

# SUSTAINABLE IMPROVEMENT OF ANIMAL PRODUCTION AND HEALTH

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## PREFACE

The world's poorest people, some one billion living mostly in Africa and Asia, depend on livestock for their day-to-day livelihood. To reduce poverty, fight hunger and ensure global food security, there is an urgent need to increase livestock production in sustainable ways. However, livestock production in developing countries is constrained by low genetic potential of the animals, poor nutrition and husbandry practices and infectious diseases. Nuclear techniques, when applied in conjunction with conventional methods, can identify constraints to livestock productivity as well as interventions that lead to their reduction or elimination in ways that are economically and socially acceptable. The challenge is how best to exploit these techniques for solving problems faced by livestock keepers within the many agricultural production systems that exist in developing countries and demonstrating their advantages to owners, local communities and government authorities.

This publication is a compilation of the contributions emanating from an international Symposium on 'Sustainable Improvement of Animal Production and Health' organised by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture in cooperation with the Animal Production and Health Division of FAO. It provides invaluable information not only on how nuclear and related techniques can be used to support sustainable livestock production systems, but also about the constraints and opportunities for using these techniques in developing countries; it also attempts to identify specific research needs and gaps and new options for using these techniques for solving established and emerging problems. As such, it is hoped that the information presented and suggestions made will provide valuable guidance to scientists in both the public and private sectors as well as to government and institutional policy and decision makers.

The Symposium comprised a plenary session and four thematic sessions, covering (i) interactions among nutrition, reproduction and genotype, (ii) livestock-environment interaction / productivity / climate (water / land / plants / heat / altitude), (iii) detection and control of transboundary animal diseases, including zoonoses, and (iv) animal product safety and food quality. The Symposium was attended by approximately 400 delegates from 100 Member States as well as representatives of international organizations including FAO, WHO, OIE and ILRI who presented and discussed strategies for the sustainable improvement of animal production and health, with particular emphasis on global food security, poverty alleviation and hunger reduction. The seriousness with which these topics were being tackled by Member States was shown in the results of their studies, presented in 53 oral presentations and 163 poster displays by an assorted group of researchers, veterinarians, policy makers, students and other animal scientists who attended the symposium.

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# OPENING STATEMENTS



## Ana María Cetto

*Deputy Director General, Department of Technical Cooperation, IAEA*

Dear Colleagues, Ladies and Gentlemen,

On behalf of the Director General of the International Atomic Energy Agency (IAEA) and on my own behalf, I have the great pleasure to welcome you to the Vienna International Center for this International Symposium on '*Sustainable Improvement of Animal Production and Health*'.

Let me start by briefly highlighting the mandate of the IAEA, and in particular, the Department of Technical Cooperation which I head. The IAEA, a specialised organization within the United Nations system, was set up as the world's 'Atoms for Peace' organization in 1957. The Agency works with its 146 (as of September 2008) Member States and multiple partners worldwide to promote the safe, secure and peaceful use of nuclear energy thus contributing to the United Nations Millennium Development Goals for social, economic and environmental development.

The IAEA has three main areas of work or pillars underpinning its mission: Safety and Security, Science and Technology, and Safeguards and Verification. The IAEA is best known for its statutory roles in nuclear safety and security and the verification of international safeguards agreements relating to the non-proliferation of nuclear weapons. It is less known, however, for its role of helping countries mobilise peaceful and safe applications of nuclear science and technology for sustainable development. The Department of Nuclear Sciences and Applications is charged with the responsibility of implementing one of the Agency's Major Programmes, i.e. Nuclear Techniques for Development and Environmental Protection. The five key thematic areas of Water, Energy, Health, Agriculture and Biodiversity and ecosystem management that were identified in the Millennium Declaration of 2000 and reaffirmed in the Agenda 21 Action Plan and the World Summit on Sustainable Development in 2002 drive the Programme. The main programmes within Nuclear Techniques for Development and Environmental Protection are food and agriculture, human health, water resources, environment and radioisotope production and radiation.

These priorities are reflected in the structure of the Department of Nuclear Sciences and Applications, which utilises nuclear and isotope techniques, alone or integrated with other technologies, to assist countries in providing unique solutions to help solve the relevant Water, Energy, Health, Agriculture and Biodiversity topics. These techniques are employed in programmes addressing agricultural productivity and wider food security, improvement of human health, increased availability of water resources, assessment and management of the marine and terrestrial environments and industrial applications.

Of course, the Department of Nuclear Sciences and Applications focuses on those nuclear techniques and technologies that are indispensable to the Agency's mission or that have a comparative or competitive advantage over non-nuclear techniques in terms of cost-effectiveness, or are complementary to non-nuclear techniques. In developing and implementing this vision, there is a need for appropriate coordination within the Agency and with Member States, flexibility in adapting Agency programmes and activities to meet changing needs and to incorporate emerging technologies, the development

of the necessary infrastructures, and the timely dissemination of information.

More than half of the Agency's activities in Nuclear Sciences and Applications are implemented through the Technical Cooperation (TC) Programme which helps to transfer nuclear and related technologies for peaceful uses to countries throughout the world. Through training courses, expert missions, fellowships, scientific visits, and equipment disbursement, the TC Programme provides the necessary skills and equipment to establish sustainable technology in the counterpart country or region i.e. Africa, Asia and Pacific, Europe and Latin America. The other half of the Agency's activities is implemented through Coordinated Research Projects (CRPs). The CRPs are research networks that stimulate and coordinate research, and foster the exchange of scientific and technical information by bringing together research institutes in both developing and developed Member States to collaborate on the research topic of interest. Currently, the Department of Nuclear Sciences and Applications is providing technical and scientific support to 901 TC projects and cooperates and collaborates in research and development activities in 77 CRPs across the globe. The research that is supported encourages the acquisition and dissemination of new knowledge and technology generated through the use of nuclear technologies and isotopic techniques in the various fields of work covered by the Agency's mandate. These programmes are supported by the FAO/IAEA Agriculture and Biotechnology Laboratory, situated at Seibersdorf, 35 km south of Vienna which provides scientific and analytical services to research projects, and training and quality assurance services in the area of technical cooperation.

To enhance cost effectiveness and efficiency, inputs from various stakeholders (Member States, donor agencies, UN organizations etc.) must be harnessed, for example, through cooperation, collaboration and information sharing. In order to take these factors into account in the development, review, and implementation of the strategy, the Agency needs a mixture of inputs from Member States and from technical experts. Of particular importance are international conferences, symposia and other meetings, which bring us to the topic of this Symposium: '*Sustainable Improvement of Animal Production and Health*'. This Symposium will address aspects of:

- interactions among nutrition, reproduction and genotype;
- effects of environment on animal productivity;
- detection and control of transboundary, emerging and zoonotic diseases;
- achieving food safety and security in the 21 century

### But why livestock?

Throughout history, science and technology have been powerful tools for human development and poverty reduction. For decades, the IAEA, in partnership with FAO, has assisted its Member States to produce more, better and safer food. The on-going 'Livestock Revolution', a demand-driven increase in livestock production, especially in developing countries, presents both opportunities and challenges. As countries experience economic growth, higher incomes and increasing urbanisation, consumers are able to diversify their diets to include more meat and dairy products. Livestock production is therefore one

of the fastest growing sub-sectors in developing countries, where it already accounts for a third of GDP and is predicted to become the most important agricultural sub-sector by 2020 in terms of added value. The increasing demand for livestock products is creating opportunities for improving the welfare of millions of poor people who depend on livestock for their livelihoods. However, changes in production, procurement, processing and retailing of food, along with environmental and food safety concerns, erosion of animal genetic resources and the threat of zoonotic and other emerging infectious diseases, threaten the potential of the poor to benefit from the on-going livestock revolution.

The food crisis — which began last year with soaring prices and food shortages — continues today in many countries. However, it has been overshadowed in recent months by the global economic

crisis. Today, one in six people in the world is food insecure; almost one billion people. The choices we make now will determine how or whether we can feed ourselves in the future. If we get it right, we can have a thriving food economy. Speaking at the end of the two-day Madrid 'High Level Meeting on Food Security for All' — UN Secretary-General Ban Ki Moon said: 'We worked hard to bring food assistance to those that most needed it in 2008. This year it is going to be harder because of the financial crisis which impacts on food security, but we must remain focused on improving food production' which is the theme for this Symposium. Increasing agricultural productivity remains one of the most effective ways to combat hunger and poverty.

Ladies and gentlemen, I wish you fruitful discussions and a successful participation at the Symposium.

## Modibo Traoré

*Assistant Director-General, Agriculture and Consumer Protection Department, FAO*

Dear Colleagues, Ladies and Gentlemen,

It is a great honour and pleasure for me to participate at this timely International Symposium on 'Sustainable Improvement of Animal Production and Health'. I wish to convey my sincerest thanks to Mr Burkart and the IAEA for the invitation and the opportunity to share the FAO, and other role-players', view on sustainable livestock production and health. I also wish to acknowledge and congratulate all those who have been involved in our successful and longstanding partnership of over 40 years since the inception of the Joint FAO/IAEA Division in 1964. As many of you are aware, the focus of this joint initiative is to support both FAO and IAEA Member States in the area of food and agriculture through the development, adaptation and transfer of nuclear and nuclear-related technologies in the areas of:

- sustainable intensification of crop production systems;
- sustainable intensification of livestock production systems;
- strengthening compliance with food and environmental safety standards through good agricultural practices.

However, Ladies and Gentlemen,

The challenges facing us in food and agriculture are enormous. The 2008 FAO report on 'The State of Food Insecurity in the World' shows that in 2007 — mainly because of soaring food prices — the number of hungry people in the world rose by approximately 75 million. With an expected increase of another 40 million people in 2008, it is currently estimated the world has 963 million malnourished people. This implies that almost one billion of the world's 6.5 billion people face hunger and are 'food insecure'. Global food security is becoming an issue for the first time since the Second World War. The UN Secretary-General Ban Ki-moon recently warned at the Madrid 'High-Level Meeting on Food Security for All' that the situation is likely get worse unless more is done to tackle the food security problem.

In the past, famine conditions usually affected only one country or region and the crisis was a result of a particular circumstance, such as drought, floods or civil unrest. The current food crisis, although overshadowed in recent months by the global economic and financial downturn, is a worldwide phenomenon and cannot be attributed to one single factor. It's the perfect storm of the increasing demand and variety (consumer preference) from emerging markets in Asia and Latin America, extreme weather cycles linked to climate change, and the diversion of food crops (maize in particular) from the human food chain to the production of biofuel. Our challenge is not only how to ensure adequate food for the current 963 million hungry people, but also how we are going to feed a world population of over 8.3 billion people by 2030. If food demand is to be met in future, increased outputs will have to come mainly from intensified and more efficient use of the land, water and plant and animal genetic potential, fisheries and forestry resources that smallholder farmers in developing and transition countries have at their disposal. Smallholder farms around the world are home to approximately two billion people, making up one third of global population thus representing a vital part of local and national consumption and international trade. A lack of water to meet daily needs is also a reality for many people around the world today. Water availability and access are key constraints to poverty reduction and food security. At the same time, action must

be taken to arrest the destruction and degradation of the natural resource base.

Currently, seventy percent of the world's poor live in rural areas and are dependent on agriculture. Although global food prices have fallen in recent months, because of years of under-investment in agriculture, coupled with the increasing threat of climate change, future food security is not guaranteed, and in fact, the situation is likely to worsen. Urgent action is needed to prevent hundreds of millions more people from slipping into hunger because of the volatile food prices, increasing energy and water scarcity and the economic and financial crunch. Beside its direct benefits, agriculture also has important linkages with the rest of the economy and creates jobs in other sectors. Increased agricultural productivity would result in lower food costs, which in turn would help reduce poverty in both rural and urban households by lowering the high proportion of their household income currently spent on food.

### But why livestock?

Approximately one billion people in the world today depend on animals for their livelihood. Animals provide protein, natural fertiliser and a cash income, and are also a source of draught power (ploughing, traction, irrigation). Because many developing and transition countries have realised high economic growth in recent years coupled with an expanding urban population, income growth is altering spending and consumer preferences. Global food demand is shifting from grains and other staple crops to processed food and high value agricultural products, such as vegetables, fruits, meat, and dairy. The increasing demand for livestock products e.g. increased demand for meat and dairy products from the growing middle classes of countries such as China and India as well as heavy demand for feedstock from the biofuel industry is creating opportunities for improving the welfare of millions of poor people who depend on livestock for their livelihoods. However, changes in production, procurement, processing and retailing of food, along with environmental and food safety concerns, erosion of animal genetic resources and the threat of emerging infectious diseases, threaten the potential of the poor to benefit from the on-going livestock revolution.

The world has about 5 billion hectares of agricultural land. Whilst 90 percent of the world's 1.4 billion hectares of arable land are increasingly being devoted to agro-export crops, biofuel and transgenic soybean to provide fuel for cars and feed for livestock, millions of smallholder farmers in the global South still produce the majority of staple crops needed to feed the world's rural and urban populations. In Latin America, about 20 million peasant production units occupying close to 60 million hectares, or 40 percent of the total arable land with average farm sizes ranging from 1–2 hectares, and producing 51 percent of the maize, 77 percent of the beans, and 61 percent of the potatoes for domestic consumption. In Africa, arable land comprises some 213 million hectares farmed by approximately 33 million smallholder farmers, representing 80 percent of all farm holdings in the region. Although Africa now imports large amounts of cereals, the majority of African farmers (many of whom are women farming less than two hectares of land), produce a significant amount of basic food crops with little or no usage of fertilisers and

improved seed. In Asia, arable land comprises slightly more than half a billion hectares is occupied by the majority of the more than 200 million rice farmers. Few farm more than two hectares of rice but they produce 91 percent of the world's production, with China and India growing more than half the total crop. Small increases in yields on these smallholder farms that produce most of the world's staple crops will have far more impact on food availability at the local and regional levels than the doubtful increases predicted for distant and corporate-controlled large monocultures on commercially managed farms incorporating such high tech solutions as genetically modified seeds.

The FAO/IAEA partnership, mandated through the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, has greatly contributed knowledge and assistance in capacity building activities for our Member States over the years. This longstanding collaboration, which began as a visionary initiative within the United Nations system, is being recognised increasingly by other international organizations as an excellent example of striving to work more closely together under 'ONE UN', to meet the Millennium Development Goals. The Joint FAO/IAEA Programme provides a synergy of resources and common aims in promoting the IAEA mandate on peaceful uses of nuclear technologies along with the FAO mandate

in food and agriculture. Furthermore, the interdisciplinary approach of the Joint FAO/IAEA Programme plays a major capacity building role in these areas and supports the efforts of FAO directly. Sustainable livestock production systems require an integrated management approach to farming practices that take account of complex interactions between soil, water and crops, their linkages to livestock and plant pests, and their relationship to the efficient use of agrochemicals. Sustainable farming systems rely not only on the conservation and efficient use of resources that protect our environment, but also on other factors such as agricultural investment, government policies and meeting consumer's demands and perceptions related to rural development and farming operations.

However, current technologies for enhancing crop productivity, animal production and health, food quality and safety, and controlling plant and animal pests and land degradation may have to be modified in response to these new emerging global issues and our efforts re-directed. It is imperative that we rise up together as an all inclusive community to meet these challenges on food security and agricultural sustainability, and I look forward to the continued strengthening of our FAO and IAEA collaboration in providing solutions to these problems.

Thank you for your kind attention.



## David Nabarro

### *UN System Coordinator for Influenza and Global Food Security*

Dr Traoré, Excellencies, Colleagues,

I am most grateful for this opportunity to address you today. In January 2009 I was appointed by the United Nations Secretary General, Ban Ki-Moon, to serve as Coordinator of the United Nations System High Level Task Force for Global Food. This Task Force was established in May 2008 in response to the urgent need expressed by United Nations Member States for coherent efforts in response to both short and long term elements of food security. The Task Force brings together the work of UN system bodies, International Financial Institutions and the World Trade Organization.

The members of the Task Force are committed to supporting national and regional responses to food insecurity through a Comprehensive Framework for Action that covers a broad spectrum — first, on reducing the vulnerability of households and communities at risk of food insecurity (as manifested by hunger and high rates of malnutrition); second, encouraging investments in sustainable and productive agricultural systems that improve the resilience of smallholder producers; third, improving opportunities for marketing with the participation of producer organizations and engagement of private sector partners as appropriate; and fourth promoting trade in agricultural products that works fairly in the interests of all communities and countries.

Why the emphasis on smallholder farmers? In much of the world they produce the majority of the food that is consumed. They tend to have been left aside in agricultural development efforts — especially so in recent decades as investment in agriculture generally has fallen. Yet more than two and a half billion people depend for their income and nutrition on the efforts of smallholder farming households, particularly on the work of women farmers. They live in rural areas and also in the outskirts of cities, farming plots of less than two hectares. Some of them depend entirely on livestock, others farm with a mix of livestock and crop production. They make difficult choices in the face of uncertainty about climate, access to inputs, disease, crop losses and their opportunity to market their produce at a reasonable price. Most of them are women whose children are at risk of nutritional insecurity: frequently they have to choose between caring for a child that is unwell, and has special feeding or health care needs, and working on their land or tending the animals. Much animal rearing is done by children themselves.

The Comprehensive Framework reflects a three track approach. The starting point is a recognition of the absolute importance of people being able to enjoy their right to food. Then the emphasis is on actions to realise immediate outcomes — hunger reduction, immediate boosts to agriculture, sound policies on export restrictions and import tariffs and balance of payments support on the one hand, and the third emphasis is on longer term social protection, agricultural development, attention to regional and global trade, and action on complex policy questions on the other.

The framework, which reflects the combined food security aspirations of the whole of the UN system, highlights the importance of strong partnerships involving producer organizations and civil society, businesses, professional groups (including agricultural extension and veterinary services), and researchers at national, regional and global levels. It emphasises the need for those representing the interests of

hungry people and smallholders in policy debates on short and long term aspects of food security.

The Task Force is chaired by the UN Secretary General with the Director General of FAO as Vice-Chair.

This meeting comes at a critical time. There is widespread concern among governments, farmers' organizations and civil society groups, reflected by members of our Task Force, that too many people are unable to enjoy the right to food and nutrition, to have the wherewithal to feed themselves and their families, and to be resilient in the face of economic shocks, climatic events or acts of violence. The UN Secretary General is deeply concerned that food insecurity and hunger are being experienced every day by at least one billion of the world's inhabitants. That is one person in six, or 14 percent of the global population, with a child dying of malnutrition every six seconds.

Much of your work is destined to improving the performance of the livestock sector. Unhealthy animal rearing practices in medium scale commercial operations can affect all who earn their living from animal rearing, especially those who keep a few animals in their back yards. They can also undermine the prosperity of the whole livestock sector, one which is growing at an extremely rapid rate. The prompt diagnosis of, and response to, diseases in animals is vital both for disease control and for assessing practices that are most likely to result in risks to animal health. This, in turn, is important not only for those who rear animals but also for the wider population given the importance of animal illness as a source for emerging disease in humans. At least two new pathogens capable of harming humans emerge each year, and 75 percent of these come from the animal kingdom. Frequently we do not know the potential pathogenicity of such organisms when they first emerge.

The work in which you are involved will have important repercussions for the short and long term health of people and their communities, and may also have implications for wider national prosperity and political stability.

Within our Task Force we work with nations as they contribute to national, regional and global partnerships for agriculture, food security and nutrition. We seek to help them mobilise and improve access to the resources that are necessary to initiate and sustain improved production, with Financial Coordination Mechanisms that gives them a better chance to access the investments they need in an integrated rather than piecemeal manner.

We will be guided in our work by the extent to which we are able to demonstrate reductions in hunger and poverty reduction as laid out in the Millennium Development Goals (especially MDG 1) through demonstrable improvements in production, agriculture-related income, and the contribution of agriculture systems to mitigation of and adaption to climate change.

I would like to focus now on the specific challenges to both animal and human health posed by influenza viruses, and the ways in which different national governments, regional bodies, political organizations and international institutions have worked together to address them.

During the last few years we have witnessed the agreement and application important standards for animal and human health to

the trans-boundary threats posed by disease. I refer specifically to the World Organisation for Animal Health (OIE) animal health standards and the Revised International Health Regulations (IHR 2005) developed by member states of the World Health Organisation. The IHR, for example, is an important intergovernmental framework and series of instruments for collective responses to infectious disease. The proper implementation of IHR 2005 depends on the full participation of national authorities and other stakeholders. Some of them question the extent to which systems for global governance on health reflect the interests of poor people and their nations: they question the value of globalised thinking and working.

A word on my own involvement in this field. I started out thirty years ago as a public health doctor working in rural communities in the Middle East and in South Asia, especially in Nepal. I focused on the determinants of resilience and nutrition in communities, and particularly on the problems experienced by women and children during the tough rainy months leading to harvest, when the demands of the fields and child care tended to compete, when money supply was tight, and when the health centres often lacked necessary medicines. For about five years I taught public health and nutrition, and for another ten years I worked as a civil servant with the British Government, in Africa and then in London. I joined WHO and served in various roles between 1999 and 2005. In September 2005 I was asked by the late J.W. Lee, the then WHO Director General, and Kofi Annan, the Secretary General of the United Nations, to move to New York. My remit was to help different parts of the UN system react to increasing political concern among Heads of State and Government, particularly from Southeast Asia, about the potential political, societal and economic impacts of a severe influenza pandemic.

I was asked to establish a temporary mechanism to ensure that the capacities of the whole UN system (technical human health and agriculture bodies, as well as our full range of social, political and economic bodies) are made available, in a coherent way, to the governments of our Member States.

During 2005 there was broad agreement on the scientific basis of work being undertaken on avian and pandemic influenza; outstanding research questions were also clear. These include a better understanding of risks associated with the movement of highly pathogenic avian influenza among poultry (particularly in ducks); the relative roles of wild birds, trade, and cross border movements in spreading H5N1 among birds; and the behaviour patterns that increase risks for human infection still needing some work.

WHO, FAO and OIE had established clear strategies for national actions to be undertaken: stamping out Highly Pathogenic Avian Influenza (HPAI) when identified — through quick and thorough action; reducing the threat to poultry through introducing biosecurity; monitoring wild birds and charting their movements so that where possible wild birds that might be infected with this virus could be separated from domestic birds; reducing the risk of human sporadic cases by limiting the degree to which humans would be in contact with infected birds, and then preparing to contain and then mitigate the next influenza pandemic when it happens.

