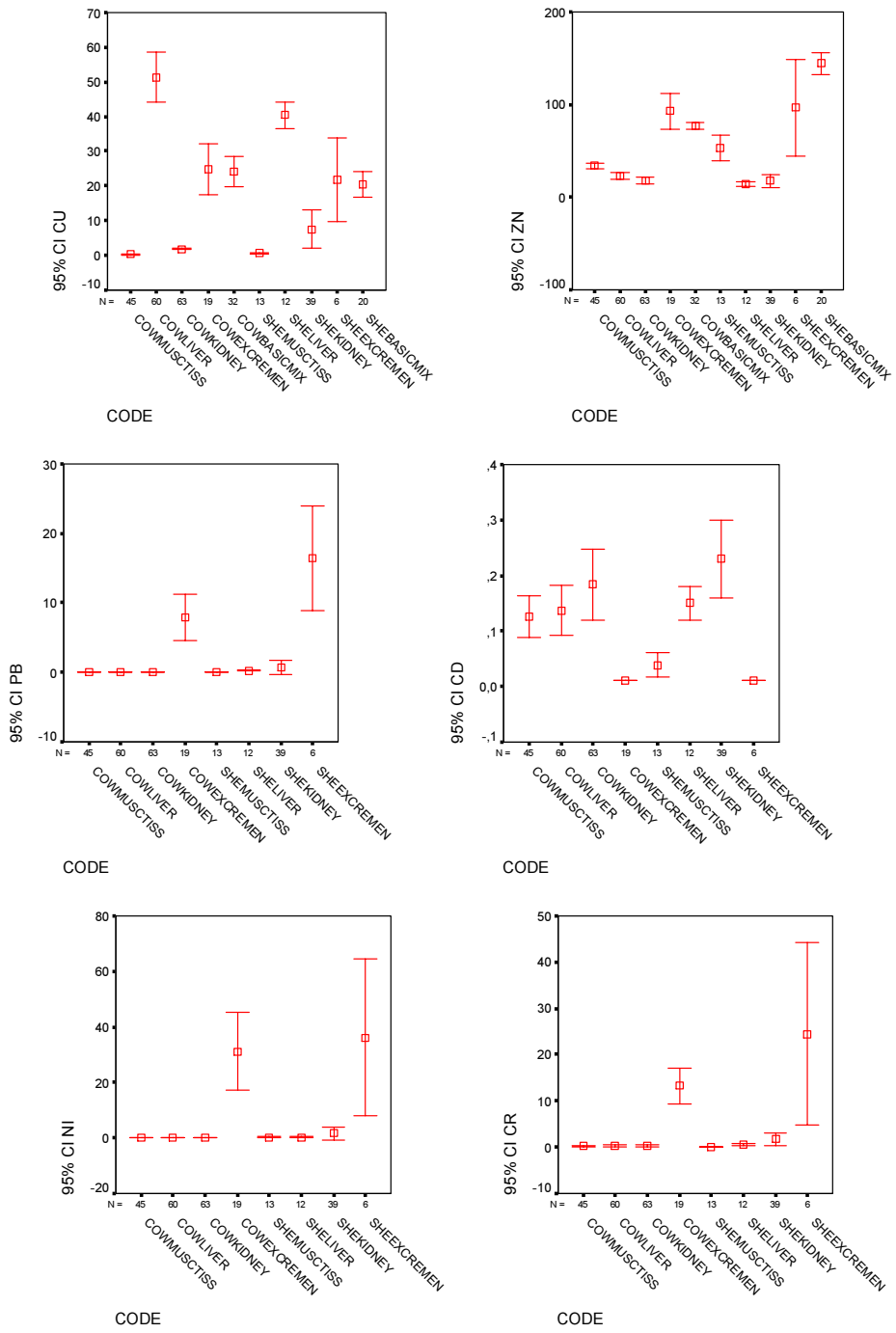

FIG 1. Box and wiskers plots of Cu, Zn, Pb, Cd, Ni and Cr. The y-axis denotes concentration in ppm for muscle tissues, livers, kidneys and animal faeces in both cow and sheep farms.