The challenge for us was to ensure that governments gave these strategies the impetus necessary for their implementation, leading to the control of HPAI and preparedness for an influenza pandemic. The technical work had to be taken forward within the momentum of the emerging political environment. As well as ASEAN, the USA, the EU, Canada and Japan took political initiatives.

Within the UN Influenza Coordination Office we sought to align different international institutions — including the World Bank, the international organizations of the UN, the regional development banks, other international, regional and local research bodies and so on — and to encourage the collective pursuit of international normal

and standards, with the specialized organizations (WHO, FAO and the OIE) charting a path for the rest of the UN system and the myriad of other organizations becoming engaged in work on avian and pandemic influenza.

From the start most of those who were involved in this work demonstrated unity of purpose and synergy of action. In general, coordination between the bilateral donors, the foundations, national governments, regional bodies and international non-governmental groups (including the Red Cross movement) was strong.

We have subsequently sought to identify the incentives that brought many disparate groups to work together. Finance was important, and the partnership mobilised over US\$ 3 billion in assistance for avian and human influenza actions between 2005 and 2009. But this — on its own — cannot explain the extent to which national authorities have worked together on these issues. The funds that have been pledged are primarily made available to governments: they have moved comparatively slowly.

An International Partnership on Avian and Pandemic Influenza was established as a basis for this cooperation. Other partnerships were organised at regional level through the European Union, APEC, ASEAN and other regional groupings. Few of these partnerships were formal: most had real impact on the alignment and ways of working of their members.

We concluded that most of the groups working together on this issue recognised the value of working together, in synergy. They found it both operationally useful and reassuring in a situation where there was considerable political urgency and need for concerted action by institutions. Stakeholders from the public, private and voluntary sectors have valued the opportunity for coherence, joint working and participation. They have worked together on disease surveillance, reporting and response. They have joined together to support the evolution of an inclusive movement that enables hundreds of different stakeholders to feel at home within it.

Pandemic preparedness work has moved forward over the last four years thanks to the efforts of this broader movement, and the effort has been tracked through annual global progress reports using information from countries. The reports, which have involved the full range of UN system agencies and the World Bank, have served as the basis for collective accountability. The reports reveal that over the four-year period there has been more rapid reporting of HPAI and more effective, sustained responses to outbreaks of the disease in poultry. The OIE is now pursuing the elimination of H5N1 in the next few years. There has also been a massive effort to initiate pandemic preparedness work which we believe has stood us in good stead as the world faces up to the first outbreak — potentially pandemic — of a novel influenza virus of this century. Once again, our preparedness is being tested by the uncertainties around which way this particular threat will go.

Our Annual Reports identify seven factors for success. These are:

- consistent political commitment;
- resources and capacity to go to scale in response to a threat;
- interdisciplinary working (particularly animal health and human health) within countries and across borders;
- predictable, prompt, fair and sustained compensation schemes for those who lose property or animals as a result of control measures;
- strong engagement of public sector, private sector and voluntary agencies;
- clear and unambiguous communication of reliable information (and sharing of uncertainty as appropriate);
- the need for a viable and scientific response strategy.

Experiences with SARS and other diseases suggest that if information is kept from people they will not feel empowered to be part of the response.

What are the incentives for success? First is the availability of good quality and accessible information about HPAI outbreaks — based on good mapping of issues, tracking of progress and risk analysis. The information that is available has been synthesised and made available to those who need it through the efforts of international organizations in response to the needs of their primary clients. Without well functioning surveillance and reporting systems we are stuck: OIE and FAO have played major roles, working with the support of a number of Member States to establish better diagnostic surveillance and reporting capacity.

A second incentive is the ready availability of instruments, services and assets needed for effective action. These include the Global Outbreak Alert and Response Network (GOARN) in WHO and the FAO-OIE Crisis Management Center for Animal Health that provide a backbone for solidarity and international action. This encourages countries and other stakeholders to be engaged — they know that dependable systems exist that can help them.

A third incentive is the existence of the right legal codes (and means for enforcement) at country level — for controlling movements of animals, for ensuring compensation when animals have to be killed and for enabling the consistent nation-wide implementation of public health functions (especially in decentralised political systems).

A fourth incentive is the widespread appreciation among the public, of the pandemic threat and the need to be prepared. Unfortunately it has not proved easy to sustain the appreciation that animals, and ways in which they are cared for, can pose a risk not only for their own health but also for human health. The risk can be reduced by changed behaviour. The information and compensation needed to encourage behaviour changes are often not sufficient. It is vital that the potential for animals to serve as the source for diseases in humans, and *vice versa*, result in better attention to the animal-human health interface — what we tend to refer to as the 'One World, One Health' movement following the groundbreaking work of the wildlife conservation movement.

A fifth incentive is empowered and professional government servants — people in government who feel that they are in a position to take the initiative in the face of a disease threat. They sometimes do not believe that their own authorities, or international authorities, are working in their interests. This is a challenge. H5N1 — and other diseases — will not be controlled through compulsion and sanctions. It doesn't work. People start to hide, they do not explain, they do

their best to avoid involvement. So it is absolutely essential to build the necessary trust for effective action.

There are a number of continuing challenges for our collective effort to control HPAI caused by the H5N1 virus and to prepare for pandemics.

The first is the continuing lack of adequate systems and capacities for data collection and surveillance, laboratory services and analysis, and for the management and use of information derived from the data. This applies to both animal and human health.

The second is the reality that some key groups (in some countries) are not fully engaged into the movement for pandemic preparedness. How to ensure that those who run the poultry industry in a HPAI-affected country see it as in their collective self interest to work together with the NGOs, the researchers, and governments on control and prevention of HPAI? This requires a continuous effort to build and sustain a movement. Movements wither away if they are not persistently supported and kept going.

The third challenge is to maintain trust. Committed professionals from countries in South East Asia worked with the Rockefeller Foundation to build the Mekong Basin Disease Surveillance Program over many years. This covers several different disease issues. It has generated trust between technicians across borders, has survived and continues to do well despite occasional difficulties at the ministerial or high political level. Similar systems are being established between Bangladesh, India and Nepal following their HPAI outbreaks in 2008 and 09.

We are all involved in this effort to build trust. We should ask ourselves, from time to time, whether we are contributing to trust as effectively as we could.

In conclusion, we who are involved in this work tend to want to implement the most appropriate (or 'right') actions. These norms must be well publicised, continuously reinforced in a very positive and embracing and open way and backed with good quality literature.

We need viable animal and human health services based on the best available technologies, and to be sure that the incentives for them to work well are the right ones. The OIE's PVS scheme offers us some valuable pointers.

It is worthwhile getting the incentives right so that pandemic preparations are successfully put in place. The reward may well be that when the next severe influenza pandemic strikes, millions of people survive who might otherwise be expected to die.

I acknowledge the contribution of my many colleagues in UN system agencies to the development of these ideas. The responsibility for the way in which I have presented them is mine alone.



## Qu Liang

*Director, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, IAEA*

Dear Colleagues, Ladies and Gentlemen,

I am delighted to see that we have been able to attract such a high calibre of livestock scientists, practitioners and managers from all over the world. Welcome to Vienna and to this International Symposium on 'Sustainable Improvement of Animal Production and Health'. I would also like to record my appreciation to the private sector for the support given to this Symposium which has enabled us to channel more resources than would otherwise have been possible into ensuring that so many researchers and decision makers from developing countries are here this week. I believe we all share a common vision — the Millennium Declaration to 'spare no effort to free our fellow men, women and children from the abject and dehumanising conditions of extreme poverty.'

Ladies and Gentlemen,

The current food crisis is unprecedented. A staggering one billion of the world's 6.5 billion people face hunger and are 'food insecure'. These are tough times for everyone, but they're especially desperate times for many poor communities and smallholder farmers in developing countries. The global food crisis has been blamed on a combination of the increasing demand and variety (consumer preference) from emerging markets in Asia and Latin America, extreme weather cycles linked to climate change, oil prices, and the diversion of food crops (maize in particular) from the human food chain to the production of biofuel. All this is true, however, in the past seven to ten years; the world has consumed more grain than its farmers have produced thus relying more and more on grain reserves. As a result, grain reserves are currently at their lowest level in the past 50 years. Furthermore, for decades there has been a decline in global agricultural scientific research and development funding in both developing and developed countries. Because it takes on average 15 to 20 years for a new piece of science and technology to be researched, developed and disseminated to end-users, the challenge facing today's farmers is how to overcome obstacles to sustainable food production and double the world's food production using less land, less water, fewer nutrients, and less technology.

The Joint FAO/IAEA Programme assists Member States of FAO and IAEA to develop improved strategies for sustainable food production through the use of nuclear and nuclear related techniques and is comprised of five sub-programmes covering: Soil and Water Management & Crop Nutrition, Plant Breeding and Genetics, Animal Production and Health, Insect Pest Control, and Food and Environmental Protection.

Briefly, the Soils and Water Management and Crop Nutrition sub-Programme assists national institutions in developing countries involved in agricultural production to develop integrated strategies and technologies using nuclear and nuclear related techniques to improve the efficiency of nutrient and water use within selected cropping systems, while conserving the natural resource base (soil, water, biodiversity, etc.). The Plant Breeding and Genetics sub-Programme assists Member States in the implementation of modern and competitive plant breeding programmes using radiation induced mutation and efficiency-enhancing biotechnologies to ensure food security through sustainable crop production. The Insect Pest Control sub-Programme assists Member States in implementing environmentally friendly and sustainable methods to control major insect pests of plants, animals and humans by focusing on area-wide integrated pest management approaches involving the sterile insect technique (SIT)

to enhance food security. The Food and Environmental Protection sub-Programme assists Member States in their endeavours to ensure the quality and safety of food and agricultural commodities and facilitate international trade. The focus is on strengthening Member State capacities for applying international standards on irradiation and on using nuclear and nuclear related analytical techniques in the management of food and environmental hazards.

Last but not least, the Animal Production and Health sub-Programme contributes to the enhancement of global food security through the implementation of sustainable livestock production systems using nuclear and nuclear related techniques. Sustainable livestock production systems require an integrated management approach to farming practices that takes account of complex interactions between soil, water and crops, their linkages to livestock and plant pests, and their relationship to the efficient use of agrochemicals. We assist Member States to improve livestock productivity through the efficient use of locally available feed resources, adequate management practices and breeding programmes for indigenous and upgraded animals, and diagnostic tools and prophylactic measures for the control and prevention of animal and zoonotic diseases.

In all areas, support and guidance is provided in the formulation and implementation of activities that underpin Member States' national, regional and global development objectives through strategic, applied and adaptive research, technology transfer, capacity building, policy advice and information management. In all our activities, the overall objective is the development and use of novel nuclear and nuclear related technologies for a more profitable and sustainable agriculture, a secure food production system and a healthier environment.

### How is this achieved?

This is accomplished by co-ordinating and supporting research, providing technical and advisory services, providing laboratory support and through scientific training and by collecting, analysing and disseminating balanced scientific, technical and policy-relevant information.

#### *Co-ordinating and supporting research*

Approximately 600 research institutions and experimental stations in Member States co-operate in 40 Co-ordinated Research Projects. Each project attempts to solve practical problems of economic significance for developing countries and involves collaboration among 10–20 institutions including those belonging to the Consultative Group on International Agricultural Research (CGIAR). Institutions in developing countries are normally awarded Research Contracts with nominal financial support, whereas those in the more developed countries participate through Research Agreements with financial support only for attendance at Research Co-ordination Meetings. These projects normally last for five years and the results are published either as an IAEA TECDOC or a special issue of a journal.

#### *Providing technical and advisory services*

The Joint FAO/IAEA Programme is also responsible for providing scientific and technical guidance and support to over 200 national and regional Technical Co-operation Projects, as well as for Inter-

regional and Regional Training Courses. These projects are financed by the Agency's Technical Co-operation Fund, FAO's Technical Co-operation Programme and through trust funds provided by donor countries and international funding agencies for the procurement of equipment and provision of expert advice and training (through fellowships and scientific visits).

#### *Providing laboratory support and scientific training*

The Joint FAO/IAEA Programme is supported in its activities by the FAO/IAEA Agriculture and Biotechnology Laboratory, situated at Seibersdorf, 35 km south of Vienna. The laboratory specialises in research, development and transfer of nuclear and nuclear related techniques including training of scientists through individual fellowships and inter-regional and group training courses in various disciplines. The laboratory also provides guidance on the introduction of analytical quality control and assurance into counterpart laboratories, and training in the maintenance of laboratory equipment and instruments.

#### *Collecting, analysing and disseminating information*

In addition to encouraging the direct transfer of skills and technology, the Joint FAO/IAEA Programme provides a variety of information services including conferences, symposia, seminars and advisory group panels, and the publication of technical and public informa-

tion documents that arise from these meetings as well as from Co-ordinated Research Projects and Technical Co-operation Projects. The Programme also maintains contact and collaboration with Member States through joint scientific publications and other publications, such as newsletters, periodic reviews, and computer databases.

Going back to Animal Production and Health, let me re-emphasise the importance of the issues raised by Dr. Traoré in his address. Approximately one billion people in the world today depend on animals for their livelihood. We know that the global food demand is shifting from grain and other staple crops to more processed food and high-value agricultural products, such as fruits, vegetables, meat and dairy products in several transition economies such as Brazil, China and India which have enjoyed high economic growth in recent years coupled with an expanding, wealthier urban population. Although we tend to associate these changes with urban centres, the same change is happening in rural areas as well. Visit any small town or village in these transition economies and some developing countries and you will see a booming business in milk and other livestock products. This increased demand can only be met through the protection of animals from diseases, the selection of animals that give more meat and milk, and the optimal utilisation of local resources whilst protecting the environment to which the Animal Production and Health sub-Programme contributes through the use of nuclear and nuclear related techniques.

Thank you for your kind attention.

# SUMMARY AND CONCLUSIONS





## I. PLENARY SESSION

Two keynote addresses were presented: Historic role of nuclear techniques in solving problems of animal health & production by Wyn Richards, and Decline in available world resources – implications for livestock production systems by Ron Leng. The relevant points that emerged from these talks were:

- The world demands more and healthier animals and animal products produced in an 'environmentally safe, clean, and ethical' way. This is imposing new challenges for animal scientists whose primary concern has been improving livestock productivity. Improving understanding and technologies in animal nutrition, animal reproduction and breeding, and animal health is critically important for food security, poverty alleviation and environment protection on a global scale. The Animal Production and Health Subprogramme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has developed, validated, and transferred to IAEA and FAO Members States sound, low cost and easy-to-use nuclear and nuclear related techniques coupled with convenient strategies to improve animal productivity and to efficiently manage diagnostic laboratories.
- These nuclear applications have undoubtedly spearheaded modern biotechnological research. For example, the most used disease monitoring technology is the enzyme linked immunosorbent assay (ELISA). ELISA assays were developed through research using radioactive isotopes (radioimmunoassay, Western blot), and many still use gamma irradiated pathogens as safe antigens (e.g. Rift Valley fever IgG and IgM ELISA's). Similarly, molecular diagnosis and characterisation techniques were founded using radioisotopic applications. In fact, the most sensitive and cost effective pathogen detection and characterisation applications (1-100 protein or nucleic acid molecules) still demand the use of isotopes. In modern biotechnology, nuclear applications will continue to play a major role.
- The IAEA was instrumental in the success of the global rinderpest eradication campaign through transfer of technologies, improvement in laboratory infrastructure and staff proficiency, and provision of methodologies and operational guidance. The laboratory groundwork laid then, now forms the basis of an increasingly successful animal health control programme in developing countries, demonstrating the sustainability of the interventions fostered by the IAEA in partnership with FAO.
- The world is faced with three simultaneous and interrelated crises that if not responded to appropriately will create chaos, i.e. climate change, increased oil demand 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. The Food and Agriculture Organization of the United Nations (FAO). FAO has indicated that one billion of the world's 6.5 billion people face hunger and are 'food insecure'.
- The primary resource depletion is fossil fuel energy as the world has been using more fossil energy than is being discovered. Several countries have opted to cover part the demand by using bio-ethanol and bio-diesel, thereby impacting on land use and grain availability, and contributing to increasing world food prices. Cereal grain availability for industrial livestock production (pig, poultry and feedlot beef) will be highly restricted and the resulting shortfall in meat production will only be replaced by expanding ruminant animal production. Grain based animal production will become increasingly expensive. There is a greater need to intensify ruminant production on crop residues by applying better

methods of treatment to improve digestibility and to prioritise protein supplementation that increases feed conversion efficiency.

- Water, the other major resource required for agriculture has also been depleted. Many of the world's large river systems are being drained for urban and industrial water supplies or for irrigating crops. Humans, animals, and plants compete for water and it is by far the most important limiting factor in livestock production. Although much research is being done, it needs to be focussed on overcoming the significant obstacles to sustainable food production in order to create the means to double global food production using less land, less water, fewer nutrients, and less technology to satisfy the expected demand.
- Global warming is causing serious deleterious effects to the environment. There is an evident increase in the incidences of extreme weather events such as storms, droughts and floods and, at the same time, changing temperatures may support the occurrence or recurrence of agricultural pests, diseases and their potential vectors.

## II. ANIMAL PRODUCTION

- Much of the activities of the Animal Production and Health Subprogramme of the Joint FAO/IAEA Division of Nuclear and Techniques in Food and Agriculture will have important repercussions on people and their communities, as it was clearly revealed through the various sessions in the symposium. To meet the demands and need of food of animal origin, to improve the efficiency of the large number of production systems in more than 20 livestock species reared under various environment and climatic conditions, and to provide sound surveillance disease programme, as well as national control and eradication programmes for transboundary and zoonotic diseases have to be the result of coordinated and joint efforts from several international organizations.
- The paramount of work ahead cannot be underestimated and therefore cannot be solved by few people or institutions. Livestock population is predicted to increase dramatically over next 40 years with an important impact on land use; but also, there is an annual loss of 150 million cattle and small ruminants from parasitic and infectious diseases and even greater losses in production from inefficient husbandry practices. These facts must be highlighted and that should be bear in mind when designing future activities. Besides this, papers and talks presented during the symposium showed that the coming world food crisis has three important factors to consider: the end of inexpensive energy era (and beginning of expensive inputs), global climate change, and global resources depletion including mineral fertilizers, irrigation water, soil fertility, and land use. Research work is highly needed to solve or alleviate much of these problems, and certainly the IAEA contribution with the development and application of nuclear and nuclear related techniques are utmost relevant; however, we must differentiate the target audience for application of nuclear techniques as the users in many cases are different from beneficiaries - but both of these are members of a much larger value chain. Research is highly needed but the results and its application in large scale has to be balanced, as proper evaluation and consideration to public needs, beliefs and cultural heritage are different within countries, regions and continents.
- Facts, data, results, and recommendations given by experts have resulted in a long list of valuable ideas, proposals, and procedures that can be used at various levels to boost livestock production, reduce hunger and improve health conditions in animals and

humans. Among them, manipulation of feeds and nutritional strategies to reduce methane production while improving body weight gain and milk yields, management practices to improve fertility and number of newborns, without high inputs, and therefore benefiting farmers with higher economical returns and the world with larger quantities of high quality food.

- Meat and dairy products are mainly produced by cattle, pigs, sheep, goat, and poultry, but there are several other species which are highly relevant for specific regions or countries, which need to be properly characterized and efficiently improved for the benefit of the local population. Among them, the buffalo is an important ruminant species in Asia that requires further research in both environmental effect on milk production and fertility using artificial insemination. Camels in the Arab world and camelids (alpaca, llama, and vicuna) in the Andean countries substantially contribute with meat, milk (camel) and fibre; yaks in China are essential animals for a vast number of people; and rodent species such as the cane rat in West and Central Africa and the Guinea pig in Peru and Bolivia are important sources of meat.
- Much of research data, methodologies, management practices, and health procedures produced and validated in the developed world cannot be easily transferred to developing countries, due to climatic, soil, genetic and cultural differences and therefore, the huge amount of valuable research has to be properly evaluated and in many situations adapted; however, in many other situations developing countries have to develop their own technology based on the existing species and breeds, and production systems. As an example, embryo transfer technology, widely available in the North, is not economically feasible under small farmer conditions despite efforts from Governments and interested parties. On this respect, the search and evaluation of locally available feedstuffs, the implementation of feeding trials, the emphasis on reduction of expensive concentrates, alternatives for oestrous synchronization, the use of artificial insemination on a fixed time without the need for heat observation, and the expansion and support of artificial insemination services were presented by many of the symposium participants.

### Session 1: Interactions among Nutrition, Reproduction, and Genotype

Three keynote addresses were presented. These focussed on (1) nutrient requirement models for livestock to improve feed utilisation and animal performance under various environments, (2) the increase on milk yield in specialised dairy breeds and its relationship with fertility, body weight, and metabolic diseases, and (3) assisted reproduction techniques to obtain higher quantity of valuable embryos from high genetic quality animals for transferring to lower genetic value females. Additionally, 63 papers by scientists from 35 countries were presented orally or displayed as posters.

The main points emerging from this session were:

#### a) *Multidisciplinary approaches for solving livestock constraints*

- Integrated and multidisciplinary research approaches are needed to identify, test and solve or at least ameliorate productive constraints in developing countries, especially in rural communities with low education and without land ownership. In many cases, isolated interventions may result in adequate biological results but fail to significantly boost domestic dairy production because farmers do not see the economic gain associated with potential bio-

logical improvements. The integration of interventions covering nutrition, health, reproduction, and management can bring more economic benefits to small holder farmers and improve dairy production. The application of integrated interventions in dairy requires the synergistic action from the government, researchers, non-governmental organisations and farmers. It requires expertise from many inter-related fields, which calls for the creation of integrated action teams at different administrative levels.

#### b) *Nutrition x reproduction x genotype interactions*

- In tropical countries, there is usually a mismatch between the cattle genotype and the environment. The major problems associated with cattle production in these regions are diseases and biting flies, water shortage, heat stress, and scarcity of feeds. Cattle production is therefore characterised by high calf mortality, slow growth rates, low fertility and calving rates, low milk yield and carcass weight, and little attention is paid to disease resistance and heat tolerance. Farmers are demanding more adapted and productive animals. In this scenario, it is of great importance to consider indigenous breeds. Disease resistance and heat tolerance are particularly valuable traits in indigenous breeds compared with exotic ones reared in the same environment. However, little is known about the genetic composition controlling these characteristics. The rapid and growing knowledge of genes and genomes in livestock obtained by the assessment of a large number of DNA markers in phenotypic recorded populations could be used to localise and further characterise candidate genes of economic interest. The analysis of mutations is in essence the discovery and exploitation of the natural variation existing in the biological machinery with the aim of increasing the frequency of favourable alleles to the benefit of livestock breeding strategies.
- After calving, high milk yielder cows are unable to compensate for the increased energy demands by increasing feed intake, resulting in a negative energy balance. The consequent catabolic status is characterised by low insulin and glucose concentrations and reduced insulin sensitivity, leading to a massive mobilisation of body reserves. Animals can lose up to ten percent of their body weight during the first weeks post partum and the occurrence of first oestrus is delayed. This metabolic condition can directly hamper growth and function of the ovarian follicle as elevated free fatty acid and the low glucose concentrations are toxic for the granulosa cells and the oocyte. Some studies have shown that a higher proportion of non-viable embryos were flushed from lactating cows compared with non-lactating cows.

#### c) *Feed and nutrients for improved livestock productivity*

- Provision of nutrients should cover maintenance and production needs to a point that the investment generates an economic return for the farmer. Animals differ in their nutrient requirements according to their inherent genetic potential and the desired level of production. Nutrient requirements for maximum production in high genetic merit Holsteins in industrialised countries are fairly well known but still there is a need to improve the knowledge on the level of response for nutrients other than energy of specialised dairy breeds and indigenous crossbred animals under tropical and subtropical conditions where feeding, management, health, and climatic conditions are much less than optimal.
- 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. Seasonal

droughts and floods, and changes in cash crop production alter or limit feed availability from local sources and therefore impact animal productivity. Optimisation algorithms can be utilised to assess maximum production or economic returns given a set of constraints, especially those related to nutrient requirements and availability and accessibility of food supplies. Thus, it is important that animal performance models are capable of accurately predicting production responses when varying the quantity and quality of feeds.

- Rice straw, maize stover, wheat, and sorghum straw are major feed sources for ruminant in many tropical countries, especially during the dry season. The nutritional limitations of rice straw may be overcome by supplementation with concentrates, urea, or green forage. Among the latter, tanniferous tree foliage and shrubs are efficiently used together with agricultural by-products, mainly crop-residues, containing low levels of nitrogen to enhance rumen microbial fermentation and hence animal productivity. Rice straw is a highly fibrous feed and with low digestibility; therefore its retention in the gastrointestinal tract is longer, and within this period the fermentation process results in higher methane production which contributes to environmental; however, the combined use of rice straw as a cheap feed source with the supplementation of concentrates activates microbial fermentation in straw, resulting in a shorter retention time and increased feed intake, the final result being higher productivity (body weight and milk yield) without increasing methane production. Tree legume forages offer a cheap alternative to concentrate diets in ruminant nutrition
- Gamma irradiation of some animal feeds like cottonseed meal decreases crude protein disappearance in the rumen, allowing part of the protein to by-pass the rumen to the intestine which is beneficial for high producing ruminants.

#### d) Selection of livestock on genetic merit

- The resumption of ovarian activity in the cow includes the growth of healthy follicles, oestrus behaviour, and ovulation. The resulting oocyte will be fertilised and a viable embryo will be attached to the uterine wall. This process requires the release and inhibition of several reproductive hormones at various times. Any disturbance of the fine-tuned biological and mechanical events leads to failures in ovulation, fertilisation, or early embryonic death. Unfortunately, selection over the years for high milk yield has been at the expense of sustained reproductive efficiency.
- Overall improvements in the genetic merit for milk production in dairy cows in the last 20 years have led to a tremendous increase in milk yield; however, milk yield maximisation has been paralleled by a decrease in reproductive performance and higher incidence of metabolic and infectious diseases during the early post-partum period. Disappointing fertility results are a world-wide concern in the modern dairy industry as high milk yield is a major factor contributing to a reduced number of calvings per lifetime, days in milk and decreased longevity. Optimum reproductive parameters considered in the past as one calving per year, 1.8 services per conception, and 60 percent conception rate are no longer valid. Current figures, even in the USA and Western Europe show average calving intervals close to 400 days, more than two AI per conception and 40 percent conception rates in specialised dairy breeds.
- There is inadequate selection and handling of top quality alpacas. This is based on phenotype and leads to a further progressive

deterioration of fibre quality. The scientific approach towards selecting animals (based on genotype) in combination with modern techniques for handling of gametes and embryos could ensure the preservation of highly valuable animals for high quality fibre. Making use of the sophisticated knowledge from assisted reproduction techniques to 'identify' genetically pure alpacas will allow obtaining 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.

#### f) Managing the ovarian cycle and the ova

- Optimisation 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 from extinction.
- *In vitro* production of bovine embryos is currently a routine technique in several countries; however, there is a consensus among animal reproduction scientists that current techniques for the culture and maturation of oocytes are still inefficient compared with 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 expected, probably due to several external (seasonality, pasture, environment) and internal factors (disease, stress).
- A challenging new concept is that it might be possible to optimise 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.
- A 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 using vitrification in combination with high concentrations of cryoprotectants has been documented. The use of several small animal models may allow evaluating 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.
- Current reproductive techniques and the complexity of the reproductive system in different species require a profound training in reproductive biology. The precise understanding of the changes in metabolic regulation 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.

### Session 2: Effects of Nutrition, Reproduction, Genetics, and Environmental Factors on Animal Productivity

Four keynote addresses were presented. These focussed on (1) managing livestock in degrading environments, (2) foundations, fallacies, and assumptions of science for livestock development, (3) interactions among nutrition, heat stress, and reproduction in tropical cattle, and (4) early stirrings of landscape genomics. Additionally, 81 papers

by scientists from 35 countries were presented orally or displayed as posters.

The main points emerging from this session were:

#### a) *Dealing with enteric methane production*

- Highly fibrous diets not only decrease feed-use efficiency, but also increase methane production in ruminants. Reducing methane emissions from ruminants therefore has implications not only for efficient animal production but also for global environmental protection. Computer applications for diet formulation must consider the need to reduce greenhouse gas emissions from ruminants.
- Mitigation of methane emission is essential to protect the environment from the greenhouse effect and at the same time improve feed conversion efficiency. Some strategies to mitigate methanogenesis are: (1) non-productive or low productive animals should be replaced with either high producing animal be they indigenous cattle/buffalo or crossbred cattle; (2) any effort made to enhance degradability of poor quality feeds (e.g. urea ammoniation of straw) results in an improvement in nutrient availability accompanied by a decrease in methanogenesis, (3) use of plants containing secondary metabolites (saponins, tannins, lignins, essential oils, etc.) that are effective against methanogenesis and ciliate protozoa.

#### b) *Grazing and feeding behaviour*

- Well managed livestock on 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. 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. On the other hand, both over- and under-grazing are likely to result in a loss of species diversity. As livestock have the ability to select plants with higher digestibility, adequate nitrogen (crude protein) and low or manageable anti-nutritional compounds, in under-grazing situations loss of diversity will be accompanied by a decline in nutritive value and palatability, and reduced ability to deal with toxins. In the case of over-grazing the decrease in diversity and nutritive value will be accompanied by a decrease in feed availability. Management of livestock, vegetation, and the interaction between the two is critical for productive of 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 productivity. 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.
- 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.

#### c) *Towards intensive management systems in developing countries*

- Consistent management systems based upon intensive inputs plus a compliant consumer market exist in industrialised countries; however, the social, physical, and economic environments in developing countries where most livestock are located are very variable, often unreliable, and distant from large markets. 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.

#### d) *Environment, heat stress and animal productivity*

- 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 technologies. 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.
- Development of low impact sustainable agriculture and a growing use of adapted breeds are of priority to most countries in the world, and are particularly important 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.
- Bulls that transmit a high tolerance to heat stress have daughters with higher pregnancy rates, a longer productive life, but lower milk yields. Continued selection for milk yield without consideration of heat tolerance will likely 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. By knowing the gene sequences of the bovine genome, identification of heat tolerance genes of *Bos indicus* breeds offers the potential of introducing these genes into less heat tolerant breeds.
- An array of refined reproductive technologies is available to better manage the reproductive performance of dairy cows. 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 timed artificial inseminations (TAI) with high fertility during summer to avoid seasonal breeding and parturitions. Optimised TAI programmes coupled with heat abatement systems reduce the impact of poor oestrus expression during summer.

### e) Breeding management: crossbreeding and embryo transfer

- An efficient way to produce milk in the tropics is the direct cross between *Bos taurus* and *Bos indicus* under well-structured crossbreeding programmes; however, the problem arises when the farmer faces the challenge to breed the crossbred animal because crossing back with *Bos taurus* the resulting cow is quite vulnerable to the harsh environmental conditions in the tropics and crossing with *Bos indicus* the offspring will be deficient in milk production. An alternative is to transfer F1 embryos to F1 dams, hence avoiding the hazards of crossbreeding. Although the technique of embryo transfer has been available for many years, there are several pitfalls at least under tropical conditions, which need to be considered. Among them, embryo transfer programmes in small community farms is difficult because the selection of recipients is restricted to a few animals in the herd and even worse if the distance between farms is large; the super-ovulatory response can be directly related to the follicular dynamics at the moment of treatment and experience 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.
- Timed artificial insemination are often advantageous to cattle producers because they reduce the time and labour required for the detection of oestrus and allow all animals to be managed in groups rather than individually. A wide variety of effective TAI programmes have been successfully developed but their use on a particular region must be validated. The selection of suitable females is of vital importance as the practitioner must ensure that cows are non-pregnant and with adequate body condition; otherwise, the treatment will fail.
- The monitoring of reproductive hormones, through radioisotopic techniques such as radioimmunoassay, in conjunction with field protocols for sampling, collection of behavioural and biological data, and the use of computer software applications, developed by the IAEA have proved highly advantageous for obtaining a better understanding of the reproductive physiology of livestock species, in identifying and ameliorating limiting factors affecting reproductive efficiency (in high-input-high output dairy production units, medium scale farms using modern technology, small-scale livestock farms and pastoral farms), in providing diagnostic tools for ensuring proper AI timing, for monitoring ovarian cyclicity, identifying anoestrus and non-pregnant females, and in assisting AI centres and services. Individual or corporate farmers, by using simple but well established and validated field and laboratory protocols, can monitor and evaluate the performance of their animals and farms. Animals can thereby reach sexual maturity and first parturition at an earlier age, offspring obtained at a higher frequency and in return, farmers can achieve higher and sustainable economic returns.
- The efficiency and sustainability of embryo transfer programmes should be critically evaluated. Data from a number of these programmes in Mexico was evaluated to determine the cost involved in the preparation of the donor and embryo recovery, the average number of embryos recovered, the percentage of animals pregnant, the production of the embryo itself. It was found that the cost for a replacement heifer was US\$ 2 640 dollars which surpassed by far the commercial cost of a crossbred heifer (approximately US\$ 900).

### f) Camelids and milk production

- Old world camelids (Dromedary and Bactrian camels) are important sources of milk in rural areas of many arid countries; however, extensive production systems cannot guarantee constant quality and quantity raw milk for the market. Some attempts to develop large scale camel milking farms in Dubai draw attention to camels as a potential source for high quality milk and meat in developing countries. Average milk production per lactation was  $2\,467 \pm 79.4$  kg ( $340 \pm 7.9$  d) with some animals able to produce up to 8 000 kg per lactation.

### g) Molecular genetics and livestock productivity

- Molecular genetics opens new horizons for changing the life processes of animals in ways that have great potential to improve their health and production. 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 shows that control of gene expression is far more complex than conveyed in current genetic models. For developing countries use of radical molecular genetics raises issues of risk, accountability, authority, power, ownership, morality, the nature of animals, and the sustainability of life for rural people in remote areas. Perhaps of greatest importance 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 are indigenous breeds with some degree of enhanced resistance compared with exotic ones reared in the same environment, especially for gastrointestinal nematode infections, diseases due to mycotoxins, bacterial diseases including foot rot and mastitis, ectoparasites such as flies and lice, and scrapie, and small ruminant transmissible spongiform encephalopathy. Therefore through genomic studies using radiolabeled nucleotides in DNA hybridisation, DNA characterisation, and hybrid mapping procedures, activities are focusing on the identification of molecular markers of economic interest. This will open possibilities to select and breed animals for enhanced resistance to disease.
- The number of available environmental data bases (NASA SRTM, NASA MODIS, Norwich CRU, etc.) is gradually increasing and their quality improving (better resolution). Most of these global data sets are freely available through the Internet and can be used for a comparable description of production environments worldwide. On the other hand, the amount of molecular data to be analysed will more than likely expand rapidly, and the next generation of sequencers will allow researchers to obtain genomic data faster and at a lower cost. Several alternative low cost sequencing technologies are under way, and the \$100 complete sequencing of individuals is about to become real.
- Researchers in molecular biology have begun to consider new approaches for analysing the large data sets produced by the 'next next' generation sequencing technologies. On the GIS and statistical side, it is necessary to stress the importance of recording geographic coordinates in any new project involving animal sampling campaigns, and to improve related software. As the association analysis process is rather straightforward, the challenge will mainly be to improve the efficiency and robustness of algorithms.

- The cultivation of genetically modified plants has increased worldwide from 1.7 (1996) to about 114 million ha. Currently, soybeans, corn, cotton, and canola are the most important genetically modified plants. They are modified mainly for agronomic traits but the second generation 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.). Both chemical analyses and animal studies have revealed no significant differences between feeds from genetically modified plants and their isogenic counterparts and hence strongly support their substantial equivalence; also no significant differences were found in digestibility and animal health.

### III. ANIMAL HEALTH

- The need to intensify and increase food production to meet the needs of the ever growing human population for nutritious and safe products demands more efficient means to diagnose and control animal diseases and to measure food and feed contaminants. The most prominent challenges that arose from the Symposium focussed on the increase in human population, new farming systems, increased movement of animals in world trade and the alterations in ecosystems brought about by climate change and the geographical distribution of pathogens or their vectors. Against this background, resource poor developing countries will increasingly be faced with an increasing prevalence of infectious diseases due to both known, as well as hitherto unknown emerging diseases. A significant proportion of the latter, over 60 percent, are likely to be zoonotic diseases, the greatest proportion of which will probably be associated with wildlife and therefore the domestic and wildlife interface is very important (e.g. HIV, avian influenza, rabies, Ebola, Rift Valley fever). This will challenge our perception of surveillance, requiring capacities at national and international levels to diagnose infections in the animals, rather than identify a problem zoonosis after it has spread to humans.

Faced with this situation, it will be important for countries and the international community to prioritise activities in terms of resource allocation and focus on:

- the most important endemic diseases;
  - the most important zoonotic diseases;
  - the most important exotic diseases;
- In spite of the many advances in our understanding of diseases and their epidemiology there are still gaps in knowledge, for instance in the global economic burden of zoonoses. Better insights are needed into the transmission pathways for opportunistic zoonotic infections, and at the same time to understand disease epidemiology in each host species, particularly wildlife reservoirs, since persistence of infection will slow down attempts to effectively control diseases. The global effort for surveillance and research on emerging infectious diseases is poorly allocated with much of the scientific endeavour being located in regions where new pathogens are least likely to appear. There is an urgent requirement for allocation of resources for surveillance of emerging diseases in Africa, Asia and South America. Such studies should include targeting at-risk people to identify emerging diseases before they become large scale problems. Zoonoses of wildlife origin are particular threats and it would therefore be useful to identify new potentially zoonotic pathogens in wildlife to forecast risk from emerging diseases. Zoonotic diseases are frequently under-reported and there is a pressing need for cross disciplinary approaches to fill knowledge gaps in research and

diagnostic needs, and define responsibilities between veterinary and public health authorities to ensure integrated surveillance and control of diseases.

- There is a comprehensive range of new generation (nuclear and nuclear associated and nuclear related serological and molecular) diagnostic techniques to detect animal diseases. The list is long and includes both direct and indirect methods. On-site diagnostics for foot and mouth disease (FMD) and avian influenza using disposable automated sample preparation units have been developed with communications systems that enable instant reporting of results from even remote locations. Moreover, handling is optimised and indeed simplified to reduce human (field and health worker) and laboratory technician exposure. Rapid, specific penicillin diagnosis has also been made possible for peste des petits ruminants (PPR) by the development of a loop-mediated isothermal amplification (LAMP) PCR.
- Global trade in livestock and livestock products will continue to increase as it is driven by demand towards an ever increasing protein-based consumption. However, biosecurity and in particular, infectious diseases, along with other factors will limit this trade. Since the greatest biosecurity risk is posed by the live animal, investment in processing capacity at or near livestock production sites and prior to export has the potential to significantly reduce biosecurity risks. Trade in the processed product and not the animal could be a way for livestock producers to trade their way out of the poverty trap in developing countries. This would be of greatest benefit in those areas currently excluded from exporting due to biosecurity concerns and disease risks in national herds.

#### Session 3: Transboundary, emerging and zoonotic diseases

Five keynote papers were presented focusing on (1) emerging and re-emerging zoonotic diseases, (2) early, complex and rapid diagnostic technologies, (3) climatic changes, seasonality and the dynamics of infectious diseases, (4) an overview of FAO's Emergency Prevention System for Transboundary Animal and Plant Pests (EMPRES) and the FAO, OIE and WHO Global Early Warning System for Animal Diseases (GLEWS), and (5) World Organisation for Animal Health (OIE) activities for the global improvement of animal disease detection and control. In the open section, 39 papers and posters were presented by scientists from 27 countries.

The main points arising from the session were:

##### a) Emerging and re-emerging zoonotic diseases

- Recent outbreaks of infections with influenza H5N1 and H1N1 and the occurrence of HIV have drawn the attention of the general public to the problems of communicable diseases; nevertheless, communicable diseases have always impacted on mankind and although they might not nowadays present such a problem in the industrialised world this is not the case in developing countries where they have been, and continue to be, a major cause of morbidity and mortality. However, the more frequent movement of people between countries together with the movement of animals have increased the likelihood that diseases can spread more rapidly to areas where they are not normally found.
- Another factor that might in the future contribute to the problems of diseases is that climate change could lead to certain diseases increasing their range and affecting countries where they have not previously been a problem. There is a wide diversity of

protozoa, helminths, viruses and bacteria that infect animals, but have the potential to infect man; these zoonotic diseases represent the majority of newly emerging diseases now occurring, and they present a considerable challenge both for diagnosis and control. Where domestic animals provide a reservoir of infection, control and eradication of the disease is often viewed as being difficult or impossible and there is a tendency to neglect such diseases. Amongst those considered in this respect are intestinal protozoa, food-borne trematode and cestode infections and vector-borne protozoan infections like human African trypanosomiasis, leishmaniasis and Chagas' disease. Molecular biology has played a pivotal role in helping define these diseases in terms of their epidemiology, pathology and diagnosis as well as contributing to monitoring and surveillance. This is exemplified by the studies on Chagas' disease that have considerably altered our understanding of both the parasite and the insect vector, allowing their genetic subdivision and relating these parameters to their geographic distribution and the sylvatic cycle of development in reservoir hosts. Molecular characterisation of the liver fluke and the snails that harbour the infective stages have enabled a more clearly defined understanding of the epidemiology of human and animal infections.

- In spite of examples such as these, there are still major problems in dealing with zoonotic diseases. Although there is usually a general understanding of their epidemiology, at the specific, local level they may not be as well defined. More attention needs to be placed on establishing appropriate multi-disciplinary networks of health professionals to cooperate in managing the various strategic and political implications of controlling zoonotic diseases. Critically, much more basic field work needs to be done in endemic areas, applying not only the modern molecular methods, but also the increasingly neglected disciplines of medical entomology and malacology.

#### b) Diagnostic technologies

- Transboundary animal diseases disrupt trade and cause enormous economic damage; for instance, the last outbreak of FMD in the UK caused losses of Euros 12 billion. Long-standing problematic TADs like FMD and classical swine fever and various food-borne diseases have been joined by newly emerging diseases like highly pathogenic avian influenza (HPAI), swine 'flu and bocaviruses. For all of them however, there is a need to provide early and rapid diagnosis using the most up-to-date technologies. This aim is being met through the OIE Collaborating Centres for Biotechnology-Based Diagnosis of Infectious Diseases. These Centres, together with their collaborating partners have achieved considerable success in developing cost effective strategies for disease diagnosis and control. Among their achievements is the development of multiplex PCR and ELISA assays for classical swine fever that can be used to assist control of the disease worldwide, including in wildlife. For vesicular diseases, multiplex diagnosis using padlock probes can identify FMD, swine vesicular disease virus and vesicular stomatitis virus. These diseases present a similar clinical picture and conventional diagnosis is complicated and time-consuming, increasing the likelihood that they could spread further. The microarray system detects and identifies all three viruses in just a few hours.
- HPAI presents a major problem, both as a serious economic threat and a potential zoonotic infection in humans and there is a need for improved laboratory techniques for both virus detection and typing. Again, padlock probes allow identification of avian influ-

enza viruses and subtyping within one platform, instead of needing to carry out 25 separate tests.

- Bluetongue has recently become an emerging disease in Europe and there was an urgent requirement for developing new virus detection techniques to replace the complicated cumbersome ones generally in use as well as providing a means for identifying the many serotypes. Primer-probe energy transfer (PriProET) with high diagnostic sensitivity and specificity now allows detection of all 24 serotypes. The necessity for rapid, on-site diagnosis in emergency situations, or in situations where laboratories have only minimal facilities is being addressed by the development of techniques such as the INVADER assay for classical swine fever and LAMP tests where results can be read by the naked eye using sophisticated portable PCR machines with built-in communications links.

#### c) Climate change and disease

- Climate change is widely acknowledged as being a significant factor likely to affect the occurrence and pattern of disease globally, and predicting what might happen in the future, or indeed, what has already happened to the epidemiology of infectious diseases is a matter of debate. The impact of animal disease in sub-Saharan Africa is considerable, overall leading to a 24 percent reduction in productivity, especially in livestock species important to the rural poor. For example, an outbreak of Rift Valley fever in eastern Africa caused losses of US\$ 130 million and resulted in a decline of 20 percent% in household income. In trying to predict what might happen we must identify those diseases that are more likely to be sensitive to climate change and what are the main drivers, i.e. temperature, rainfall, drought etc. How will these changes be manifest — by increased incidence and prevalence of infection or by increased virulence? Some pointers to climatic effects are already known. Outbreaks of anthrax are associated with heavy rainfall and drought and high temperatures; PPR is linked with the onset of the rainy season or dry, cold periods; African horse sickness (a vector-borne disease) is associated with a combination of drought and heavy rainfall; West Nile virus (another vector-borne disease) has been linked with severe droughts in Europe, the Middle East and the USA. A warmer, wetter world is likely to favour vector transmitted diseases and pathogens with intermediates hosts like helminths and gastrointestinal parasites. Among the effects of higher temperature could be an increase in vectorial capacity and range expansion e.g. bluetongue virus and its vectors in Europe. Nevertheless, predicting what is likely to happen in the future is not an easy process; the changes will be complex e.g. range shift, range expansion, population amplification. Socio-economic factors are also likely to impinge upon the process as there may be alterations in the environment that change farming practice and livestock production. Also, predictive models might not be sufficiently accurate when targeted at the local level.