A COMPARATIVE STUDY ON DIFFERENT SURFACE DECONTAMINANTS ON CHEVON CARCASS QUALITY TO ACHIEVE PRODUCT SAFETY

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Goat meat (chevon) is the costliest meat in Indian sub-continent due to its widen acceptance amongst the consumers. Behind this acceptance, its virtue over other meats, mainly because it is devoid of any religious restrictions is considered as one of the important factors to endow with nutrition to the human population. Indian sub-continent is having the highest number of goat 124.36 Million and about 36 percent of this population is slaughtered annually. Consumers demand safe and hygienic meat and it is the onus of the technocrats or the scientists to provide guarantee in this regard. It has been reported by a number of researchers that a major part of food borne interic infections could be transferred from the body of the animal to the meat. To avoid this situation a several technique of surface decontamination have been tried with varying degrees of success. Out of these, the chlorinated water was found to be effective and easy method; but, in course of time, its bleaching effect, preparation of its standardized solution and transfer of its certain odour to the carcass and subsequent to the meat put this technique on a question mark and obligated the scientist to take up this aspect of work for further analyses.

With all these in perspectives and also to identify a simple, convenient to adopt, cheap and eco-friendly with hazardous left over effect and in consistent with the existing legislature, this work was outlined, where hot water (80° C), lactic acid, chlorine solution and acidified sodium chlorite solutions were used as surface decontaminants on chevon carcass. The work was designed to have a comparative study on the effect of different surface decontaminants, namely, hot water (80°C for 2 minutes), 2% lactic acid for 30 seconds, 1200 ppm acidified sodium chlorite (ASC) for 5 seconds and 50 ppm chlorine solution for 5 minutes in a form of dip and spray on the surface of dressed chevon carcass for 0, 24, 48 and 72 hours of storage. The parameters studied were total plate count (TPC), presumptive total coliform count (TCC), pH, Water Holding Capacity (WHC), Extract Release Volume (ERV) and Thiobarbuturic Acid Reacting Substance (TBA).

All the treatments in the said experiment reduced TPC and TCC. Lactic acid dip and hot water dip were most effective in reducing TPC with no significant difference between them (1.32 & 1.26 log/cm^2 respectively). ASC and hot water in dip could diminish TCC but did not vary significantly (1.33 & 1.30 log/cm^2 respectively). No treatment affected muscle pH, WHC, TBA, ERV, appearance, smell, tenderness and overall acceptability of treated chevon carcasses significantly. All the decontaminants were found to be effective in diminishing the surface microbial load in chevon carcasses without distressing the keeping and eating quality adversely but the potentiality of lactic acid and hot water were more effectual in flagging TPC. When TCC is considered, the best treatment was found to be the hot water and ASC. The primary aim of the work was to identify the most competent and cheapest decontaminant having the ample potentiality to uphold the wanted keeping and eating qualities, the hot water was found to be the apposite one for hygienic and wholesome goat meat production. The findings are more noteworthy due to the fact that the hot water is very simple to get hold of, and it do not have any residual or impact in terms of transferring any uncalled-for odour to the product and thereby the product safety, its excellence and the question of food security are away from any question. The entrepreneurs could be advocated to embark on this technique in larger way, without any extra economical contribution to take on it in their small ventures of chevon meat production. Thus it could be a very effective decontamination methodology, suitable for numerous small and marginal meat traders who deal with minimum number of slaughtered animals but are having a direct access to the consumers with their ready produce.

ESTABLISHMENT OF ANTIMICROBIAL RESIDUE MONITORING PROGRAMME FOR FOOD OF ANIMAL ORIGIN IN SRI LANKA

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Antibiotic drugs are often used both therapeutically and prophylactically in animal production, and are necessary for many production systems. However, the presence of unacceptable levels of antimicrobial residues in animal products may lead to direct effects on the consumer, such as allergies and toxicities such as dose-independent idiosyncratic reactions that can be triggered due to chloramphenicol residues [4]. Indirect adverse reactions include the promotion of antimicrobial resistance [2, 5]. Further, the parent drugs and their metabolites of the nitrofuran group of antimicrobials are known to be carcinogens [1].

In order to promote awareness on food safety and quality assurance, it is necessary to monitor antimicrobial residues in animal products. This can be done only by having well equipped laboratories and validated techniques [3]. Sri Lanka, as an export country for cultured shrimp, needs to comply with EU regulations. The establishment of the residue monitoring programme in Sri Lanka was commenced in 2002 at the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya.

Three techniques have been established in Sri Lanka for monitoring antimicrobial residues in food of animal origin. The modified EU Six Plate Test (SPT) is a bioassay technique, which screens six groups of antimicrobials, namely; penicillin, aminoglycosides, fluoroquinolones, macrolides (erythromycin), tetracycline and sulphonamides. Food commodities are screened for chloramphenicol residues using a commercially available ELISA kit (Euro Diagnostica, Netherlands), which is a microtiter plate, based competitive enzyme immunoassay. A HPLC-DAD technique has been established to detect nitrofuran metobolites in shrimp including the primary metobolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazon.

Since July 2002 a total of 1712 samples including 900 chicken samples and 812 shrimp samples were screened for antimicrobial residues using the SPT. Since November 2002, 1027 shrimp samples from export consignments have been tested using ELISA. In 2007 the HPLC technique was established and 85 shrimp samples have been tested. Out of the 900 broiler meat samples tested by SPT, 52 samples (5.8 %) showed positive results while all the shrimp samples tested were negative. Out of the 1027 shrimp samples tested using ELISA, 2 samples (0.2 %) were positive. All the samples tested using by HPLC were negative for nitrofuran metabolites.

There is clear evidence that the frequency of residues occurrence in the samples tested decreased as the project progressed due to increased awareness among farmers on restrictions imposed on using antimicrobial agents in animal production. Trace back procedures were adopted in situations where residue violations were observed in order to initiate action to prevent reoccurrence through the appropriate and responsible use of antimicrobials, and efforts were taken to ensure sustainability of the project. Further, steps are now being taken to comply with ISO 17025 Certification in order to obtain the status of laboratory accreditation.

The laboratory established at the Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka is now recognized as an Independent Reference Laboratory for monitoring antimicrobial residues in food of animal origin. The laboratory service for the analysis of food samples for antimicrobial residue monitoring is now extended to producers and quality assurance divisions of regulatory authorities.

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DEVELOPMENT OF A RELIABLE METHOD FOR THE DETERMINATION OF ANTIMICROBIAL RESIDUES AGENTS IN POULTRY MEAT

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Enrofloxacin is a fluoroquinolone with broad antibacterial spectrum and high bactericidal activity against pathogenic bacteria found in diseased animals [1]. Ciprofloxacin is the active metabolite of enrofloxacin in several species [2, 3]; so, this study must be capable of distinguishing between these drugs.

Our objective as the first step of this project, conducted under IAEA Coordinated Research Project/Task N°: C3-NIC/5/007 02 01 was to develop a simple, sensitive and reliable method for the simultaneous determination of residues of two quinolones (enrofloxacin and ciprofloxacin) in poultry tissue. High Performance Liquid Chromatography (HPLC) with fluorescence detection was used as mean of detection taking advantage of fluorescent properties of these compounds. The analysis was performed on a Waters Alliance 2695 Separation Module and Waters 2475 fluorescence detector. The analytical column was a NovaPak C-18 (5 µm, 3.9 mm x 150 mm). 5 g of sample was weighed into a 50 mL centrifuge tube. The analyte extraction was performed with 5 mL of acetonitrile after stirring with a vortex mixer (2 min), followed by a 1850 g centrifugation process for 10 min. The organic phase was transferred to another centrifuge tube and evaporated under nitrogen flow at 40° C until dryness. The residue was re-suspended in 1 mL of a buffer (Glycine 0.1M, NaOH 0.1M, pH 9.1). The solution was filtered through a 0.45 µm filter into LC vials and then is ready for injection.

The average recoveries of enrofloxacin and ciprofloxacin from poultry samples at the level of 50 µg/kg were in the range of 74.5% and 114.0%, respectively. The decision limit was 101 µg/kg for enrofloxacin and 105 µg/kg for ciprofloxacin and the detection capability was 103 µg/kg for enrofloxacin and 111 µg/kg for ciprofloxacin. Typical chromatogram is shown on figure 1.

Considering the results obtained, we conclude that the method described for analysis of quinolones in chicken is fitted for purpose and is suitable for Official Monitoring proposes in Nicaragua and other developing countries. The next step of this project is to extend the method capability to detect other quinolones and also different classes of antimicrobial drugs.