#### d) Wildlife and emerging transboundary diseases

- Over half of the occurrences of emerging infectious diseases during the past ten years have been caused by zoonotic infections and nearly three quarters of them originated from wildlife, a trend that has been observed for the past 50 years. Their emergence is often driven by environmental changes that affect ecological systems, sometimes brought about by man-made agricultural changes. Diseases and pathogens that are exchanged between humans, wildlife and livestock should receive more attention in

the form of targeted, active surveillance, in order to improve timely detection, identification, molecular typing, and monitoring. Understanding the human-wildlife-livestock interface is being achieved by the establishment of collaborative networks that ensure capacity building, wildlife disease surveillance and studies of migration pathways to understand disease ecology and pathogen maintenance. For instance in the case of avian influenza the FAO plays a leading role in a scientific taskforce comprising over 100 individuals, governmental and non-governmental organisations that provide science based guidance for decision and policy makers

#### e) *Improving animal disease detection and control: role of the OIE*

- Ensuring food security is a goal that will not be met unless there are coordinated efforts to ensure that livestock productivity is maintained by the improvement of disease detection and control. Central to this tenet is the OIE that has the mandate of improving health all around the world. The OIE stands as the arbiter of the legal framework for controlling regulations on international trade and is one of three reference organisations responsible for international standards. Its objectives are to ensure that there is international collaboration in promulgating proper livestock health standards for international trade and animal disease surveillance, thereby contributing to food safety and security. It also provides the expertise for ensuring that there is international collaborative effort to control animal diseases and improve the resources for national veterinary services.
- These aims are achieved through the publication of international standard monographs on animal health, diagnostic tests and vaccines that describe in detail the various needs for ensuring livestock health and welfare. To ensure competency in the delivery of veterinary services OIE operates a Performance of Veterinary Services (PVS) tool that seeks to ensure components such as human resources, technical capability, interaction with stakeholders and access to markets are fulfilled. Over 170 Reference Laboratories in 32 countries facilitate disease diagnosis, supply reference reagents, provide training and organise proficiency testing. A further 30 Collaborating Centres assist in coordinating collaborative studies and harmonising procedures for animal disease regulations. The OIE network is enhanced by Twinning Projects that link an OIE Reference Laboratory or Collaborating Centre with a national laboratory, thereby improving expertise and providing sustainable diagnostic capacity to OIE standards.

#### f) *Coordinating responses to the problems of transboundary diseases*

- Transboundary diseases not only inflict direct economic losses but also affect trade since sanitary measures are often put in place to restrict the movement of animals and sale of livestock products in affected countries. In order to enable more effective response to TADs that directly impact on hunger, malnutrition and poverty, FAO created EMPRES, and in the area of animal health provides the information, training and emergency assistance to help control livestock diseases, including newly emerging diseases. Early warning is a vital component of the strategy and FAO. OIE and WHO have developed the GLEWS that will coordinate the response of the three organisations. In this way a more effective response to outbreaks of disease, especially those with zoonotic potential, can be realised.

- Peste des petits ruminants (PPR) is just one of the diseases that is a priority for EMPRES; since its discovery in Africa in the 1940s the disease has spread throughout Africa, the Middle East and Asia, so that over one billion small ruminants are at risk from this disease. FAO aims to progressively control PPR by stressing the importance and impact of the disease to national governments and other stakeholders and conducting epidemiological and socio-economic impact studies. The introduction of appropriate diagnostic tools will lead to a strengthening of surveillance and more effective emergency responses.
- For FMD a sophisticated Progressive Control Pathway, implemented in 2009, has been developed to assist the progress of individual countries in managing disease on the way to establishing freedom from infection. It is planned to have in place an active programme covering all seven major endemic regions from 2010, hoping to achieve in most endemic countries reduced circulation of the FMD virus by 2020, thereby offering safer trade from an increased number of countries.

#### g) *Making the right choices for disease control*

- By improving animal and human health, food safety and quality and facilitating access to trade for developing countries the Millennium Development Goals will become a possibility. One way of assuring this is to provide the means to accelerate the development and distribution of the means to control animal diseases in both Europe and the developing world. This is the aim of the European Technology Platform for Global Animal Health (ETPGAH) whose agenda sets out to address the views of stakeholders on the research and development needs in the short, medium and long-term.
- A number of thematic initiatives have been identified that will progress these aims. In the first place, diseases will be targeted based on appropriate prioritisation models that have been peer-reviewed and accepted by funding agencies. In Europe, the emergence of new disease conditions, either natural or by bioterrorism is an issue that requires immediate consideration. Since wildlife diseases rank first as a source of new pathogens, the introduction of sophisticated technologies as screening assays is a priority especially where zoonoses are involved to ensure that such pathogens are rapidly identified.
- Research funding will be targeted towards the priority diseases, but efforts would be made to identify existing gaps in our knowledge and understanding to enable more effective implementation of the strategy. New technologies will need to be assessed to ensure that they are used to their full potential and provide maximum benefits. There is very little information on the current research effort in priority diseases, nor on the availability of products from manufacturers of animal health products, including vaccines, pharmaceutical and diagnostic kits so it is necessary to identify those that are available. Finally, although the major aim of the ETPGAH is to provide adaptive technologies, it is vital that fundamental research in areas such as host-pathogen interaction, immunology, epidemiology genomics and bioinformatics is supported to ensure the development of new tools can be sustained

### Session 4: One Health

Three keynote speakers presented papers dealing with One World One Health and there were a further 27 papers and posters in the general section presented by participants from 19 countries.

The main points arising in this session were:



#### a) *One world One Health: what can be learned from the farm to the fork?*

- The dynamic interaction and convergence of animals, humans the environments they occupy has created a situation in which the health of each group is interconnected. This is exemplified by the fact of the 1 500 or so diseases known to affect man nearly 60 percent are brought about by pathogens that have crossed the species barrier. The need to increase animal productivity together with the increased trade in animals and animal products may well be one of the most important risk factors with regard to infectious diseases. It is clear therefore that current attitudes of the animal health, public health and food industries to the management of disease will have to undergo a paradigm shift if the challenges of providing healthy animals together with food security, food safety and optimal health for people are to be met. The 'One Health' initiative seeks to improve collaborative interaction between the veterinary and medical professions to meet these challenges.
- In testing food, the emphasis should be on carrying out strategic testing based on the likely risk and there should be more effort in integrating data for agriculture, food and animal health. In the case of wildlife this is a particularly under researched area and it will be necessary to look further into the impact wild animals might have. There should be improvements to food control systems to allocate funds to sensible data gathering, including disease burden estimations. There also needs to be a combination of active surveillance and new typing systems to enable source attribution. Overall, risk can be reduced through efficient interventions.
- International regulations governing zoonotic diseases are already in place through the WHO International Health regulations and the OIE Terrestrial Animal Health Code. For major animal diseases the GLEWS enables the sharing of information between FAO, OIE and WHO. GLEWS is triggered by a number of events such as high mortality or morbidity in animals and/or humans, an unusual first occurrence or events associated with an unknown pathogen. It also considers the likely risk of international, transboundary spread that might lead to restrictions on international trade or travel.
- The International Food Safety Authorities Network (INFOSAN), a global WHO/FAO undertaking in collaboration with OIE promotes the exchange of food safety information, assists countries strengthen their capacity to manage food safety risks and responds to international food safety problems. Some 174 countries belong to INFOSAN.

#### b) *One World One Health: is there a need for a global research agenda?*

- Although there are efficacious tools for control of zoonoses, few have proved sustainable in resource poor countries. Since they impact on public and veterinary health, livestock production, and international trade and community development their management and control must be cross-sectorial in their inception. However, cooperation between these different sectors is often not encouraged for various reasons including institutional barriers and perceived 'mandates' of responsibility. Of considerable importance is identifying the sector that is most likely to benefit from control of zoonoses, and indeed, who will pay for the activities?

- Zoonoses tend to be neglected diseases; there is no common indicator of the effects, and they are often under-reported due to the poor capacity of developing countries to recognise and diagnose them. There is no measure of the effect, measured in disability adjusted life years (DALYS) for even the most common zoonoses such as anthrax or cryptosporidiosis and almost no global assessment. Wildlife reservoirs are of critical importance in control of zoonoses but there is insufficient knowledge of their ecology and epidemiology in the various host species affected. However, it is clear that the persistence of disease in wildlife reservoirs will hamper efforts to eliminate disease.
- Various One Health initiatives have been adopted including MED-VET-NET the European Network of Excellence for Zoonoses research. Med-Vet-Net's aim is to develop a network of excellence for the integration of veterinary, medical and food scientists, in the field of food safety, at the European Level, in order to improve research on the prevention and control of zoonoses, including food-borne diseases. The Network will also take into account the public health concerns of consumers and other stakeholders throughout the food chain. ProMed-mail is the global electronic reporting system for outbreaks of emerging infectious diseases and toxins. A central purpose of ProMED-mail is to promote communication amongst the international infectious disease community, including scientists, physicians, epidemiologists, public health professionals, and others interested in infectious diseases on a global scale. It encourages subscribers to participate in discussions on infectious disease concerns, to respond to requests for information, and to collaborate in outbreak investigations and prevention efforts.

#### c) *Integrating laboratory results from animal food and human investigation*

- Several sources allow for the introduction and transmission of food contamination, from feed – farm – slaughterhouse – processing — retail — consumption and at each point in the chain, the stakeholder is responsible for safety of the product. Therefore coordination of control actions is required with good communication between the many stakeholders. Information is needed on the source of contamination, the route of transmission, the most effective means of controlling or containing the problem and what is the likely public health burden. Various tools will be applied including suitable diagnostics, epidemiological modelling and decision support tools.
- There are many stakeholders in ensuring food safety, including ministries of agriculture and health, veterinary authorities, public health authorities as well as reference laboratories and the food industry itself. A central coordinating body can ensure that there is appropriate collaboration and communication between these diverse organisations, providing information on all aspects of identifying, tracking, controlling and disseminating data from different sources. Integrated surveillance should encompass food, human and animal data and there should be a network among the various laboratories involved, including data sharing and standardisation of methods and definitions.
- The food trade is increasingly globalised; products seemingly originating from one country can be found to have ingredients that have been sourced in several other countries, possibly from a different continent. Hence there can be a rapid spread worldwide by the movement of food. This requires international oversight and this is ensured by FAO/OIE/WHO through GLEWS and INFOSAN.

## Session 5: Achieving Food Safety and Security in the 21st Century

Three keynote speakers presented papers and 13 scientists from 10 countries presented their findings orally or with poster demonstrations.

The main points arising in this session were:

### a) *The global market for livestock and livestock products and biosecurity*

- The enormous increase in demand for livestock and livestock products had been driven by increasing urbanisation and the shift away from cereal based diets. This has been accompanied by a movement in production from temperate and drier areas to warmer, more humid and disease-prone environments. Large scale industrial production near urban centres is increasingly common, often with emphasis on pigs and poultry. There is expected to be a significant shift of production to developing countries, with these contributing over half the milk, and two thirds of the global meat production by 2030.
- In relation to food security and the global market in livestock, the greatest concern is for the likely risks of disease being introduced to the national herd in the receiving country by the importation of live animals. This compromises the ability of developing countries to trade internationally, especially where veterinary services are inadequate. An alternative approach is to trade in processed products produced close to production areas. By investing in managing biosecurity at the processing stage the risks from disease would be mitigated. This would provide considerable benefit to those areas that are presently excluded from trading due to biosecurity concerns. This approach has had some success in Kenya and Ethiopia and deserves wider application.

### b) *The future of aquaculture and its role in contributing to food security*

- Aquaculture started as a fresh water food production system in Asia, but has since spread to all continents and other aquatic ecosystems so that now a wide variety of both fresh water and marine species are produced. The range of enterprise covers both small scale, family based operations up to commercial, international industrial scale production. Aquaculture is still greatest in China and the Asia Pacific region, but in other regions, the scale of production, although much lower, is often of greater value.
- The importance of aquaculture is seen in relation to the decline in capture fisheries over the last 30 years. Many species are disappearing due to overfishing and perhaps a third of species are no longer available. Hence aquaculture is the fastest growing food sector in the world as the growing global population demands more aquatic food products. The annual growth rate over the last 50 years averages about 9 percent and is now valued at US\$ 70 billion. The present trend is for continuing intensification of farming activities and increased diversification. There will need to be new production systems in which there will be more influence by the markets, trade and the consumer. Regulatory procedures will require better management practices.
- Aquaculture is limited in sub-Saharan Africa, its contribution being only <1 percent and the per capita consumption of fish has dropped. There is plenty of potential for development and growth of aquaculture and it is a high priority for this region. There will need to international cooperation and a focus on regulation, sus-

tainable development and diversification while at the same time diminishing the impact on wildlife habitats.

- Aquaculture will be faced with various environmental constraints as a result of global climate change caused by increased temperatures, variable weather patterns, and the availability and quality of freshwater. Pollution and diseases will always be threats.
- Disease results in lower productivity but it also incurs costs to the producer in the form of the preliminary diagnosis, then control will require additional use of microbial compounds with the possibility of residues in the final product that compromises product marketing. Environmental degradation of the rearing area might also occur. The most important aspect of disease is the possibility of spread through trade, both national and international. Although local pathogens are the most prevalent form of disease outbreaks, introduction of exotic pathogens through trade is a reason for epidemics e.g. the spread of white spot disease of shrimps. However, endemic pathogens, internal transfers of live aquatic animals and factors such as poor biosecurity, water quality and bad husbandry are more common causes of outbreaks than international trade. The OIE, through the Aquatic Animal Health Standards Commission provides guidelines on health measures to ensure the safety of international trade and prevent the transfer pathogens for aquatic animals.

### c) *Production of biopharmaceutical compounds in plants*

- Plants have a number of advantages as producers and delivery vehicles for antigens and other compounds. Antigens are more stable and protected against degradation and, in addition, no cold chain is required. To date nearly 200 heterologous proteins have been expressed in plants, including hormones, antigens, enzymes, growth factors and antibodies. Approximately a quarter of them are for veterinary use.
- Producing proteins in plants is cost-effective; it is cheap compared with systems based on tissue culture or milk from transgenic animals and the yields are higher than in these systems. Cereals have been used quite frequently, with yields in rice and corn in the order of 1–2 g/kg. The maintenance costs required for large scale production are low, but the cost advantages of oral delivery are the main advantages. Edible vaccines remove the need for trained personnel to administer injections, the cost of materials is lower and animals can be vaccinated via their normal feed; there is no need to gather them specifically for injection.
- Since large quantities of antigen can be produced, oral doses can be greater than those considered for injection. Oral delivery will stimulate a serum antibody response and also stimulate substantial mucosal immune responses. Hence, plant-based vaccines are particularly suitable for combating intestinal pathogens and those targeting other mucosal surfaces. Proteins expressed in grains can be stored in a dehydrated and stable condition for several years at ambient temperatures. Since plant based vaccines are sub unit vaccines, the safety concerns of live vaccines are removed.
- The market in veterinary pharmaceutical products is valued at US\$ 15 billion and market conditions are favourable for developing new products for the livestock market, particularly for respiratory and gastrointestinal diseases. Amongst areas of opportunity are the development of multivalent vaccines for poultry diseases to replace current vaccines that require injection and to seek new avenues of development such as in fish farming where diseases are not currently controllable by vaccination.

#### d) Support for vaccine development

- The Global Alliance for Livestock Veterinary Medicine (GALVmed) established with funding from the UK Department of International Development seeks to identify gaps in the availability of vaccines or drugs in developing countries. It has a target list of two avian diseases, three diseases of swine, four of small ruminants and four of cattle. These include major transboundary diseases like PPR, contagious bovine pleuropneumonia (CBPP), African swine fever (ASF), Newcastle disease and avian influenza. *Theileria*

*parva*, the cause of East Coast Fever is the first disease that GALVmed is providing the means for a sustainable vaccine. Supplies of the original vaccine are now depleted and GALVmed is supporting the preparation of new material at the International Livestock Research Institute (ILRI) to ensure continuity of control of the disease. The aim is to register the vaccine in Kenya, Uganda, Tanzania and Malawi and transfer the manufacture and distribution to the private sector to enable long term sustainability. It is anticipated that improvements in the production process will lead to price reductions.



# PLENARY SESSION



# Nuclear and Related Techniques for Solving Problems of Animal Production and Health: Development Context and Lessons Learned

J.I. Richards<sup>1</sup>

## ABSTRACT

In reflecting on the role nuclear and related techniques have played in resolving problems of livestock kept by the poor in developing countries, the author acknowledges previous reviews in this area which dealt with the use of these techniques in generating new information on the elaboration of metabolic pathways, in unraveling the complexity of disease transmission, in understanding feedbacks in the endocrine system in relation to reproductive and metabolic hormones, and in attenuating parasites for vaccine production. Clearly the early benefits, perhaps with the exception of vaccine irradiation work, had more intellectual/scientific than economic/practical value. This short review, however, considers developments to date from the perspective of both the users and beneficiaries of such knowledge. It is only in the last 30 years or so that new knowledge and innovative technologies based on nuclear and related techniques have been used in the field to address challenges facing livestock keepers. Interestingly, these developments occurred in parallel with new knowledge in the physical and biological sciences — particularly in the use of plastics in bio-medicine, in fluid transfer and analytical throughput systems, in the computerisation of analytical technologies, in the measurement/detection of ultra-low levels of radiation in immunology, and in molecular biology. Also, there has been a palpable increase in interest of the business sector in developing robust and/or field-oriented technologies for use in the South e.g. nuclear and other marker-linked assay systems for the measurement of a wide range of key antigens and antibodies. In reviewing these developments, the author has attempted to place them within a development context and to draw out lessons learned particularly from the perspective of their contribution to solving problems of animal health and production and contributing to achievement of the Millennium Development Goals. Ten lessons are identified for consideration by FAO/IAEA, donors, policy makers, research managers and researchers to make the use of nuclear and related techniques more applicable and available for users, and to ensure the benefits of such techniques address the needs of poor livestock keepers. They include: a less reductionist approach to research; a more holistic approach to research on livestock systems; more diverse public and private research partnerships; longer, more sustained research trajectories; more support for extension/technology transfer; better communications (and resources) to facilitate wide-scale adoption

of research products; more emphasis on monitoring and evaluating the outputs of research on livestock production and livelihoods; and greater advocacy of evidence for the key contribution of livestock and livestock research for development.

**Key words:** *livestock, research, poverty, nuclear, innovations, lessons.*

## INTRODUCTION

This paper does not detail the history of using nuclear and related techniques in livestock development since this has been done previously by acknowledged experts in the field (Mulligan 1976; Thatcher et al., 1986; Robertshaw, 1986; Wright et al., 1986; Poppi, 1986; Annison and Leng, 1991). Rather, an attempt is made to locate the use of nuclear techniques within an agricultural developmental context and to highlight some lessons learned from previous experiences which may enable future investments in the development and application of such techniques to be put to better use in resolving problems of animal production and health, particularly as they impact on the needs of the poor. This paper also attempts to put the work on livestock of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture into a global context.

The publications emanating from the work of this Division are not included in the list of references provided as they are too numerous to cite and are widely accessible through the Division's web site at <http://www-naweb.iaea.org/nafa/aph/index.html>. They were, however, reviewed in the process of preparing this paper which benefited also from an internet search to capture global contributions in this field. However, it should be emphasised that unlike most other papers in these Proceedings, this is not a conventional research paper — but a review which attempts to identify lessons learned from past experience in developing and applying the techniques concerned.

The development context under which innovative nuclear and related techniques are employed is described including the challenges faced by livestock keepers in developing countries: poverty, hunger and food security, climate change etc; the special case of Africa; the emerging role of the new economic powers (BRIC countries — Brazil, Russia, India and China); and the increasing demand for livestock products at the global level. The paper also describes the processes used in identifying appropriate research; the sustainability of R&D support; and the dissemination pathways traditionally employed to relay innovative research products to target beneficiary groups.

The lessons learned from the foregoing are grouped into 10 discreet but related issues. These are directed at a range of stakeholders involved in nuclear applications for livestock development

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— policy makers, donors, research managers, researchers, academic institutions, public/private extension agencies, civil society, farmers associations, providers of goods and services, knowledge managers, the media, consumers etc.

## CONTEXTS

### World Hunger, Health and Poverty

With grain yields currently increasing at 1.1%/annum and the world's population growing at 1.2%/annum the ability to satisfy food needs are gravely threatened. Concurrently, there is increased demand for feed grain to satisfy the ever-increasing demand for animal protein especially by the new burgeoning economies, where grain is increasingly being used as raw material for fuel energy, and reserves of grain and other food staples are at an unprecedented low. Consequently, Malthusian predictions are not unthinkable, particularly in Africa. The latest reports from FAO and other global contemporary information sources indicate that the number of hungry people in the world increased in the past two years by 75 million to 963 million. Among the causes cited are: decreasing areas of productive farm land for food, higher input prices, greater demand for and increased price of food and climate change (FAO, 2002 and 2008a; World Bank, 2008a; Holmes and Nabarro, 2009). Consequently, every day, some 16 000 children die from hunger-related causes — one child every five seconds (FAO, 2002; Black and Bryce, 2003; UNICEF 2008; Bread for the World, 2009). In essence, hunger is the most extreme form of poverty, where individuals and families are unable to grow, do not have access to, or simply cannot afford to satisfy their most basic need for food. This is particularly disastrous for families suffering from HIV-AIDS, malaria, dysentery etc (WHO/UNICEF, 2004). The food security issue has become so severe that it is impacting on world stability with food riots in over 40 countries over the last two years (Population Reference Bureau, 2006; Brown, 2009).