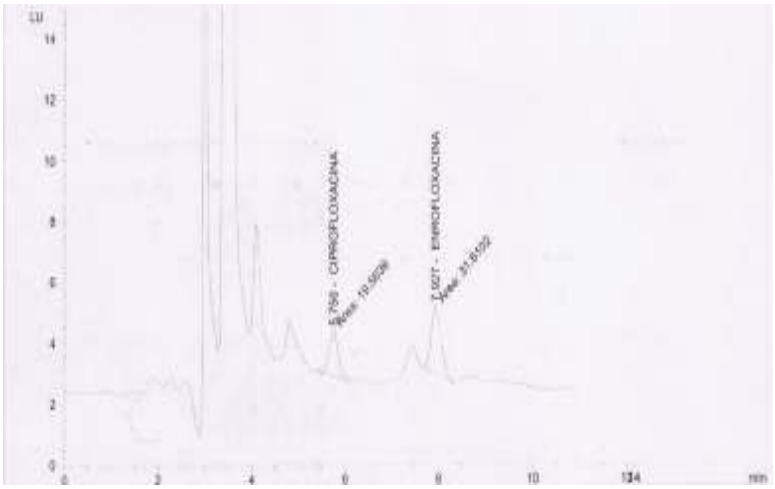

FIG. 1. Typical chromatogram of enrofloxacin and ciprofloxacin at 50 µg/kg.

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ADVANCES IN SARCOCYSTIOSIS DIAGNOSIS IN SOUTH AMERICAN CAMELIDS IN PERU

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Sarcocystiosis in south-American camelids has a high prevalence, about 94% and affects alpacas and llamas by producing cysts either in skeletal muscle or heart muscle and is caused by *Sarcocystis aucheniae* and S. *lamacanis.* The approach of this investigation was to identify the species involves, their pathogenicity, protein structure and the antibody kinetics of animals raised under field conditions, in isolation and under experimental infection and immunization by ELISA and Western blot analysis. Also a PCR assay was developed for diagnosis of the disease in live animals, because so far this parasitic disease is diagnosed at the post mortem examination in the slaughterhouse. PCR product was sequenced and registered in the GeneBank for S. *lamacanis*.

To learn about antibody kinetics under field conditions a group of 30 alpacas and their offspring raised in the Andean region of Huancavelica was sampled. Blood samples were collected and assayed by ELISA for 6 months after lambing. Similarly, a group of alpaca crias kept in isolation in Lima was monitored for antibodies.

A group of 20 pregnant alpacas raised in the Andean region of Puno was transferred to our station in Lima and raised in sarcocystis-free pens. These alpacas were monitored for antibody to sarcocystis by ELISA and blood parasitemia by PCR for 2 years. A group of alpaca crias was immunized using a bradyzoite protein suspension, and blood samples were collected for monitoring antibody by ELISA and western blot. In order to identify genes coding surface antigens, bradyzoites of S. *aucheniae* were collected for total RNA and mRNAisolation. From this mRNA a cDNA library will be constructed, using ligation, transformation and sequencing.

Alpacas from Huancavelica assayed by ELISA showed antibody (93.3%) up to 4 months after parturition, and then 100% of them were positive. This result indicates the high rate of the infection in field conditions. A 30% of their offspring showed antibody up to 2 months of age being 100% by 6 months of age. This indicates that there is an early exposure of the crias, which may be between the second or third month of age. A similar group of alpaca crias born in Lima and raises under isolation remained negative during the 6 months observation period.

Those pregnant alpacas born and raised in Puno for 6 years and then transferred to Lima and kept under isolation showed antibody (100%). The PCR was positive in 16 (80%) and decreased after 7 months of isolation, when 17 (85%) remained positive, and turned negative by 20 months of isolation, however the remained ELISA positive.

The group of crias of alpacas for the immunization trial had antibody at birth and these declined by the second month of age. The treatment group was immunized with a bradyzoite protein suspension subcutaneously by the second month of age, and booster dose was administered 4 weeks later. A control group received a saline solution. Blood samples were collected prior and after immunization. The antibody optical density (OD) was 0.553 for the immunized group while in the control group was 0.158 ($P < 0.05$). After the booster dose the OD increased in the immunized group to 1.318 while in the non-immunized group was of 0.142. This finding shows that there was a immune response induced by the immunogen. Both groups were challenged by an oral dose of oocysts and changes in the antibody response were detected. Western blot analysis showed that the antibody recognized sarcocystis proteins of 34 and 31 kDa mainly, which generally were detected by 4 and 5 weeks after either immunization or experimental infection.

The PCR was targeted at the ssu rRNA of S. *aucheniae*, and an amplicon of 420 bp was amplified. This was sequenced and showed to correspond to S. *aucheniae* when DNA was isolated from bradyzoites of cystis of the skeletal muscle. When DNA was isolated from microcysts located in the heart muscle, this nucleotide sequence showed to correspond to a new species, *Sarcocystis lamacanis*. Due to its high rate of infection in the Peruvian camelids the control approach for this parasite should focus on the vaccine development, either recombinant vaccine and education.

CONTROL OF AUTHENTICITY OF TYROLEAN MILK BY STABLE ISOTOPE MEASUREMENTS

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Consumers are willing to pay elevated prices for specific product qualities, e.g. for food from a certain region, therefore the declaration of origin of these products needs to be controlled. Conventionally this is done by estimating the flow of goods and by controlling the documentation accompanying the products. However, this means are often not sufficient to detect intentional deception.

The measurement of the stable isotope composition of products offers the possibility to investigate the product itself. The stable isotope ratios of the elements H, C, N, O and S are varying geographically due to diverse environmental conditions (e.g.: climate, geology, soil, altitude, geography…) thus creating individual patterns for each region. These patterns are transferred in different ways into plants and animals originating from a certain region. Therefore analysis of the stable isotope pattern is a potent tool for geographic differentiation.

Tyrolean milk is regarded as a high quality good produced under strict regulations in a special (alpine) environment. To protect Tyrolean milk from incorrectly declared milk originating from other regions/countries, stable isotope investigations have been carried out over a period of one year on samples from all regions of Tyrol. Samples have been measured for isotopic composition of H, C, N and O.

The observed variation in the isotopic pattern of the Tyrolean milk within the year can be explained by different feeding regimes during summer and winter. Comparison of the isotopic pattern of the Tyrolean milk and milk samples of the same age from other regions gives evidence for significant differences in the isotope ratios. As the investigated “non-Tyrolean” samples have been produced in neighbouring regions that should have similar isotopic signals due to comparable environmental conditions, presumably it should be even easier to distinguish between milk from farther regions and milk from Tyrol.

THE EFFECT OF SUNLIGHT ON THE SURVIVAL OF *SALMONELLA* SPECIES FROM THE POULTRY PRODUCTION CHAIN ENVIRONMENT IN ZAMBIA

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This study investigated the effect of simulated sunlight on the survival of Salmonella isolates on surfaces under both clean and dirty conditions. Isolates from Zambia were compared to a strain of S. Enteritidis with known characteristics, which was isolated from poultry in the UK. Cells were suspended in 20 µl droplets on stainless steel surfaces and placed in an incubator maintained at 25 oC and a relative humidity of between 50 and 70%. Survival after a period of 12 h light and 12 h dark or 24 h dark was measured. Differences were analysed for significance using a one-way analysis of variance (ANOVA). Results show there were a significantly higher number of cells surviving on surfaces after 24 h in the dark when compared to that of populations exposed to a 12 h light/ 12 h dark cycle. Significantly more cells also survived exposure to sunlight under dirty than clean conditions. Under field conditions exposure of contaminated surfaces to sunlight could be used in place of chemical methods of control as a cheaper way to reduce Salmonella contamination of surfaces.

TABLE I. BACTERIAL STRAINS USED IN THIS STUDY

Strain	Description	Source
LA5	Salmonella Enteritidis (PT4)	Chicken
ZA-1	Salmonella Enteritidis (PT7)	Poultry processing plant
ZA-2	Salmonella Enteritidis (PT7)	Poultry processing plant
ZA-3	Salmonella Enteritidis (PT7)	Poultry processing plant
ZA-4	Salmonella Heidelberg	Poultry processing plant
ZA-5	Salmonella Heidelberg	Poultry processing plant
ZA-6	Salmonella Heidelberg	Farm C
ZA-7	Salmonella Heidelberg	Farm C
ZA-8	Salmonella Heidelberg	Farm D

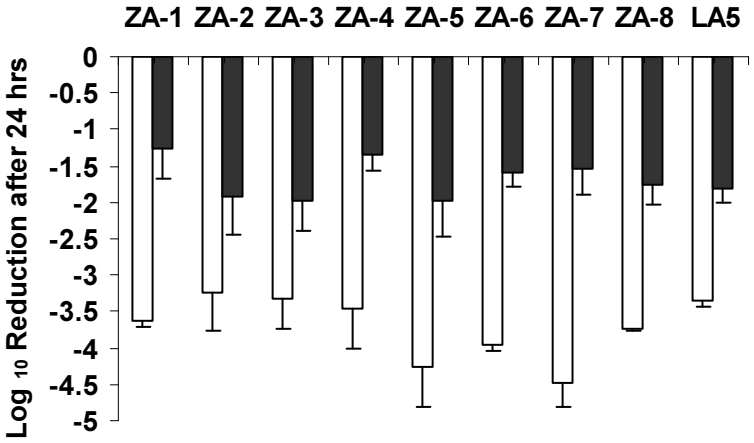

Figure 1. The effect of exposure to sunlight on the reduction in numbers of Zambian Salmonella strains compared to UK strain LA5. Cells were suspended in PBS and placed on a stainless steel surface then exposed to a 12 h light/ 12 h dark cycle (white bars) or kept entirely in the dark (black bars). Bars represent three experiments performed in triplicate with standard error.