World food prices remain high despite small recent decreases (FAO, 2008b). One major cause for soaring food prices has been the rapid growth in demand for biofuels, which has diverted land from food production. Rapid economic growth and urbanisation too, particularly in Asia, have resulted in a high demand for meat and consequently for maize and soybeans for livestock feed; and in some parts of Africa, improved economic growth has increased the demand for staples such as maize. Meanwhile, growth in agricultural productivity especially in developing countries continues to remain static or is declining. Growing water scarcity and negative climate change effects are also increasingly affecting food production and prices. Poor and food insecure households are among the hardest hit by rising food prices and subsequently by the current global economic recession.

The Green Revolution in Asia in 1960–1980, and increased investments in agricultural research and development (R&D) in the North up to the early 1980s contributed to a doubling in global grain and livestock production which exceeded population growth needs — and prompted the slogan 'end of famine forever'. The consequent 'mountains' of stored grain, meat and dairy commodities caused much public criticism and political concerns in the North. In response, overzealous policies were put in place to limit production e.g. restrictive milk quotas in the European Union (EU) in 1983 to lower the butter 'mountains'. Concurrently, much of the excess was 'dumped' in the South; this inhibited local production and compromised trade and investment in agriculture there. A quarter of a century later, just as demand for food is exceeding supply, the stores are empty and many former farmers have found alternative employment.

Investments in global agricultural R&D also fell dramatically beginning in the early 1980s from two percent growth year-on-year to 0.6%/annum from 1990 onwards; the relative investment in livestock R&D was skewed even worse. Experience shows that research products in the agricultural sector take a generation or so to filter through to farms at best, so the consequence of this underfunding in agricultural R&D means there are fewer potential technologies available for farmers to use than would otherwise be the case.

### Developments in the BRIC Countries

The economies of the four BRIC countries have one thing in common — fast growth. They are undergoing rapid industrial and agricultural expansion, have strong consumer and export growth, enjoy substantial Foreign Direct Investment, and are in the process of becoming much larger forces in the global economy (Mityayev, 2009; Kumar and Fodea, 2009; Beijing Review 2009).

Brazil's wealth derives from an abundance of mineral resources, agricultural products such as coffee, soybeans, sugar, oranges, cocoa and tobacco, livestock products including beef, poultry and leather footwear; and wood products such as pulp, paper and plywood. Equally rich in natural resources, the vast territory of Russia, the world's largest country in terms of area, is endowed with great reserves of oil, natural gas, metals and timber, which together account for more than 80% of exports. On the other hand, the economy of India is booming as a result of its huge population, which provides a variety of service skills such as a thriving information technology (IT) sector. It is also the world's largest milk producer — the majority of this being generated by 1–3 cow/buffalo herds. And China's dominance in global manufacturing is due to a vast low-cost labour market and a dynamic entrepreneurial approach.

### The Special Case of Africa

Africa is the poorest continent on earth, and the only one, despite all the technological advances that are filling stomachs and pockets elsewhere, that has actually grown poorer over the last 35 years. Approximately two-thirds of sub-Saharan Africa's population is dependent on agriculture and the sector is responsible for generating one-third of the continent's GDP. With its vast arable land, Africa has the potential to feed its citizens and to be a significant global exporter of food. After independence, investments in R&D continued to improve production and productivity of crops, livestock, forest products and fish. There was also a significant increase in monoculture, and greater specialisation and intensification; and there were attempts to sustain productivity and overcome trade barriers. Yet today, Africa must import much of its food and rely on food aid to feed many of its people. Most countries in the continent are now poorer than they were 50 years ago; half of sub-Saharan Africa's over 600 million people live on an average of US\$65 cents/d — and the majority of poor subsistence farmers lack the money to buy seeds, fertilisers, tools and basic resources; and for numerous reasons, mostly associated with risk, financial institutions are reluctant to provide credit to small farmers.

The issues farmers face are enormous — access to water and shelter; prices of and access to basic foodstuffs and agricultural inputs; huge levels of mortality and morbidity in the human and animal population, some but not all due to avoidable conditions such as contaminated water; lack of protection from infectious pests/diseases; more frequent and severe droughts and floods exacerbated by climate change; the continuing debilitating effects of malaria, yellow fever and AIDS, and a host of energy-sapping parasites all exacerbated by poverty and hunger; problems of governance, land



ownership, security, political/social unrest; institutional corruption; national and individual debt; national and regional coordination difficulties; coping with climate change including more frequent and severe droughts; and now suffering the worst of the global food and financial crises (Delgado, 1995; Cleveland, 2007; World Bank, 2008b, ONE International, 2009). Many of the richer and better educated Africans see a dismal future too — about 40% of Africa's privately held wealth is held offshore.

Efforts by the world's rich countries to solve Africa's problems over the past few decades have largely been of a reductionist nature, and have been spectacularly unsuccessful; those countries that have prospered have tended to do so by their own efforts and addressed issues in a more holistic way.

Development assistance in the form of appropriate technologies in agriculture can help provide the technical expertise to move smallholder farmers out of poverty, but this has to be accompanied by an effective extension/advisory service, better access to inputs and markets, and a revitalisation of national agricultural research systems. Unfortunately, international and national development assistance for agriculture has declined dramatically over the last two decades. This despite the fact that the World Bank estimates that growth in the agricultural sector in Africa is four times as effective at reducing poverty than growth in other sectors (World Bank, 2008). Yet over the past 2–3 years, Official Development Assistance (ODA) to agricultural development in Africa has begun to increase; and as part of the African Union's Comprehensive Africa Agricultural Development Programme (CAADP <http://www.nepad-caadp.net/>) African governments have pledged to commit ten percent of national budgets to agriculture and reach the goal of a year-on-year increase of six percent in agricultural productivity by 2015. In addition, the 2008 G8 Summit in Hokkaido and the January 2009 UN Summit on Food Security signaled the urgency of both short-term and long-term needs in food security and agricultural productivity. Also, The UN's Comprehensive Framework for Action (CFA) lays out a set of actions for donors and international financial institutions to act upon (Holmes and Nabarro, 2009).

### Global Livestock Issues — Past, Present and Future

The livestock sector is the fastest growing agricultural sector and has been predicted to continue growing at high rates for the foreseeable future. Global demand for meat and milk has more than doubled in the developing world over the last 20 years — driven largely by the new affluence in BRIC countries. This 'livestock revolution' is largely satisfied by semi-industrial production systems involving cereals as the primary energy source — in direct competition with more direct human demands.

Livestock production has been shown to make an important contribution to national economies as well as to increasing incomes and security at the community and individual levels. Mechanisms to escape from poverty are inextricably linked to livestock. Consumption of livestock products has important health benefits and a modest amount of animal protein in the diets of African children appears to improve their mental, physical and behavioural development (Neumann and Bwibo, 2003). As a proverb in the Horn of Africa goes: *if the animals die, then the people will die too*. Approximately one billion poor people in the developing world rely on livestock for their livelihoods: for their nutritional wellbeing, for social standing, for financial security, for draft power, for dung for fuel and fertiliser, for their hides and skins etc.

However, despite the many new developments in animal nutrition, breeding, husbandry practices, reproduction and animal health over the last 50 years — some of which have filtered down to

the developing world, there are still annual losses of 50 million cattle and water buffalo, over 100 million sheep and goats and countless poultry from parasitic and infectious diseases (FAO, 2009). Many more succumb from inadequate feed and water supplies, poor husbandry practices, inappropriate policies and ignorance.

A major new challenge to livestock today is the conflicting evidence and consequent media frenzy regarding their contribution to climate change (Cline, 2007; Pachauri and Reisinger, 2007; Stern, 2006, IPCC, 2007; Nyong, 2007; Noble, 2007; Human Development Report, 2007/2008). According to a recent report from the UN's Food and Agriculture Organization, livestock production, dominated in the West and increasingly in BRIC countries by large-scale factory farming, is responsible for 18% of the world's greenhouse gas emissions (FAO, 2006), a bigger share than that contributed by all of the world's transport. Yet the global livestock population is predicted to increase dramatically over the next 40 years: cattle from 1.5 to 2.6 billion; and sheep and goats from 1.7 to 2.7 billion. Simultaneously, grazing intensity is projected to increase by 50% globally by 2030 — with substantial land degradation potential. As a consequence, livestock are receiving a very bad press in the North and there is pressure to reduce consumption of livestock products and/or increase their price through some form of carbon taxation. Yet as stated above, livestock production provides an essential pathway out of poverty. Rich and poor worlds are thus colliding when it comes to the value of livestock production and consumption of livestock products. But who is advocating for the key contribution of livestock for bettering the lives of the poor?

Science therefore, and the generation of objective evidence through the use of nuclear and other techniques, can serve as an honest broker in the complex and often controversial debate over livestock and environmental issues. The 'truth' may be inconvenient to some, but clear empirical evidence is needed in this discussion. The global agricultural research community needs to develop a more comprehensive, integrated agenda to provide crucial, objective evidence on the trade-offs between food security, livelihoods and the environment. It is surely not beyond man's wit to protect the livelihoods of poor livestock keepers while also conserving environmental resources. The foregoing begs the question why livestock research findings have not had a greater impact on livestock development and poverty reduction.

### LESSONS LEARNED FROM RECENT USES OF NUCLEAR TECHNIQUES

#### Effective Development Occurs when the Poor are Empowered to Articulate their Needs and Concerns

Whereas many people are familiar with the eight Millennium Development Goals, the one which most recall is the one which addresses a monetary target — "to reduce by one-half those who subsist on less than US\$1/d". But what are the true indicators of poverty? Most subsistence farmers do not have the opportunity to earn money from their small land holdings or to earn a wage from labouring; and subsistence farmers represent the majority of poor farmers in most African countries, e.g. in Rwanda, over 90% are subsistence farmers. Their basic human needs include access to food, water, shelter and clothing. Another measure of poverty, the UN's Human Development Index, looks at quality of life factors including access to education, health systems and credit. Others consider human security indicators — whether people have the assets or skills to survive shocks such as poor rainfall, while others stress the importance of empowerment and participation in decision making, including the right to information and knowledge. Therefore, when (or if) scientists undertake

market research in relation to the issues affecting the poor, they need to specify which poverty indicator is being addressed by the research and not restrict the vision statement to improvements in agricultural commodity or productivity indicators which may not automatically be beneficial to the poor.

In the North, indicators of successful agriculture and livestock research at the project level are normally increased production on a per ha or per investment basis. These indices are in common use and many of the research benefits accrued are marketed through public/private extension systems to commercial farmers, and the extra income earned goes to improving the livelihoods of the farming community. Unfortunately, this model has been transferred wholesale to the South — and is largely inappropriate for two main reasons. Firstly, the vast majority of small-scale crop-livestock farmers and pastoralists seldom if ever receive advice from government extension agents. There are many reasons for this — but in general, the number of farmers far outweighs the capacity of the extension services and so they are generally not fit for purpose. With very few exceptions, the extension systems are generally grossly underfunded and ineffective; the criteria of success are not appropriate and incentives to adopt new technologies or to innovate seldom exist. And secondly, animals are often kept for reasons not understood by Western influenced researchers, e.g. for good reason, the Maasai herders in East Africa believe that a cow/bull should not be bred to be heavier than the ability of two men to lift it to its feet during a drought. How this contrasts with the breeding philosophy from the North.

### The Development and Transfer of Nuclear Techniques have to be Appropriate for Need and Local Circumstances

Up until the 1970s nuclear techniques contributed largely to learning — biochemical pathways, and physiological mechanisms in animals and man, and the use of radiation to produce attenuated vaccines. Thereafter, there was greater emphasis on the application of these techniques to problem solving in animal feeding, reproduction, breeding, genetics, animal health etc. Examples include:

- *nutrition and feeding*: the use of isotopes (deuterium) in the investigation of metabolism in the rat in the 1930s; the use of stable ( $^{15}\text{N}$ ) and radioisotopes ( $^{14}\text{C}$ ,  $^{32}\text{P}$  and  $^{35}\text{S}$ ) in characterising biochemical and metabolic pathways in the 1950s–1980s; the development of feeding standards for most livestock species in 1960s; and in some applications such as in evaluating new diet formulations from mixtures of feeds for livestock in marginal environments, e.g. various radioisotope markers in *in vitro* systems such as the Rusitec in the 1980s and 1990s.
- *reproduction*: the use of competitive protein-binding assays (based on  $^3\text{H}$ ) for various steroid and protein hormones in the 1960s and 1970s to the use of rapid ( $^{125}\text{I}$ -based) radio-immunoassays for quantifying levels of progesterone, luteinising hormone, follicle stimulating hormone etc. in biological fluids in the 1970s–1990s to assist in detecting oestrus and diagnosing pregnancy in farm animals and improving the efficiency of artificial insemination (AI), natural service and animal husbandry practices. Whereas these were widely adopted over this period in many developing countries and found to be extremely useful tools by researchers in support of farmer needs, their subsequent transformation into enzyme marked immunoassay technologies resulted in much wider use as pen-side tests.
- *animal health/disease diagnosis*: the use of ( $^{32}\text{P}$ -based) DNA probes and the development of enzyme linked immunosorbent assays (ELISA) for a range of key livestock diseases including

rinderpest, foot and mouth disease, brucellosis, peste des petits ruminants, Newcastle disease etc in the 1990s – early 2000s have been enormously successful in contributing to national and global eradication and control of these diseases (viz. rinderpest). Recent collaborative work with international diagnostic centres has also produced kits which distinguish vaccinated from naturally infected animals — a key development in animal health practice in the developing world; these procedures are being proposed as candidates for commercial kit application. Also, a range of exciting new related and complementary technologies, including genetic modification (GM) and RNA interference are being assessed for their potential.

- *insect and other pests*: the eradication of disease vectors which affect livestock — such as tsetse flies and New World screwworm — where gamma radiation or X rays are used to sterilise male flies prior to mass release through use of the sterile insect technique (SIT) has also been used successfully in North Africa and on the island of Zanzibar to eradicate these pests. And the use of stable isotopes to follow the migration of wild birds is an important component in the control of the avian influenza virus.
- *structural and functional genomics as associated biotechnologies* will become really important to agriculture in the future as they begin to unlock the mysteries of disease and the characterisation of breed traits. One goal will be to accelerate disease research by enhancing the genomic tools used to explore how bacteria cause disease. Also, sequencing of the cow genome (Elsick et al., 2009) provides new information about mammalian evolution as well as bovine-specific biology and points the way to research that could result in more sustainable food production. Whereas these are molecular diagnostic developments, they are often characterised as nuclear-related because of the pioneering work in DNA technology using  $^{32}\text{P}$  and  $^{35}\text{S}$  markers and their continued use in labeling genetic markers e.g. in Southern blots.

Whereas the successful use of these techniques in increasing basic knowledge of biological systems cannot be denied, their successful application in addressing the needs of the poor cannot be argued so strongly, at least as yet. Critics question whether these specific developments have addressed user needs or simply the interests of the scientific community — and whether adequate market research was undertaken up-front to identify and prioritise problems facing poor livestock keepers and in ‘marketing’ the products adequately as is the norm in private sector research.

### The Most Useful Applications of Nuclear and Related Techniques Occurred as a Consequence of Parallel Innovations in Other Sciences

Particularly crucial were developments in immunology (Wu, 2006), in plastics chemistry, in radiation detection, in liquid delivery systems (Martin, 2001), in miniaturisation, in throughput speed technologies, in complementary enzyme marked systems (Engvall and Perlman, 1971), in DNA chemistry and in genomic sciences (Elsick et al., 2009) etc. These developments would not have become marketable products without the key involvement of the private sector, particularly the equipment manufacturers and the diagnostic companies who saw unique opportunities in the application of much of the above particularly in the field of human medicine both in the North and South. There is still a key role for blue-sky research and research into potential ‘marriages’ between nuclear techniques and other innovations in science and technology. Some of the developments in genomics illustrated in these Proceedings exemplify these exciting innovations.

### Nuclear Techniques are Likely to Further Significantly Influence Development only when:

- animal science researchers collaborate more closely with those in other disciplines within agricultural systems — crops, pests, residues etc, and with other sectors which affect the systems in which poor livestock keepers operate — human health, education, infrastructure, policy development;
- the agricultural systems within which livestock and their keepers co-exist are better understood;
- researchers address both long term and contemporary issues which affect livestock keepers e.g. access to food, adaptation to climate change, disease control, sustainable feed resources, simple breeding practices, pen-side diagnostics etc.

### The Different Needs of Target Users and Beneficiaries are Better Understood

Research needs to be balanced and considered — between the demands for better livestock trade by commercial farmers, to the increased livestock production/productivity and associated livelihoods benefits of small farmers, to the improved production of livestock under challenging environmental conditions faced by subsistence farmers and pastoralists. The target groups for the information generated through the application of nuclear techniques also need to be defined and addressed. Researchers need to be clear whether they are developing technologies for *users* or/and for *beneficiaries* — accepting that both are members of much larger value chains of institutions that span the farm-to-fork continuum.

### The Benefits of Wide-scale Use of Nuclear Techniques in Development are Effectively Communicated

Based on the outputs of the FAO/IAEA's Coordinated Research Programmes over the last 30 years, the Joint FAO/IAEA Division has generated an excellent series of publications. There is also an 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, there is little objective evidence of the impact of this information on livestock production or practices.

One major reason for this may be poor communications practices. Public sector research has a great deal to learn from private sector approaches to the research process, including better communication. The private sector traditionally devotes as many resources to communication (product marketing, publicity, branding, promotion, sales etc.) as it does to the research and development of a product. And prior to the R&D activity, significant investments are made in communications (market research) to ascertain exactly what clients need. Compare this with the tiny investments made by the public sector in communications and it becomes more understandable why public sector research findings are not more widely adopted. The situation has been exacerbated in many developing countries by collapse of national extension services during the debt crisis of the 1990s; and most of these have still not been rebuilt.

To improve the situation, there is need for root and branch change in the mind sets of research institutions and donors involved in development. Despite the fact that researchers are now encouraged to add value to their research products through working on-farm, by writing extension products and even advising farmers directly, they have not been trained in this area, there are no financial or career development incentives to go the 'last mile', and there

are too few to address the multitude of clients. Thus, there appears to be an urgent need for public sector research to employ and train professional communicators and marketing specialists, and allocate appropriate budgets to enable them to undertake this work within and outside current extension systems. As an indicator of the importance assigned to this sector, some donor research programmes now allocate up to one-third of their research budgets to communications to facilitate greater out-scaling of relevant research findings to value chains.

Communication strategies also need to be part of research programme planning and implementation. Indeed, in a recent report by Perera (2006) who reviewed the successes and missed opportunities in the work of the Joint FAO/IAEA Programme, there was an acceptance by many decision makers interviewed that better access to appropriate information by animal scientists and field practitioners was essential if the impact of innovations on poverty reduction are to be realised.

The internet search made for this review also highlights a communication issue in need of attention. Approximately 90% of the references resourced originated from, or referred to, FAO/IAEA Research and IAEA Technical Cooperation projects. The remaining 10% were from the USA, India, Israel and Brazil — and most of these came from their respective Atomic Energy establishments. Whereas it may be comforting for the Joint FAO/IAEA Programme to know that its work is at the centre of activities in this field, it does illustrate a problem that is consuming the attention of many donors — lack of evidence of widespread application of research findings and innovation to non-nuclear research laboratories and field operations. This is not uncommon at all. For instance, in managing the UK Department for International Development's (DFID's) numerous livestock research projects in Africa, Asia and Latin America ([www.lpp.co.uk](http://www.lpp.co.uk)) and more recently its Research Into Use (RIU) Programme ([www.researchintouse.com](http://www.researchintouse.com)) the author became increasingly aware of the strong disconnect between generators and users of new technologies, practices or policies, and also between the users and beneficiaries, irrespective of whether these research products had nuclear labels or not.

### Need for Champions to Advocate a More Significant Role for Livestock in Development

There is general agreement that the livestock R&D community needs to become more vocal in advocating evidence-based support for the contribution of livestock and livestock research to development. Regarding 'advocate to whom?' the answer must include the media, parliamentarians and other decision makers, donors, the general public, the private sector — institutions not normally targeted by researchers. All animal scientists have a role to play in lobbying and in potentially challenging government on policies with evidence based on objective scientific data. They also have a responsibility to inform the press appropriately and prevent the catastrophic media blunders such as the recent misuse of the term 'swine flu' and its extreme consequences on the pig industry world-wide (El-Awady, 2009) However, while accepting that the generation of such evidence is a keystone to effective advocacy, it must be acknowledged that the publication records of many southern scientists in peer-reviewed journals leaves much to be desired — and some question the value of further donor investments in research in the South until this record is improved.

Issues in need of advocacy include: i) increase ODA for agriculture and livestock; although 75% of the world's poor live in rural areas in developing countries and depend on agriculture for their survival, a mere four percent of ODA goes to agriculture and less than one

percent to livestock, ii) in sub-Saharan Africa, GDP growth originating in agriculture is about four times more effective in raising incomes of extremely poor people than GDP growth originating outside the sector, iii) a dynamic 'agriculture for development' agenda can benefit the estimated 950 million rural people in the developing world who live on less than US\$1/d, most of whom are engaged in agriculture. Agriculture needs more prominence across the board. At the global level, countries must deliver on vital reforms such as cutting distorting subsidies and opening markets, while civil society groups, especially farmer organisations, need more say in setting the agricultural agenda.

### Partnerships Strengthen the Development Effort

These include in research, in development, with industry and with marketing. The work of the animal health group within the Joint FAO/IAEA Programme has been exemplary in this regard. For instance, by engaging with global organisations and initiatives such as FAO's Global Rinderpest Eradication Programme (GREP), with the World Organisation for Animal Health (OIE), and the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES), the diagnostic and training elements provided by the Programme contributed to international coordination mechanisms to verify freedom from rinderpest and promote its global eradication while providing technical guidance to achieve these goals. Such partnerships have also enabled the Programme to be influential at the global scale in the control of foot and mouth disease, trypanosomiasis, brucellosis and peste des petits ruminants.