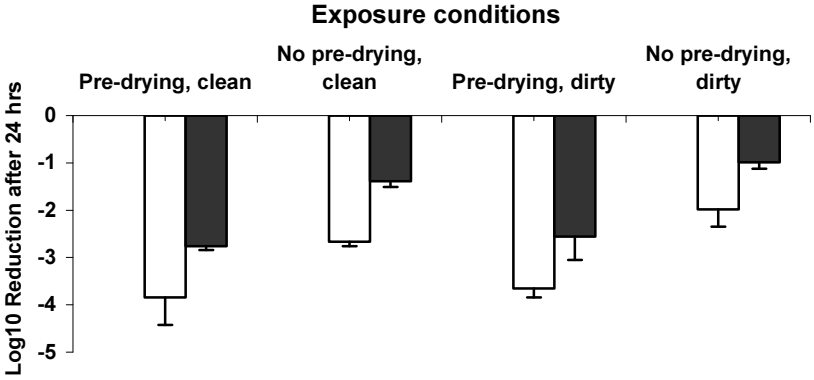

Figure 2. The effect of exposure to sunlight on Salmonella Enteritidis PT4 (LA5). Droplets containing bacteria were either allowed to dry for 2 h before exposure to light (pre-drying) or exposed immediately (no pre-drying) to a 12 h light and 12 h dark cycle. Cells were suspended in either PBS (clean) or PBS containing 1% bovine serum albumin (dirty) conditions. Bars represent three experiments performed in triplicate with standard error.

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EVALUATION OF LEAD AND CADMIUM RESIDUES IN MIXED BROILER FEEDS AND SOME RAW MATERIALS

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Safety food requirements for both man and animals are the main goals of the risk evaluation process. The investigation of the chemical contamination level of feeds is part of the HACCP process (1). Broiler feed samples were harvested from a chicken farm during 2006-2007: corn meal, wheat, soybean meal, full-fat soya, starter, grower and finisher. Pb and Cd content as well as their toxic risk for animals were evaluated. The mean values of the Pb and Cd determined by an atomic absorption (AA) method with a flame AA spectrophotometer are presented in Table I and II and Figure 1 and 2.

TABLE I. AVERAGE CONTENT OF PB RESIDUES IN FEEDS/RAW MATERIALS (MG PB/KG AT 12% HUMIDITY)

Sample		n	$\overline{X} \pm s_{\bar{x}}$	
			2006	2007
Corn meal		5	0.48 ± 0.03	0.63 ± 0.02
Wheat		5	2.03 ± 0.04	0.18 ± 0.01
Soybean meal		5	1.05 ± 0.04	1.06 ± 0.01
Full fat soya		5	1.77 ± 0.03	1.09 ± 0.01
Mixed feeds	Starter	5	1.11 ± 0.03	0.92 ± 0.01
	Grower	5	1.08 ± 0.03	0.70 ± 0.01
	Finisher	5	1.47 ± 0.02	0.43 ± 0.01
TOTAL	Average	35	1.28 ± 0.03	0.72 ± 0.01
	Min.-Max.		0.48 - 2.03	0.18 - 1.09

TABLE II. AVERAGE CONTENT OF CD IN FEEDS/RAW MATERIALS (MGCD/KG AT 12% UMIDITY

Sample		n	$\overline{X} \pm s_{\bar{x}}$	
			2006	2007
Corn meal		5	0.053 ± 0.002	0.155 ± 0.002
Wheat		5	0.162 ± 0.002	0.025 ± 0.001
Soybean meal		5	0.124 ± 0.002	0.180 ± 0.001
Full fat soya		5	0.024 ± 0.001	0.180 ± 0.001
Mixed feeds	Starter	5	0.110 ± 0.003	0.081 ± 0.001
	Grower	5	0.060 ± 0.002	0.115 ± 0.001
	Finisher	5	0.112 ± 0.002	0.111 ± 0.001
TOTAL	Average	35	0.092 ± 0.002	0.121 ± 0.001
	Min.-Max.		0.024 - 0.162	0.025 - 0.180

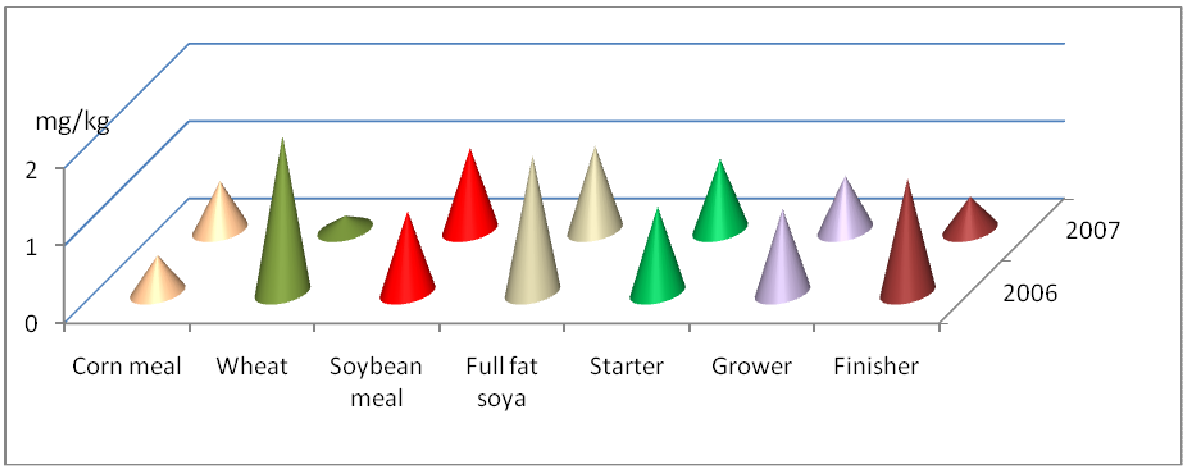

FIG. 1. Average content of Pb in feeds/raw materials (mgPb/kg at 12% umidity)

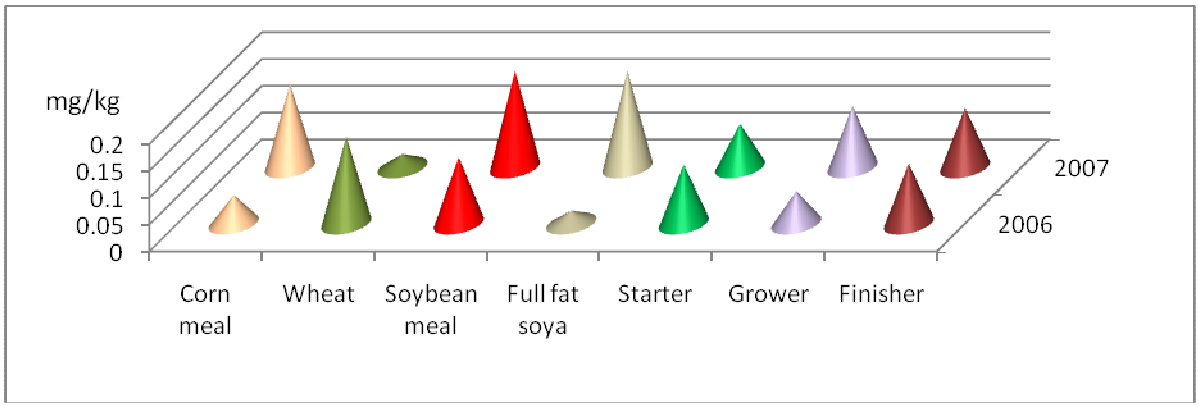

FIG. 2. Average content of Cd in feeds/raw materials (mg Cd/kg at 12% humidity)

The broad range of the Pb and Cd residue levels in raw materials is due to their various sources from all over the country. The investigated feeds had a low content of Pb and Cd as compared to those of the feedstuffs from the highly contaminated areas of Romania(2) The Pb and Cd residue levels were found under the admitted maximal limits established by the ANSVSA Order no. 18/2007: for lead, the maximal limit established for feedstuffs is 10 mgPb/kg and for mixed feeds is 5 mgPb/kg; for cadmium, the maximal limit established for feedstuffs is 1.0 mgCd/kg and for mixed feeds is 0.5 mgCd/kg [3].