Even more innovative is the development of the 'OneHealth' programme between FAO and WHO which addresses zoonotic diseases from both human and animal perspectives. Whereas such partnerships have generated positive outcomes, the world's agricultural R&D institutions remain largely autonomous at heart and there is need for agreement both on their 'territorial' mandates and strategies — and for greater collaboration especially at the programme/project level. This includes the UN specialised agencies, the CGIAR and the global and regional fora for Agricultural Research for Development. Other global initiatives such as the Livestock Community of Practice [www.cop-ppld.net/testsite](http://www.cop-ppld.net/testsite) for Pro-poor Livestock Development being facilitated by the International Fund for Agricultural Development (IFAD), is an inclusive partnership of livestock practitioners, managers, researchers and other actors involved in livestock development (Community of Practice) through which there is exchange of experiences, management of relevant knowledge and support for learning as an instrument to achieve better uptake of knowledge. And as for research priorities, the Inter-Agency Donor Group for Livestock Production and Animal Health Research (IADG — see [www.cop-ppld.net/](http://www.cop-ppld.net/)) website) meets annually to address how it might coordinate funding activities to significantly increase the level of adoption of new livestock technologies, practices and policies and justify the expenditure of public funds in generating such information. This Donor Group identified the top priorities for future livestock research for development as process issues in support of previous technical investments in research: i) communications, ii) advocacy, iii) research into use, and iv) vaccine delivery systems.

### R&D Processes are Significantly Improved

The fixation by donors and research implementers to employ disciplinary biased and short duration (three-year) projects to address development issues is inappropriate. History reveals that successful projects are more holistic in nature, have an agricultural systems perspective and a longer time trajectory (minimum five years). Equally

frustrating to development is the inability to disseminate and transfer new technologies, practices and policies to users as evidenced by the work of the RIU programme. However, the FAO/IAEA Programme has an unique partnership model between the research-oriented activities of its Coordinated Research Projects and the technology transfer and scaling-up services offered by the IAEA and FAO Technical Cooperation Departments. Whereas this partnership could be greatly strengthened, it provides a research and transfer model for other agencies and donors to follow. There is also increasing evidence to show that focusing research on the needs of the several institutions within the farm-to-fork value chain results in greater adoption and impact than when the research findings are directed solely at poor farmers. Finally, it is evident that greater involvement of the private sector in research activities and/or as value chain recipients of new knowledge improves the likelihood of research being adopted and generating value at the community level or wider.

### Monitoring and Evaluation Processes are Employed routinely to Assess Progress and Performance

Despite the rhetoric surrounding the use of M&E practices in development, there is a general reluctance by the scientific community involved in development to willingly engage in this area — both in monitoring the progress of a project or process and in evaluating achievements to date or at end of project. Direct evidence is normally available on the *outputs* generated as a consequence of projects involving the use of nuclear and related techniques in support of livestock research. These might include statistics on the technologies transferred such as individuals trained and data on the successful harmonisation of in-country laboratories (practices and performance) which conform to international standards. There is normally less evidence of the benefits to livestock keepers at the project level such as might be generated through the wide-scale use of technologies such as ELISA in support of livestock disease eradication or control; or at the programme level, particularly when such technologies are employed through partnership agreements with specialist global institutions. Some more tangible evidence sometimes exists of increases in livestock trade as a consequence of the use of such techniques in the control of trade-related diseases such as foot and mouth disease and rinderpest. However, despite these successes, objective evidence on the benefits of such programmes to the livelihoods of poor farmers is seldom available and there appears to be satisfaction in capturing information 'solely' on the benefits to livestock themselves. Whereas such achievements are laudable, there is an unfortunate reluctance to go the 'last mile' and assess the benefits to livestock farmers.

Regarding nuclear technologies in support of reproduction or animal nutrition, again there is much subjective evidence about improvements in livestock productivity at the local (project) level as a consequence of investments in the development and use of nuclear and related techniques but the benefits to poor livestock keepers remain largely speculative. Thus in response to the query: "has research on nuclear techniques contributed to achieving any of the MDGs or else contributed to wide-scale increases in livestock production/productivity", there is no hard evidence for the former and little for the latter. Consequently, efforts to assess the value of investments in nuclear techniques for the benefit of livestock farmers are few and far between (Vose, 1994; Dargie, 1989). With such a dearth of information, donors are understandably investing larger proportions of their project 'spends' on M&E to ensure the effectiveness of project management and to justify the expenditure to taxpayers particularly in these days of fiscal constraints.

## CONCLUSION

Nuclear and related techniques remain as cutting edge tools in scientific discovery — and need to be developed further and be applied more to address contemporary issues in agricultural development. However, appreciating the drivers which impact on poor livestock keepers and understanding the farming systems context under which they work is central to any effective agricultural R&D effort — with or without the use of nuclear techniques.

This review has identified 10 lessons to enable greater effectiveness of nuclear techniques in resolving problems experienced by poor livestock keepers in the field. There is a need for more diverse partnerships; a less reductionist approach to research, and a more holistic approach to research on livestock systems; a longer perspective — more sustained support to technology transfer; better communications (and resources) on the application of nuclear techniques on agricultural practices to discreet groups is at the heart of wide scale adoption of research products; the need for greater advocacy on the key contribution of livestock and livestock research for development — a responsibility that all animal scientists need to assume since there are many doubters; more emphasis on monitoring and evaluating the outputs of research on livestock production and livelihoods. Sadly, whereas there may well have been benefits to both livestock and their keepers at a local level there appears to be very little objective evidence available to confirm this on a broader scale. Notwithstanding, the products of research on and with nuclear and related technologies are vital parts of the armoury required to generate technologies and knowledge to satisfy the world's basic needs for food; they need and deserve a better shop window.

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# Decline in Available World Resources: Implications for Livestock Production Systems

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## ABSTRACT

The world is faced with a triple crisis: climate change, peak oil (the end of inexpensive energy) and global resource depletion. These are interrelated and interactive which makes the subject to be discussed in this paper extremely complex. The certainties are that there will be great changes to contend with in the future in order to produce and deliver food to maintain the present world population, let alone a balanced diet for everyone. The global financial meltdown is seen as having a critical role in determining future animal production strategies. The age of scarce and therefore high energy costs now dawns, and will be marked by the decline of oil and all that depends on it, including financial capital. It heralds large changes in the financial and related political structures. Without rationalization of the use of oil there seems to be little chance of continuing growth in industrialised or developing countries. Inexpensive oil allowed food to be produced cheaply but this will change greatly as oil prices rise and create the potential for major disruptions in food availability. Peak oil will affect other resource availabilities. Agriculture has received inexpensive chemicals and fertilisers on which high crop yields have been predicated, including: nitrogenous fertilizers manufactured from natural gas and phosphates reserves which have peaked. The world is now dependent on extracting phosphate fertilizers from low grade rock phosphate at high energy costs. Irrigation waters from aquifers have also been depleted and rivers overused. The advent of peak oil with the ultimate high cost of fuel and therefore power for irrigation will clearly cause a return of vast areas of highly productive crop land back to rain-fed cropping, pasture or desert with major loss of food productivity. Soil erosion and fertiliser run off from cropping systems are also major concerns as tillage and crop management have eroded the top soil of large areas of land that will inevitably lead to decreased crop yields. The dependency of the industrialised countries on imported oil has seen a headlong development of biofuel from sugar cane and maize mainly in Brazil and the USA respectively and bio diesel from plant oils, creating major cereal food/feed grain shortages. The expectation is that world cereal grain availability for livestock production will be highly restricted, with a major decline in industrialised or commonly termed factory farming of livestock. Herbivores are likely to be used more extensively with time, particularly the ruminant and the rabbit. 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 change, leading to at times more intense drought and or flooding rains. In some areas lack of synchrony of river flows (from glacial melt) with

irrigation requirements may reduce multiple cropping areas. Warming also carries with it the risk of decreased crop production as rice yields decrease by 10% for every °C rise in night time temperatures. Resource depletion threatens any attempt by countries to grow their economies and has the potential to lower world crop production by direct or various flow-on effects. It is suggested that the world is now entering a time where intensive animal production will become increasingly expensive as competition for food, feed and fuel develops. The animal production industries based on herbivores will need extensive development, exploiting a wide range of waste by-products of agriculture or biomass from land not dedicated to food or biofuel production. The high cost of fuel will also see a gradual return to animal power in agriculture. The downside of this will be the increase in ruminant livestock with potential increase in enteric methane production. A new prospect for limiting methane production in fermentative digestion indicates that this could be reduced substantially in the future.

**Key words:** *climate change, peak oil, resource depletion, biofuels, agricultural by-products, ruminants, methane emissions.*

## INTRODUCTION

Campbell (2005) speaking in Edinburgh at the Association for the Study of Peak Oil stated: "The first half of the age of oil now closes. It lasted 150 years and saw the rapid expansion of industry, transport, trade, agriculture and financial capital, allowing the population to expand six-fold. The financial capital was created by banks with confidence that tomorrow's expansion, fuelled by oil-based energy, was adequate collateral for to-day's debt. The second half of the age of oil now dawns, and will be marked by the decline of oil and all that depends on it, including financial capital. It heralds the collapse of the present financial system, and related political structures".

These predictions have been ignored by politicians, at great cost, particularly to developed countries and the implications of peak oil on future strategies for agriculture and animal production have been little discussed (Leng, 2002). To delve into changes that are likely to the world's livestock production systems without considering the complex and interacting factors that affect the outcomes makes this task seemingly impossible at the time of writing. However, the present food and financial crises have brought out some highly informative recent publications (Brown, 2009; Nellemann et al., 2009; Scherr and Sthapit, 2009).

Deepening economic turmoil is prioritising resources in the industrialized world; the emphasis is on decreasing the 'politically unacceptable', loss of jobs by increasing consumption and therefore resource utilisation, in order to return countries to growth (or business as normal). But 'kick starting' economies and increasing

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development in emerging economies, without decreasing demand for fossil energy, may rapidly reverse economic recovery by increasing fuel prices (Campbell, 2005). Historically, global economic growth has never occurred without a simultaneous increase in the use of energy (Hirsch, 2009). All nations of the world use oil, but for a few nations, coal is an important component of the fuel mix. Unless economic policies ensure a decreased demand for fossil fuels relative to production, there seems little prospect of a permanent recovery from depression in industrialised countries and little chance of substantial growth in the underdeveloped economies. The second half of the age of oil has major implications for world agriculture in both industrialised nations and in the emerging economies, particularly of India and China (Brown, 2009).

Livestock production systems will be differentially affected depending on the level of industrialisation and therefore dependency on fossil energy resources. The changes that occur in industrialised nations will inevitably impact on the less well developed countries. For instance political decisions such as subsidising first and second generation biofuels will have significant effects on food availability on world markets and therefore the availability of food for emergencies created, for example by adverse weather conditions or militant actions, particularly in heavily populated countries (Runge and Sanauer, 2007).

In the following discussion it is argued that herbivorous animals, particularly ruminants, but also small herbivores, fed on agro-industrial or under utilised feeds will need to dominate the meat and milk production requirements of future generations. However, animal production is not simply a matter of producing food; it is also relieving the ill health of resource poor people. Poor health often results from essential nutrient deficiencies in people mainly on cereal based diets which animal products can rectify. In 2000, one billion people were chronically undernourished and the number is growing. Animal products, even in tiny quantities, support physical and intellectual development of young people and pregnant mothers (Waterlow, 1998).

## LIVESTOCK FARMING

Livestock farming began with early hunters and gatherers as they settled into crop production and harnessed livestock for transportation and eventually the heavy tasks such as tilling the land. Pastoralists as they became farmers used ruminants as a source of power, and milk and meat production were secondary issues. Similarly birds and pigs were initially scavenging animals, feeding on left over or spilt food, by-products and natural fauna. Instinctively people recognised the nutritional role of animal products which provided essential amino acids and micronutrients, deficient in cereal based diets.

It was only with the advent of inexpensive energy and therefore feed (energy and protein) that livestock production diverged from its integrated roles in food production to industrialised production systems. Inexpensive grain and/or energy allowed the economic development of a number of intensive meat and milk production systems including grain-based dairy and beef production, intensive production of carnivorous (highly energy dependent) and herbivorous fish (highly grain dependent) and other smaller and specialised systems such as intensive rabbit and guinea-pig meat production.

In the non-industrialised nations, animals mostly remained an integrated component of farming, but with the availability of inexpensive grain, peri-urban pig and poultry production developed. The shift in the relationship between fossil fuel energy and food and meat or milk production began around 1970 with major changes in the cost of oil. For 20 years prior to 1970 a bushel of wheat cost about the same as that of a barrel of oil but the equivalent cost increased four-fold in recent times (Brown, 2009). Before there was abundant inexpensive fuel, world population was constrained below one billion people: it increased dramatically to 6.7 billion over a few years as food production was ramped up by mechanisation and increasing farm size, irrigation, improved seed varieties, improved agrochemicals such as herbicides and increased fertilizer availability and inexpensive distribution systems. At the same time cheap energy also aided improved healthcare and modern medicines decreased infant mortality and increased longevity. Together these allowed populations to grow at a fast rate and the world population is predicted to be eight billion within two to three decades.

There has, however, been a price to pay for the green revolution; farmers and businessmen were inevitably exploiting finite or non renewable resources. A number of these resources, just as with oil, appear to have peaked and their reduced availability in the future will have detrimental effects on the world's ability to feed its population and provide balanced diets, particularly for the resource poor of any country.

## FOOD AND FEED RESOURCES TO MEET A WORLD POPULATION OF 8–10 BILLION PEOPLE

The food requirements and the resources to meet a projected human population of 8 billion people in the decade 2030–2040 appear to be unattainable. The required resources have been estimated by Vance (2001) and are shown in **Table 1**. As oil becomes scarce it appears that food production costs will escalate and increasingly divert a higher proportion of income in the developed countries to maintenance energy of people including their food and transport costs. In the less rich societies, the cost of food may develop into

**Table 1. Agricultural production and resource use (Vance, 2001).**

Item	1960	2000	2030–2040
Food Production (M Tonnes x10 <sup>-9</sup> )	1.8	3.5	5.5
Population (10 <sup>9</sup> )	3	6	8
Irrigated Land (% arable)	10	18	20
Cultivated land (ha x10 <sup>-9</sup> )	1.3	1.5	1.8
Water stressed countries	20	28	52
N fertiliser use (Tg)	10	88	120
P fertiliser use (Tg)	9	40	55-60



major famine risks and all countries will need to develop their own food security measures

### Climate Change is Likely to Reduce Overall World Crop Yields

The most significant effects of climate change on agriculture arise through changes in weather patterns and instances of droughts and inundating rains (see Stern 2007). Increasing temperatures in tropical countries can have a slowing effect on photosynthesis and hence plant growth. For example, rice yields in Asia are declining by ten percent for every degree Centigrade rise in night-time temperatures (Peng et al., 2004). A recent report (CGIAR, 2007), on the effects of increased environmental temperatures on wheat production in the Indo-Gangetic Plain, indicates a massive decrease in yields. This area produces 15% of total world wheat annually, about 90 million tonnes. Under the climatic conditions expected to prevail in 2050, this wheat mega-environment will shrink by just over half, mainly through shortening of the growth period as a result of heat stress early and late in the wheat growing season. This threatens the food security of about 200 million people (CGIAR, 2007).

The predictions on climate change are for increasing rain in some areas but less rain in others and rainfall patterns will become more variable and storms more intense (Stern, 2007), leading to increasing crop failures. Already in 2009, catastrophic falls (20–40%) in food production appear to be probable because of drought in countries that are major food producers and or exporters (de Carbonal, 2009). Some countries that normally have food surpluses are already imposing export restrictions on grains because of high international prices and if de Carbonal's observations are correct, food prices will soar. Whether the widespread incidence of drought this year is a result of climate change is debatable but it is difficult to dismiss the possibility. Recent models suggest that global warming is likely to reduce world agricultural output by between 16% and 3%. However, the effects will not be spread evenly with productivity in the tropical, developing countries likely to be reduced disproportionately by 21–9% (Cline, 2007) The spread of estimates are associated with the uncertainty of the benefits of increased atmospheric carbon dioxide on plant growth (carbon fertilisation). Global warming will also open up land for grain production in the Northern Hemisphere, but far away from

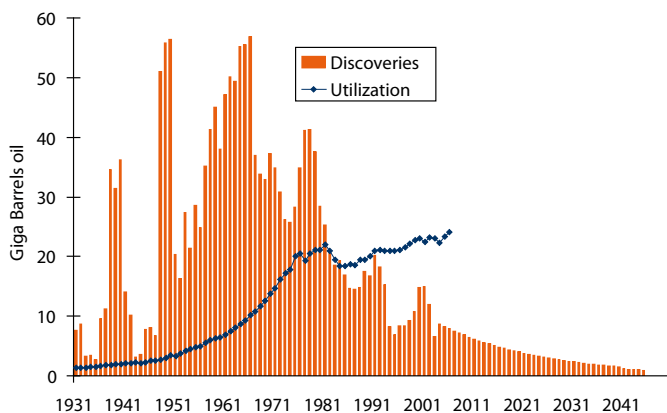


Figure 1. World oil discoveries and the trend in world utilisation of oil (from Campbell 2005).

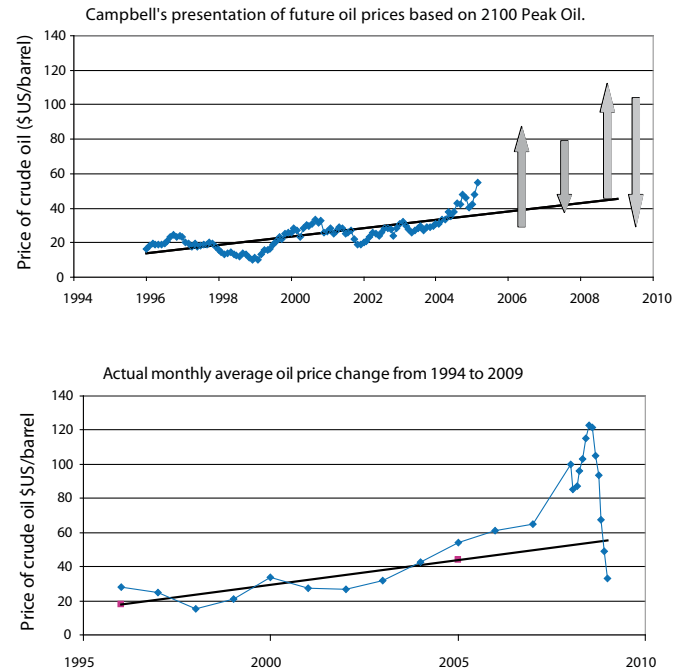


Figure 2. The change in the price of oil from 1994 and the predictions of recession and recovery according to Campbell (2005).

the main centres of population. However, the warming of the Arctic region appears to have potential to disrupt the monsoon that would have enormous social and economic impacts for the two billion people of South Asia (Pearce, 2009). Melting of the permafrost, may also result in more methane produced from decomposing organic matter and dissociation of methane hydrates, which together may be the cause of the recent upturn in atmospheric methane after a decade of stabilised concentrations (Pearce, 2009).

## MAJOR INFLUENCES ON FUTURE LIVESTOCK PRODUCTION SYSTEMS

### Peak Oil

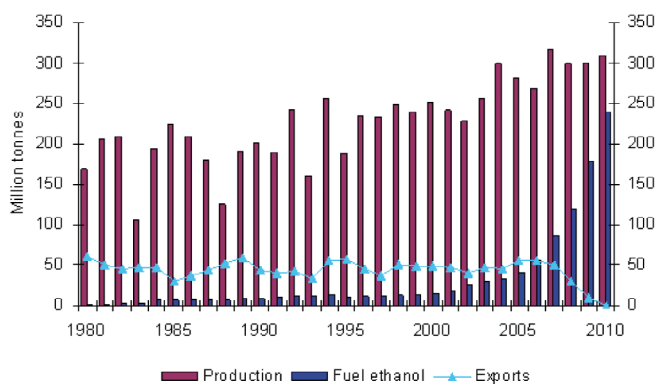
It must be emphasised that fossil fuel availability is likely to be the main driving force for change in present animal production systems. The most important aspect at this time is that oil production and oil demand are finely balanced and production has out-paced new discoveries since 1981 (Figure 1) and in all probability oil production has peaked (for discussion see Brown, 2009). The upward trend in oil price has been building for several years and Campbell in 2005 predicted vicious cycles of price shock–recession–demand fall–price collapse–recovery–price shock. This potential pattern is being borne out by the global financial downturn being experienced at the time of writing. Campbell's predictions are shown in Figure 2 with the actual price of oil to this year. The 'easy to extract' fossil fuels have been depleted and increasing world demand for fuel will inevitably force world fuel prices to rise (Campbell and Leherrere, 1998), but with a saw-tooth pattern over time, as periodic recessions lower demand and price, allowing both to rise again thereafter (Campbell, 2005). Interacting factors also indicate that food availability and food prices will be compromised directly or indirectly by flow-on effects of oil depletion (Leng, 2002, 2005), possibly the most important being

the diversion of land from food crops to production of biofuel and the variable effect on food costs (Runge and Senauer, 2007).

### Industrial Biofuel Production and Food Grain Availability

The twin threats of peak oil and global warming have resulted in politically driven development of large-scale production of biofuel from sugar cane in Brazil, and maize in the USA. Both countries have huge importation costs for oil. The USA uses 25% of the world's oil production, but imports 70% of its needs, making it extremely vulnerable to world oil supply (Beddor et al., 2009). Biofuel production from cereal grains with competition for food and feed has major implications for human welfare and livestock production worldwide (Runge and Senauer, 2007). It is not the intention here to focus on the net efficiency gains in transport fuel, however the amount of energy returned in ethanol from growing maize and processing the starches through to alcohol is hotly debated but barely positive (Patzek and Pimentel, 2006; Patzek, 2007; Pimentel et al., 2009). Even the advocates for this industry fail to estimate a net energy gain in the manufacture of ethanol that is much greater than a gain of energy represented by the by-products of the industry (distiller's by-products). These are, however, important resources for the animal feed industry, as the protein is in a form that increases the efficiency of ruminant production based on agro-industrial by-products. The latter are likely to be a major resource for future meat and milk production from ruminants (see later) provided a problem associated with antibiotic residues can be overcome (Philpott, 2009).

The industrial production of biofuel is creating major conflicts over food for humans, feed for animals and feedstock for liquid fuels. The Earth Policy Institute predicts that ethanol production claimed 50% (or 140 million tonnes) of US grain in 2008 (Brown, 2009). The balance between maize exports and maize used for ethanol in the US indicates the extent of the potential effects on world food supplies (see **Figure 3**, from Brown, 2007). However, combinations of the financial meltdown and recent lower fuel prices have emphasised the irrational and unfolding disaster of agofuels; one-fifth of the ethanol industry's production capacity in the USA — most of it less than five years old — has shut down (Staff, 2009).



**Figure 3. Production of maize in the USA and the calculated availability of grain for export and ethanol production.**

### Decline in Mechanised Agriculture and Food Production

Increasingly expensive fuel will have a major influence on the use of traction power, with the developing countries embracing more reliance on this with considerable effects on crop yields. Mechanisation in agriculture was a critical component in increasing crop yields (see Verma, 2002). The ability of farms to move from one or two crops per year to two -three crops a year is dependent on precision timing of farming practices such as land preparation, seeding, irrigation, herbicide application and harvesting times. Such farming practices also require high fertiliser inputs and are best suited to conservation practices such as no-till. To time these inputs effectively will be much more difficult using animal traction. This will reduce, in places, the number of crops that can be taken annually and also will make impossible some conservation practices, such as direct drilling which are not easily done with draught animals. Draught animals may also require some of the food grown but as mature animals, their feed requirements can usually be met from crop residues. In addition, the effects of vagaries of weather that may result from global climate change will not easily be rectified when animal power is the main source of cultivation energy. For instance 'catch up' in cropping procedures may not be possible for flood or drought related delays in planting or harvesting of a crop.

### Other Resource Depletions with Implications for Agriculture

Water is the most potent resource for plant growth. Agriculture accounts for 70% of human water use and two-thirds of the available fresh water is used for irrigation (Revenga et al., 1998). There is a growing body of opinion that water may ultimately limit world food production (Postel, 1999). However, this area has been discussed earlier and is therefore not continued here. Major factors that will interact with the peak oil and global warming in reducing world food production have been discussed previously (Leng, 2005) and more recently by Brown (2009) and Nellemann et al. (2009). They include:

1. Availability of water as affected by:
  - increasing demand with increasing population;
  - changing weather patterns having adverse effects on crop production through global warming and the incidence of drought and inundating rains;
  - over-use of river water;
  - reduction of water run-off or reduced synchronisation of run-off water as mountain ice decreases, particularly where it supplies major water sheds such as the Gangetic plains or for that matter the Murray Darling basin in Australia and Californian croplands watered by the Sierra Nevada;
  - aquifer water draw-down for industrial and irrigation purposes (e.g. the aquifers under the Texas high plains)
2. Availability and cost of nitrogenous fertilisers. These are produced directly from fossil fuels which will rise in cost and inevitably result in reduced fertiliser application, particularly by resource poor farmers and farmers in marginal areas who may not apply fertiliser in areas where crop failure is a risk.
3. Availability of other fertilisers whose cost will be determined by extraction and transport costs. Mineral fertilisers such as phosphates are mined and high quality ore deposits are now approaching peak supply (Dery and Anderson, 2007).
4. Availability of crop land that is increasingly being lost to agriculture through erosion, sea level rise, construction, salination and pollution.

5. Changing and increasingly unfavourable environment for plant growth in vast areas of the present food bowls of the world through climate change (Stern, 2007).

The future impacts of peak oil, global warming, resource depletion and an on-going financial crisis are difficult to predict and cannot be realistically incorporated into any quantitative model, as none exist that assesses agriculture using an holistic approach and that takes into account future constraints (foreseen and unforeseen). Possible future reductions in cropping areas and crop yields have been tentatively predicted by Nellemann et al. (2009). These data are presented in **Figure 4**. The tripling in the world grain harvest since 1950 was due to the ability of farmers, with modern seed varieties and access to fertilisers, pesticides and mechanised conservation technology, to increase the number of harvests produced per year in Asia, together with increased yields generally. Examples of this are the double or triple cropping of rice in southern China, southern India, and South-east Asia (Brown, 2009). A gradual return to draught power can be expected as fuel becomes expensive with world depletion. This will also signal the need for more labour in agriculture.

In addition it appears that by 2050 significant sea level rise will inundate some coastal lands and river deltas (UNEP, 1989), often the most productive cropping areas. The amount of land consumed by the sea can only be guessed at with the present uncertainties of global warming scenarios. However, these latter factors appear to represent greater threats to world food production than those considered by other authors and some estimates are included in **Figure 4**. If drought, such as is being experienced in a number of countries at the present time (de Carbonnel, 2009), returns in the next 20 years and the reduction in food production is superimposed on the other potential contractions of crop yields (**Figure 4**), famine (see also Cribb, 2007) is likely to effect more people in more countries and the developing countries will not be the only countries affected.

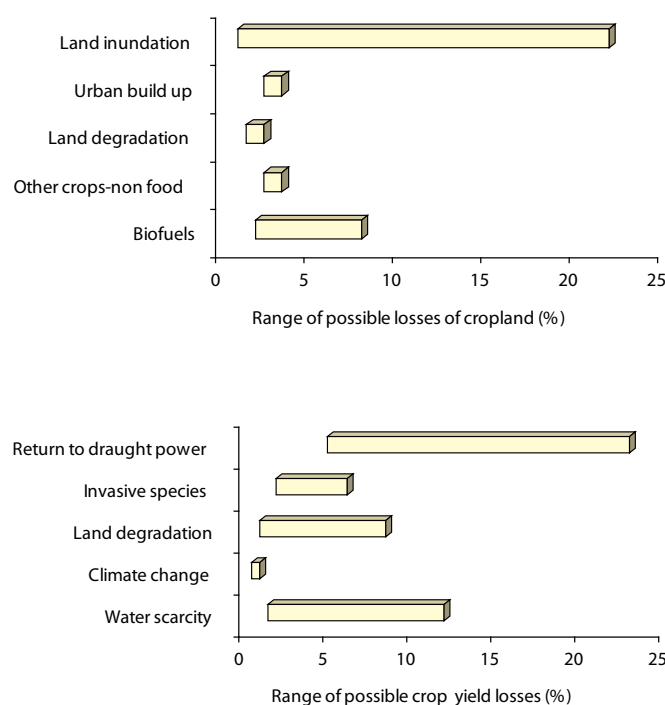
### Feed Grain Availability and Price are the Major Factors in Future Animal Production

Eighty percent of the world's food supply is derived from cereal grains consumed directly or as meat and milk from animals fed mainly grain, including fish (Pimentel et al., 2009). Worldwide food production per capita has actually declined over the last two decades and world production has lagged behind world demand in most recent years (Pimentel et al., 2009). The Livestock Revolution (Delgado et al., 1999) was predicated on surplus world grain supplies and that the relative price of grain would not rise significantly in 50 years. The quantities of feed grain needed (calculated to be over 900 million tonnes) to meet the projected meat and milk production demands in industrialised and developing countries will clearly not become available.

## WHERE TO WITH FUTURE ANIMAL PRODUCTION SYSTEMS?

### Industrialised Countries

The confluence of changes discussed above, together with financial constraints that are impacting the world, indicates that in the industrialised countries, the first and rather rapid change will be a changed pattern of use of discretionary funds by people to reduce expensive food in their diet. Expensive animal products may see people moving down the food chain, reducing their excessive consumption of meat, but retaining funds for transport. A major reduction in intensive farming of animals fed grain appears to be inevitable. This will initially be



**Figure 4. Possible ranges of cropping land losses and reduction in crop yield by 2050 (adapted from Nelleman et al., 2009).**

**Note: the values for land inundation and return to draught power are estimated by this author.**

at the expense of feedlot beef which, because of the low efficiency of conversion of grain to meat, will be more costly relative to pig and poultry production. Poultry and inland herbivorous fish farming may also be initially favoured because of the highly efficient utilisation of concentrates which is greater than that associated with pig production.

Reduction in meat consumption, particularly in the developed countries, should free up large amounts of grain and supply may keep pace with food grain production, but the chances are that grain for the biofuel industry will increase. However, the by-products of maize ethanol will make up some of the shortfall of feed for all classes of livestock (Babcock et al., 2008). As meat production is scaled back, ruminant production may be re-emphasised but with a greater dependence on grazing and the use of crop residues in a system that uses polyculture to reduce the needs for fossil fuel embedded inputs and minimise their 'long shadow' (Steinfeld et al., 2006).

In parts of Europe where rabbit meat is popular the potential to use non-concentrate feeds may see this industry expand based on cellulosic biomass. Future agriculture in industrialised countries will be forced to return to ecologically sound farming practices that produce food, meat and milk locally and with a minimum of artificial inputs using modern (yet to be designed) and energy-efficient strategies and machinery that uses the minimum of fuel.

Milk consumption will also contract with major effects on the systems that use high yielding cows that must be fed concentrates to achieve their potential milk yield. The need to produce milk from cellulose biomass will de-emphasise industrial milking units for more sophisticated systems employing cattle with lower yields but the potential to use lower quality feeds and provide meat in addition to milk. In the longer term the changes will be towards those predicted for the developing countries (see below).

The huge amounts of by-product feed from industrial alcohol production from grain (distillers dried grains and solubles referred to as DDGS) provides a source of nutrients for all animal industries. It appears that these can be included in the typical beef feedlot diets, up to 30% and some will find their way into pig and poultry diets (Babcock et al., 2008). It is estimated that annual DDGS production in the USA will surpass 40 million metric tonnes as early as 2011 (Tokgoz et al., 2007). However the high costs of drying and or transport of wet material are serious limitations to their use, except in the vicinity of distilleries. Residues of antibiotics in the by-product may also be a future constraint.

### Developing Countries

Developing countries have embraced some of the systems of intensive livestock production. These have usually been based on the models of the West but generally situated in peri-urban areas where imported grains were cheaply and easily accessed. As the price of oil and grains increase there will be a substantial reduction in these industries. These will be replaced in countries such as Vietnam by development of rabbit production based largely on forage resources from land that is not cultivatable (Preston and Van Thu, 2008). The development of rabbit feed resources from water spinach grown on waterlogged or inundated land is a very good example of likely development (see Pok Samkol et al., 2006). Farming of herbivorous fish such as carp in poly-culture is likely to be increased but high water demands may limit its growth. The high cost of fuel in developing countries is likely to enforce a return to animal power mainly from large ruminants but horses and donkey numbers will surely also increase. This scenario occurred in Cuba following the loss of cheap fuel from the former Soviet Union in 1990 (Henriksson and Lindholm, 2000). Milk and meat production will continue, mainly using agro-industrial by-products but the high cost of imported feed emphasises the need for efficient use of locally available resources to increase production per animal, increase feed conversion efficiency, and replace the decrease in white meat production.

### Ruminants Fed Forages Offer the Most Reliable Source of Protein in the Future

Forty per cent of the Earth's land surface supports the majority of the world's 3.3 billion cattle, sheep, and goats. Most of these pastoral areas cannot be economically cropped; half are moderately degraded; and five percent are severely degraded, particularly the communal lands. Forage requirements of the large livestock population in nearly all developing countries appear to exceed the sustainable yield of rangelands and other forage resources such as agro-industrial by-products (Brown, 2009). On the contrary, Savory (2009) suggests that grazing livestock have been vilified as a major cause of climate change when they are a vital part of the solution. With an holistic approach, a major reversal of desertification can be achieved, that can draw down greenhouse gas by sequestration in organic soil carbon (see Savory, 2009 for discussion).

The nutritional value of forage is the prime limitation to ruminant production in most developing countries. Poor nutrition leads to low levels of production and increases the time for animals to grow to maturity or slaughter weight, increasing the quantity of feed needed. This often suggests that feed quantity is a primary limitation to livestock production. Simply supplying nutrients that are deficient in these roughages (generally a source of fermentable nitrogen, various minerals and a source of by-pass protein) to ruminants under poor management can increase productivity many fold, and since young animals grow more quickly and reach the age of slaughter sooner,

they use considerably less feed and more animals can be fattened on the same land area. The scientific basis of feeding supplements to ruminants fed on poor quality forages has been discussed in a number of papers (Preston and Leng, 1987; Leng, 1990; Leng, 2004), and the efficient use of such feeds is a major way to increase animal protein for human consumption in the future.

### Crop Residues Must Become a Major Resource for Livestock Production in the Future

The world produces just less than two billion tonnes of cereal grains which is accompanied by about the same yield of straw. Straw has a number of uses; it is fed to ruminants, mostly without appreciation of production responses that could be achieved with treatment and supplementation; it may be burned to facilitate multiple cropping practices and it is, in places, harvested for other commercial purposes or ploughed back into the land. Much speculation has centred on the prospects for producing second generation cellulosic ethanol fuel from straw. However the logistic constraints of moving the huge amounts of straw to centralised processing plants appears to be a formidable barrier. This is fortunate because it appears to be one of the few feed resources available to increase animal protein production in the future. There is a major case to be made to retain straw organic matter as soil carbon (Lal, 2007). Nevertheless the major long-term action of straw on soil carbon is through its least digestible components i.e. lignin. This is 100% excreted by the animal and therefore if manure is returned to the land there is little reason why straw cannot be fed to livestock provided the land is not exposed to erosion by maintaining a cover of biomass.

### Crop Residues can Support Surprisingly High Levels of Ruminant Production

Crop residues particularly straw can support moderate to high levels of production in ruminants provided efficient means of treating the straw to enhance digestibility and any deficiencies of nutrients in a diet are corrected. If additional by-pass protein is then provided, levels of production and efficiency of use of biomass for growth and milk production are greatly improved (Leng, 1991 and 2004). The improvement in utilisation of straw by ruminants by adhering to these simple principles has been well demonstrated (Preston and Leng, 1987). In India, milk production (largely from cows fed straw), has escalated by the application of good nutritional principles among other applications (Banarjee, 1994). In the northern wheat belt of China cattle growth rates on straw with enhanced digestibility approached 0.9 kg/d or 75% of the rate that could be achieved with similar animals fed grain-based feedlot diets (Cungen et al., 1999). At these growth rates the numbers of animals that can be fattened on the same quantity of untreated straw increases 10–13 fold (**Table 2**). In multiple cropping areas, the wet season rice crop of straw is mostly wasted but with preservation methods that also improve digestibility, this rice straw is now being harnessed to efficiently feed ruminants (personal observation).

At the present time there is enormous need to implement known treatment and nutritional strategies to improve straw use by ruminants. All countries, must quickly begin to put in place known technologies to use crop by-products efficiently for ruminant production and at the same time relieve the pressure on over-grazed pastoral lands. Mechanisation of straw treatment/preservation is the first step for development of supplementation strategies. Cheap grain removed the stimulus for massive application of roughage fed ruminants, but with good support farmers can fill the coming vacuum of animal protein supply by directing the available resources into

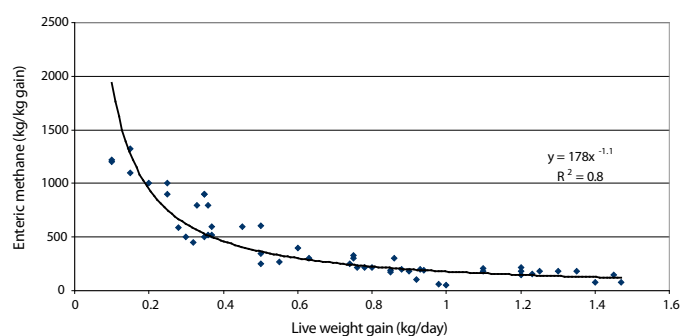
**Table 2. The potential of balanced supplementation to increase meat production from young cattle fed low quality crop residues treated to increase digestibility. The calculations are based on the data from research in Hebei, China as reported by Dolberg & Finlayson (1995).**

Cottonseed supplement fed [kg/day]	0	0.25	0.5	1.5	2.0	2.5
Lwt gain [g/day]	63	370	529	781	829	892
Straw consumed to produce 100 kg Lwt [tonnes]	6	1.1	0.92	0.56	0.48	0.46
Cottonseed cake consumed [tonnes] to produce 100 kg Lwt	0	0.1	0.1	0.14	0.22	0.24
Number of animals that can achieve an extra 100 kg of Lwt on 6 tonnes of straw	1	5+	6+	10+	12+	13+
Protein meal requirements [tonnes] to allow 100 g Lwt gain per group of animals fattened	0	0.5	0.6	1.4	2.6	3.1
Conversion of protein meal to live weight [g Lwt gain/g feed concentrate]	–	1.2:1	0.93:1	0.48:1	0.26:1	0.31:1

the efficient feeding of ruminants. The vast majority of ruminants, which number some 1 800 million large ruminant equivalents, are low producing and can be upgraded to moderate to high levels of production with modern technology. There are two billion tonnes of straw that could be converted into animal products with a feed conversion efficiency of about 10:1. This could produce 200 million tonnes of live animals annually which could support four billion people at 25 kg/year. Thus with information transfer and political will, ruminant production systems could be the major source of animal protein in the future.

### The Downside of Ruminant Production from Poor Quality Roughages

The majority of the world's ruminants are in developing countries. Globally, ruminant livestock produce about 80 million tonnes of methane annually, accounting for about 28% of global methane emissions from human-related activities (Johnson and Ward, 1996). In developing countries, the majority of ruminants are supported on forages of intermittent or poor nutritional value. In general, growth rates, milk production and reproductive rates in these systems are extremely low compared with the genetic potential of these animals (mostly about 10% and rarely exceeding 30%). Mostly (with exceptions in particular areas), cattle grow to maturity or slaughter weight between four and five years, cows produce their first calf at four to five years and then, on average, every two years. Milk production is often below 1 000 litres/lactation (Preston and Leng, 1987). Cows may be kept largely to produce draught oxen and in some specialised systems they are kept for the production of dung (which is valued as a fuel) and a number of other minor purposes (e.g. as an investment, for recreation and for religious purposes). Slow growth, low milk yield and poor reproductive performance result in poor feed conversion and a large methane output relative to product output (Leng, 1991). The benefits of high growth rates as a means of reducing methane production per unit of meat production have been confirmed from direct measures of methane output (Figure 5). Provided growth rates (in cattle) are between 0.7 and 1 kg/d, methane production will be minimised and these upper levels of growth are being achieved with cattle fed crop residues (see for example Dolberg and Finlayson, 1995). In addition at these growth rates it is possible to produce quality meat for all the major markets of the world. Thus an answer



**Figure 5. The relationship between Lwt gain of cattle and enteric methane production/ kg of gain (see Klieve and Ouwerkerk (2007) for sources).**

to world meat shortages, when industrialised production systems become too expensive, is to develop ruminant production systems from crop by-products in industrialised countries and ensure a large input into research and extension in the developing countries to achieve the levels of production at minimal cost to the environment and without increasing livestock numbers.

### Potential of Nitrate as Fermentable N Source for Ruminants Fed Poor Quality Forage

Recent studies suggest that the fermentable nitrogen requirements of ruminants on diets based on low protein cellulosic materials can be met from nitrate salts (Trinh et al., 2009), and this potentially reduces methane production to minimal levels (Leng, 2008). Trinh et al. (2009) demonstrated that with adaptation, young goats given a diet of straw, tree foliage and molasses grew faster with nitrate as the fermentable N source as compared with urea. This is a major step forward in ruminant nutrition which should create a paradigm shift in animal protein production. If methane production is lowered significantly when nitrate is fed in low protein diets consumed by ruminants, it will remove a major barrier to replacing much of the monogastric production lost because of the unavailability of feed grain in the future.

In the same way that arguments are developed to support future ruminant industries the supply of animal protein can be enhanced using rabbit fed forages (Lukefahr, 2007). Their major attributes include ability to utilise cellulosic biomass efficiently, coupled to a high fertility with ability to breed every six weeks producing multiple offspring.

## CONCLUSIONS

Ecological, bio-diverse, local agriculture combined with holistic land management that can reverse desertification and increase carbon stored in land is part of the solution to global warming and food scarcity. The world's farmers will steadily adopt these procedures as the required resources become scarce and prices move upwards. The alternative is that their land will be rendered infertile. Under these conditions even the developed countries will recognise that priority must be given to ruminants and other herbivores that transform biomass into food resources with minimum jeopardy to the environment.

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## SESSION 1

# INTERACTIONS AMONG NUTRITION, REPRODUCTION AND GENOTYPE



# Interactions Between Nutrition, Heat Stress, and Reproduction In Cattle Within Tropical/ Subtropical Environments

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## ABSTRACT

A series of approaches are presented to partially alleviate harsh environmental stresses on productivity and fertility of lactating dairy cows. The first area involves the use of various environmental facilities and management to provide heat abatement in order to enhance performance. A second complementary approach is utilisation of classical genetics that also includes introduction of heat tolerant genes into less heat tolerant breeds. A variety of reproductive technologies and their application to manage seasonal periods of infertility associated with heat stress are documented. Lastly, integration of nutritional and reproductive management strategies to improve postpartum immune competence, health and reproductive performance are presented.

**Key words:** *Heat tolerance, timed artificial insemination, seasonal infertility, selenium supplementation.*

## INTRODUCTION

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, socio-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, embryo development, and placental-foetal growth.

## Modification of the Environment

Implementation of heat abatement facilities can enhance both pregnancy rates and milk production. Heat abatement is dependent upon optimising heat exchange via convection, conduction, radiation and evaporation. Collier et al. (2006) reviewed extensively the dynamics of environmental management and subsequent impacts on the lactating dairy cow. The system to be used depends upon the local environment (e.g. arid to tropical) and includes the use of shades (reduction in solar radiation), sprinklers and fans under shade struc-

tures (enhances evaporative cooling from the skin surface), fans and sprinklers in the holding areas and/or exit lanes from the milking parlour, fans and sprinklers in free-stall facilities (e.g., cooling cows along the feed lines with sprinklers and fans) and evaporative cooling systems (i.e. cool the air that ultimately surrounds the cow). Although a shade structure partially alleviates heat exposure from solar radiation, there is no alteration in air temperature or relative humidity; consequently, additional cooling strategies are required for lactating cows in a tropical/subtropical environment. A benchmark reference point for lactating cow status is a surface skin temperature of 35°C. Below this temperature all four routes of heat exchange are possible, and the micro environment to sustain a skin temperature at or below 35°C avoids reductions in milk yield.

These types of environmental management systems need to be optimised for the region of application and integrated with the production potential of the area. For example, in many tropical areas, the period of stress most often extends for an extended period of the year and is coupled with diseases, parasites, and low nutritional inputs. Obviously, a system under this environment needs to incorporate a management plan that not only protects animals from periods of thermal stress but provides more stringent health care, well-being and nutritional inputs to reach the production potential of the animal unit in the system. Such systems involve increased capital investment to allow maximal performance of high-producing animals. A system of environmental management comprised of intermittent cooling with sprinkling and forced ventilation throughout the heat stressful period in Israel, improved conception rates (Wolfenson et al., 2000). However, fertility levels were not restored to levels typical of what is found during the winter months. Complete elimination of heat stress by intensive and frequent use of the sprinkling and ventilation cooling system was able to achieve summer conception rates comparable to that recorded in winter. Thus short periods of hyperthermia appear to have compromising effects on reproductive processes.