The investigated feed samples did not have toxicological risk for the broiler chicken in the farm they were harvested from.

References:

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[3] Ord. ANSVSA (Romanian National Veterinary and Food Safety Authority) no. 18/2007.

THE PERSISTENCE OF *SALMONELLA ENTERITIDIS* PT4 LA5 ON SURFACES AND NON SURFACES FROM THE POULTRY PRODUCTION CHAIN ENVIRONMENT IN ZAMBIA

C. Nyeleti

Zambia

EFFECTS OF COMBINED TREATMENT OF GAMMA IRRADIATION AND ADDITION OF SEAWEED EXTRACT ON READY-TO-EAT MEAT

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The demand for ready-to-eat meat products is gradually growing due to their convenience. Ready-to-eat meat products are manufactured and frozen in a processing factory, distributed and sold in the frozen condition (below -10^{o}C). However, hygienic quality can be sometimes threatened by the growth of food-borne pathogens such as *Listeria monocytogenes*. Many researchers recognized and reported that gamma irradiation at low doses, below 10 kGy, killed most microorganisms without deterioration of food quality [1]. However, several adverse effects such as lipids oxidation and softening, caused by ionizing radiation have prevented this technology from being extended. For preventing oxidation, use of an antioxidant has been considered and practically applied in some products. Recently, natural antioxidants have been used to scavenge free radicals and to inhibit lipid oxidation owing to their safety and wholesomeness [2].

The objective of this study is to evaluate the combined effects of the gamma irradiation and the addition of the natural extract from *Undaria pinnatifida* on the microbial safety and the quality of the ready-to-eat hamburger patties.

The effect of combined treatment on the microbial safety of hambuger patties is shown in Table I. By the gamma irradiation at the dose of 2 kGy, the number of colony forming units (CFU) in hamburger patties was decreased from 3.48 Log to 3.07 Log. But, when the *Undaria* extract was added, the initial CFU in the patties was decreased to 3.17 Log, and no viable colony was detected at the absorbed dose of 2 kGy. These results suggested that the lower irradiation doses could be used for killing the pathogens in meat with the addition of the *Undaria* extract.

TABLE I. EFFECT OF COMBINED TREATMENT ON THE MICROBIAL SAFETY OF HAMBURGER PATTIES

Irradiation dose (kGy)	CFU after the irradiation treatment (Log CFU/g)	CFU after the combined treatment of irradiation and addition of *Undaria* extract (Log CFU/g)
0	3.48[a]	3.17[a]
2	3.07[b]	ND[*]
4	ND	ND
6	ND	ND

[a-b] in the same column means the values are significantly different ($P < 0.05$).

* Viable colony was not detected at detection limit < 10^2 CFU/g.

To investigate the effect of the *Undaria* extract on lipid oxidation caused by the gamma irradiation, TBARS measurements were carried out (Fig. 1). TBARS values were increased from 1.35 to 2.10 at the absorbed dose of 8 kGy. But, when the *Undaria* extract was added at the concentration of 1%, TBARS value was only 1.11 at the same dose. These results shown that the hamburger patties added with the *Undaria* extract showed lower lipid oxidation compared to the patties without the extract by the irradiation.

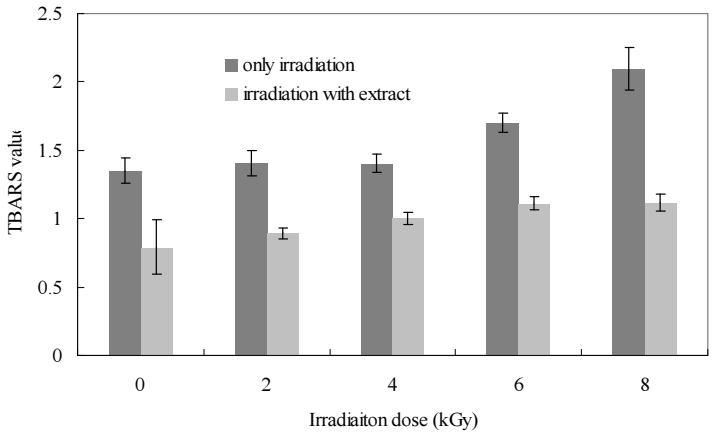

FIG 1. TBARS values of patties added with the Undaria extract by gamma irradiation

These results suggested that addition of the *Undaria* extraction could improve the microbial safety in meat and it decreases the lipid oxidation caused by the irradiation.

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