## Genetic Strategies to Increase Milk Production and Reproductive Efficiency

Conventional crossbreeding between *Bos taurus* and local *Bos indicus* cattle (F<sub>1</sub>) has been a strategy to improve resistance to thermal stress but always lowers milk yields in the F<sub>1</sub> generation compared with the *Bos taurus* purebred dairy cow. An alternative breeding programme to improve local cattle (e.g. *Bos indicus* cattle) is upgrading to *Bos taurus* dairy cattle (e.g. Holstein). As the percent *Bos taurus* breeding increases, the need for environmental management

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becomes greater. Nevertheless, lower percentage upgrades often out-produce local native cattle with minimal management inputs. Fluctuations in environmental conditions (climate, feeding, management, etc.) from year to year at the same tropical location can be important in determining the preferred genotype of dairy cows. McGlothlen et al. (1995) showed during a 27-year period that in 14 good years, upgrading of Butana cattle (*Bos indicus*) to over 70% Holstein or Ayrshire in Sudan resulted in greater annual milk yield/cow. However, in 13 poor years highest yields were obtained from three-breed crossbreds, 3/8 Holstein, 3/8 Ayrshire, and 2/8 Butana.

Within the Holstein breed in the USA, the Predicted Transmitting Abilities (PTAs) for milk yield and heat tolerance were determined from 172 411 sires and 10.5 million cows (Bohmanova et al., 2005). Heat tolerance PTAs of sires ranged from -0.48 to 0.38 kg milk/temperature humidity index (THI) unit above 72/d; milk yield PTAs for sires were between -8.9 and 7.9 kg/d. Based on estimated heat tolerance PTAs, the 100 most and 100 least heat tolerant sires were examined. Bulls that transmit a high tolerance to heat stress have daughters with higher pregnancy rates, a longer productive life, but lower milk yields (Bohmanova et al., 2005). Continued selection for milk yield without consideration of heat tolerance likely will result in greater susceptibility to heat stress. Conversely, selection of bulls for heat tolerance will likely result in a decrease in milk yield. This is to be expected because as average production per cow increases the metabolic heat output increases making cows more susceptible to heat stress.

Since genetic variance for heat tolerance exists in dairy cattle, there is the likelihood that specific genes controlling heat tolerance can be introduced into the gene pool of the population. One such gene is the slick hair gene (*slick hair*) originally described in Senepol cattle, subsequently identified in Carora cattle, and introduced into Holsteins by crossbreeding (Olson et al., 2003). The gene has been mapped to chromosome 20 (Mariasegaram et al., 2007). Animals with the dominant allele have a very short and sleek coat. Holstein (75%) x Carora (25%) crossbred dairy cows in Venezuela with slick hair coats had lower body temperatures in heat stress conditions than those with the wild-type hair coat (38.58°C < 39.09°C) and produced more milk (6 389 > 5 579 kg, 305-d milk yield; Olson et al., 2003). The superior thermoregulatory ability associated with the slick phenotype is apparently the result of increased convective and conductive heat loss and decreased absorption of solar radiation. During an experimentally imposed heat stress, slick haired lactating Holstein cows (i.e. out of Holstein cows sired by 75% Holstein and 25% Senepol bulls heterozygous for the slick hair gene) had lower vaginal temperatures and respiration rates than wild-type lactating cows (Dikmen et al., 2008). In either the indoor environment of a free-stall barn with sprinklers and fans or the outside environment during the heat stress period, sweating rates were greater for the slick-haired cows (indoor: 57 vs. 43 g/hm<sup>2</sup>; outdoor 82 vs. 61 g/hm<sup>2</sup>). Lactating cows with the slick-hair gene had a greater sweating rate which is deemed very important during hot stressful periods; since at an air temperature above 30°C, 85% of heat loss from the skin is through evaporation. Consequently, the slick hair gene is a candidate gene for incorporation into dairy cows that would improve regulation of body temperature and production potential in heat stress environments.

Differences between thermal adapted breeds and non-thermal adapted breeds extend to early developmental stages of the embryo (Hansen, 2007). *Bos indicus* embryos are less adversely affected by elevated temperature in culture than Holstein or Angus embryos. Furthermore, *Bos taurus* x *Bos indicus* embryos, in response to an *in vitro* heat shock, have a higher rate of blastocyst development acquired through *Bos indicus* genes that contribute to the pres-

ence of thermotolerance factors from the oocyte or imprinting of certain embryonic paternal genes. For example, embryos produced by insemination of Brahman oocytes with Angus semen were more thermotolerant than embryos produced by insemination of Holstein oocytes with Angus semen. However, there was no difference in thermotolerance between d four embryos produced by insemination of Holstein oocytes with Brahman semen versus embryos produced by insemination of Holstein oocytes with Angus semen. With the known gene sequences of the bovine genome, future identification of heat tolerance genes of *Bos indicus* breeds offers the potential of introducing these genes into less heat tolerant breeds.

Hansen (2007) reviewed various strategies to improve embryonic survival in dairy cattle during heat stress, and utilisation of timed embryo transfer was effective in studies at both Florida and Brazil. Embryos transferred into recipients at 7–8 d after either an oestrus or injection of GnRH, to induce a programmed ovulation, will by-pass the thermosensitive periods of the oocyte or early embryo. Pregnancy rates are enhanced with embryo transfer during periods of heat stress because transfers are made with embryos considered transferable that were not exposed to heat stress or were developmentally competent to be transferable. Natural embryos that were cryopreserved from superovulated donors or fresh embryos produced *in vitro* improved pregnancy rates but cryopreserved embryos produced *in vitro* did not enhance pregnancy rates.

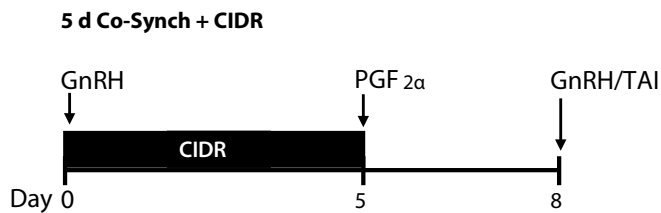
Several studies have documented the beneficial effects of adding insulin-like growth factor 1 (IGF-1) to bovine embryos produced *in vitro* on both enhancing the rate of blastocyst development and reducing the magnitude of elevated temperature effects on inhibition of blastocyst development and apoptosis (Moreira et al., 2002; Hansen, 2007). Furthermore, *in vivo* embryo transfer of *in vitro* produced embryos that were cultured with IGF-1 increased pregnancy and calving rates during heat stress but not during the non-heat stress seasons.

## Reproductive Management to Improve Seasonal Herd Fertility

An array of refined reproductive technologies is available to better manage the reproductive performance of dairy cows (Moore and Thatcher, 2006). Synchronised timed artificial inseminations (TAI) prior to the heat stress period will improve herd pregnancy rate. The strategy is to obtain as many pregnancies in the entire breeding herd before the infertile heat stress period begins. Since high temperature causes embryonic death during the first three cleavage divisions, embryo transfer of more advanced healthy embryos at d seven will bypass the early heat sensitive period to partially restore pregnancy rates, as described above. Development of vitrification procedures for storage of *in vitro* produced embryos, which develop normally following post-transfer of the embryo, will increase the impact of embryo transfer during the heat stress season. The following three reproductive management systems can contribute to seasonal breeding programmes.

### Timed Artificial Insemination in Dairy Heifers

A major limitation for using AI in dairy replacement heifers is the time and effort connected with daily oestrous detection. Systems for timed artificial insemination in dairy heifers have resulted in poor responses in pregnancy per timed artificial insemination (P/TAI). An efficient system for TAI in heifers will allow producers to synchronise a large number of fertile heifers either just before the hot season, or during the hot season to sustain calving patterns throughout the year, or to synchronise a breeding programme so that subsequent



**Figure 1. Diagram of the 5 day Co-Synch + CIDR protocol for the timed insemination of dairy heifers. Heifers received an intra-vaginal CIDR® insert and injection of GnRH on day 0; 5 days later CIDR insert was removed and PGF<sub>2α</sub> administered; on day 8, GnRH was administered and heifers received timed AI.**

parturitions are occurring at a time when seasonal availability of nutrients is optimal.

Two replicates of heifers ( $n = 203$  and  $n = 214$  for replicates 1 and 2, respectively) from a subtropical environment in North Central Florida were synchronised for a first TAI with the 5-d Co-Synch + CIDR (controlled internal drug release insert containing progesterone) protocol (**Figure 1**) with one injection of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>); (Rabaglino et al., 2010). Non-pregnant heifers at 30 d after first TAI were resynchronised for a second TAI following the same protocol initiated on the same day when the diagnosis of non pregnancy was performed. A single sire was used for the first TAI in both replicates and the second TAI of the first replicate, whereas three additional sires were used for the second TAI of the second replicate. Three and two technicians performed the AI for the first or second services, respectively.

Pregnancy per TAI for the first TAI was 60.3% at 32 d (251/416) and 58.2% (242/416) at 60 d. Pregnancy loss between 32–60 d of gestation was 3.6% (9/251). There was no significant effect on P/TAI of replicate number, technicians, or the interaction replicate number by technician.

For the second service, P/TAI was 52.1% (86/165) at 32 d and 47.3% (78/165) at 60 d. Pregnancy loss was 4.8% (8/165). Pregnancy per TAI to the second TAI at 60 d was 58.4% (45/77) for the first replicate and 37.9% (33/87) for the second replicate. Difference in P/TAI for second service was significant ( $P = 0.01$ ; OR: 0.30; 95% CI: 0.12–0.76) between replicates. The effect of service sires tended to affect ( $P = 0.10$ ) P/TAI to the second TAI, and they were 11.1% (1/9), 50.0% (14/28), and 36% (18/50) at 60 d after AI for sires 1, 2, and 3 of the second replicate, respectively. There were no differences in P/TAI between technicians and the interaction of technician by replicate number was not significant.

From a reproductive management perspective, the overall percent of heifers pregnant after two synchronised inseminations encompassing a 64 d period was 77%. The programme requires no heat detection and involves two eight-day periods with heifers being handled three times in each period. Our practical experience is that pregnancy rates are acceptable when done in the summer months probably because the heifers are not lactating. The lower P/TAI to the second TAI compared with the first TAI tended to be due to the sires. Herd- or animal-level factors affect fertility in dairy heifers (Donovan et al., 2003), and most herd level variation is caused by variation among inseminators and service sires (Ron et al., 1984). The five-d Co-Synch + CIDR protocol (**Figure 1**) is an efficient reproductive management programme to achieve acceptable P/TAI in dairy heifers. Cost per heifer to implement the programme is \$US 16 for purchase of GnRH (2), CIDR and PGF<sub>2α</sub>.

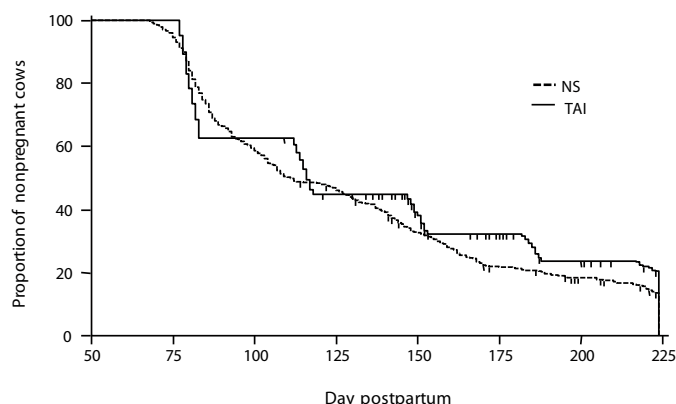
### Timed Artificial Insemination versus Natural Service with Bulls

Both expression and detection of oestrus are often suboptimal in dairy herds due to high milk production and seasonal periods of heat stress, which impair reproductive efficiency. Despite considerable advantages for AI, a significant number of dairy producers use natural service (NS) for their breeding programme or in part of their breeding following several initial artificial inseminations. A common perception among dairy producers is that NS is comparable to AI because human errors in oestrous detection are avoided with bulls. However, a comparison of reproductive performance between NS and TAI, two breeding systems where efficiency of oestrous detection is not a factor, is lacking. A study was undertaken to compare reproductive performance of lactating dairy cows bred by natural service (NS) or timed AI (TAI; Lima et al., 2009).

The study was conducted between November 2006 and March 2008 in a commercial dairy farm of 2200 Holstein cows located in north central Florida (subtropical). Cows were housed in free-stall barns with fans and sprinklers for forced evaporative cooling during the warm season. Cows ( $n = 1055$ ) were blocked by parity and enrolled to receive either NS or TAI. Cows in both groups were pre-synchronised with two injections of PGF<sub>2α</sub> given at 42 and 56 d postpartum. At 14 d after the last PGF<sub>2α</sub> injection, cows in the TAI group were enrolled in an Ovsynch protocol (d 0 GnRH, 7d later PGF<sub>2α</sub>, 56 h after PGF<sub>2α</sub> injection a second dose of GnRH was given, and 16 h after the second GnRH cows were TAI). Cows in the TAI group were resynchronised with an intravaginal insert containing progesterone inserted 18 d after TAI and removed seven d later, when GnRH was given. Cows were examined by ultrasonography on d 32 after TAI; non-pregnant cows received PGF<sub>2α</sub> and GnRH 56 h later followed by TAI 16 h after the GnRH injection. Non pregnant cows in TAI group were re-inseminated up to five times using the same scheme.

Cows in the NS group were exposed to bulls 14 d after the second PGF<sub>2α</sub> injection, and ultrasonography was performed 42 d after exposure to bulls to determine pregnancy status. Nonpregnant cows in the NS group were re-examined by transrectal palpation combined with ultrasound every 28 d until diagnosed pregnant or 223 d postpartum, whichever occurred first. Cows diagnosed pregnant in TAI or NS were re-confirmed 28 d later to determine pregnancy loss. All bulls underwent a breeding soundness evaluation and were rested for 14 d after 14 d of cow exposure. The bull:cow ratio in the NS group was one bull per 20 non-pregnant cows

The proportion of pregnant cows in the first 21 d of breeding did not differ between groups (NS, 34.2%, 175/512; TAI, 37.4, 203/543). The overall 21-d cycle pregnancy rate, which included a total of eight and five service opportunities for NS and TAI, respectively, was not different between groups (25.7 and 25.0% for NS and TAI, respectively). However, the daily rate of pregnancy differed ( $P = 0.05$ ) between groups and was 15% greater (Adjusted Hazard Ratio = 1.15; 95% Confidence Interval = 1.00 to 1.31) for NS than TAI (**Figure 2**), which resulted in fewer median days open (111 vs. 116 d). The survival curves did not differ until 150 d postpartum, when they began to separate. Nevertheless, at 223 d postpartum, which was the end point of the study, the proportion of pregnant was greater ( $P = 0.001$ ) for NS than for TAI (NS = 84.8% and TAI = 76.4%). Cow d at risk for pregnancy were not different between NS and TAI, being 30978 and 29424 d, respectively. The greater proportion of pregnant cows observed in the NS group at the end of the study is attributed to differences in breeding dynamics between groups (**Figure 2**). In the NS group, bulls had the potential for daily detection of oestrus and breeding of non-pregnant cows. On the other hand, due to the TAI re-synchronisation scheme, non-pregnant cows in this group



**Figure 2. Survival curves for proportion of non pregnant cows by day post partum for cows bred by natural service (NS) or timed artificial insemination (TAI) in the first 223 days postpartum**

required 35 d to be re-inseminated and thus the number of d to become pregnant increased. However, within this scenario, up to 223 d postpartum cows in the TAI group had only five opportunities to be bred compared with a potential eight times for cows in the NS group. A greater number of non-pregnant cows in the NS group had earlier opportunities to be bred than TAI cows under the same 21-d cycle pregnancy rate; consequently the final outcome for median time to pregnancy favoured the NS group. Primiparous cows had a greater proportion of pregnant cows and daily pregnancy rate than multiparous cows at 223 d postpartum. The THI was the criterion used to determine effect of season (warm or cold) on reproductive performance. The maximum daily THI was categorised as cool, when  $THI < 72$  or warm when  $THI \geq 72$ . The THI on the day of the first TAI or the first day of exposure to bulls was used in the statistical analysis. Cows receiving their first breeding during the cool season had increased ( $P < 0.01$ ) pregnancy in the first 21 d of breeding (41.2% vs. 27.7%), 21-d cycle pregnancy rate (27.5% vs. 22.5%), and daily pregnancy rate (AHR = 1.22; 95% CI = 1.06 to 1.41).

In spite of applying a heat abatement system during the warm season, pregnancy responses were decreased. However, it is important to indicate that no interaction between treatment (i.e., NS and TAI) and heat stress was observed for the proportion of cows pregnant in the first 21 d of breeding or pregnancy rates.

In conclusion, NS and TAI are two breeding systems that can be used strategically to minimise problems related to detection of oestrus, but the extended inter-insemination interval in TAI reduces

daily pregnancy rate because these cows have fewer opportunities for breeding. Of course, use of TAI with semen from bulls with high daughter pregnancy rates and transmitting ability for milk yield is an advantage compared with unproven NS bulls.

### Timed Artificial Insemination of Grazing Lactating Dairy Cows

A TAI programme is a reproductive management platform for grazing lactating dairy cows to establish a seasonal breeding period such that cows can be inseminated prior to the heat stress season with subsequent parturitions occurring at a time of abundant pasture for grazing. Sufficient advancements have been made in controlled breeding to obtain optimal pregnancy rates and such a programme was conducted in two Florida commercial dairies (Ribeiro et al., 2009). For the period of March and April, average and range THI were: 65 and 60 to 70, respectively.

Objectives were to evaluate reproductive performance of grazing dairy cows subjected to different presynchronisation and resynchronisation protocols. Lactating cows ( $n = 1264$ ) in two dairies were blocked by parity, breed (Holstein, H = 458; Jersey, J = 185; and Holstein/Jersey Cross, C = 621), and d postpartum, and then randomly assigned to one of four treatments in a 2 x 2 factorial experiment: 1) Presynch: two injections of  $PGF_{2\alpha}$  given 14 d apart and starting the TAI protocol 11 d later; 2) G6G: injection of  $PGF_{2\alpha}$  followed three d later by gonadotrophin releasing hormone (GnRH) and starting the TAI protocol 6 d later. The TAI protocol consisted of GnRH on day 0,  $PGF_{2\alpha}$  on d 5 and d 6, and GnRH+TAI on d 8. On d 12 after the TAI, half of the cows in each presynchronisation received one of the two resynchronisations: 1) resynchronisation control (RCON) cows were observed daily for oestrus and inseminated starting on d 19 after TAI; 2) resynchronisation CIDR (RCIDR) cows received a CIDR from d 12–19 after the TAI and were observed for oestrus and inseminated between d 19 and 35. At d 35, cows were exposed to bulls for a 65-d period so that the entire breeding period for the programme was 100 d. Pregnancy diagnoses by ultrasound were done at 30 and 60 d after first TAI, 30 and 65 d after the resynchronised AI, and at 30 d intervals following introduction of bulls.

Pregnancies per AI (P/AI) for the treatment groups are presented in **Table 1**. The P/AI following the first TAI (P/TAI; 44.3%), to resynchronised detected oestruses (P/RAI; 16.8%), and to the bulls (P to Bulls; 21.4%) did not differ between the four treatment groups. The overall pregnancy rate for the 100-d breeding season was 82.5%. The only statistical difference detected between treatments was that pregnancy loss following first service TAI between d 30 and 64 of pregnancy was lower for the Presynch protocol (8.1%, 25/310) than the G6G protocol (12.9%, 40/309;  $P < 0.05$ ). Holstein lactating dairy cows had lower P/TAI at each of the three breeding periods (i.e.

**Table 1. Effect of presynchronisation (PRE) and resynchronisation (RES) treatments on pregnancy (P) rates of grazing lactating dairy cows in a 100-d breeding season.**

PRE	RES	N	P/TAI		P/RAI		P to Bulls		P at 100 days	
			n	%	n	%	n	%	n	%
Presy <sup>1</sup>	RCont <sup>2</sup>	318	142	44.7	62	19.5	61	19.2	265	83.3
Presy <sup>1</sup>	RCIDR <sup>3</sup>	314	143	45.5	48	15.3	61	19.4	252	80.3
G6G	RCont <sup>2</sup>	316	132	41.8	56	17.7	82	25.9	270	85.4
G6G	RCIDR <sup>3</sup>	309	140	45.3	45	14.6	65	21.0	250	80.9
Total		1257	557	44.3	211	16.8	269	21.4	1037	82.5

<sup>1</sup>Presy = presynchronisation; <sup>2</sup>RCont = resynchronisation control; <sup>3</sup>RCIDR = resynchronisation CIDR; See text for specific protocols

Timed AI, Resynchronised AI, and Bull) such that overall pregnancy at the end of 100 d was lower for Holstein (71.4%, 324/455) than Jersey (89.7%, 165/184) and Crossbred (88.5%, 547/618). Mean milk production for Holstein, Jersey and Crossbred cows milked twice daily was  $28 \pm 0.7$ ,  $18.7 \pm 1.5$  and  $22.2 \pm 0.8$  kg/d, respectively.

### Nutritional Effects on Reproductive Efficiency

Clearly reproductive performance of today's high producing dairy cow is sub-optimal, and reproductive management systems have been developed for both intensive and extensive management systems as demonstrated above. Coupled with increases in milk production are major advancements in housing, nutritional management and health programmes that need to be collated with reproductive management programmes to sustain or enhance reproductive performance. This is considered essential because the next generation improvements in herd pregnancy rates are to increase reproductive competence of the lactating dairy cow during the periparturient and postpartum periods prior to the time of designated breeding. Nutritional strategies have been developed to improve health and reproductive performance with the feeding of specific nutraceuticals. A nutraceutical is defined as a product isolated or purified from feeds that is demonstrated to have a physiological benefit or provide protection against chronic disease. There are several nutraceuticals such as fatty acids, certain minerals, and vitamins that appear to have beneficial effects on production (i.e. reproduction and milk production) and health responses of lactating dairy cows.

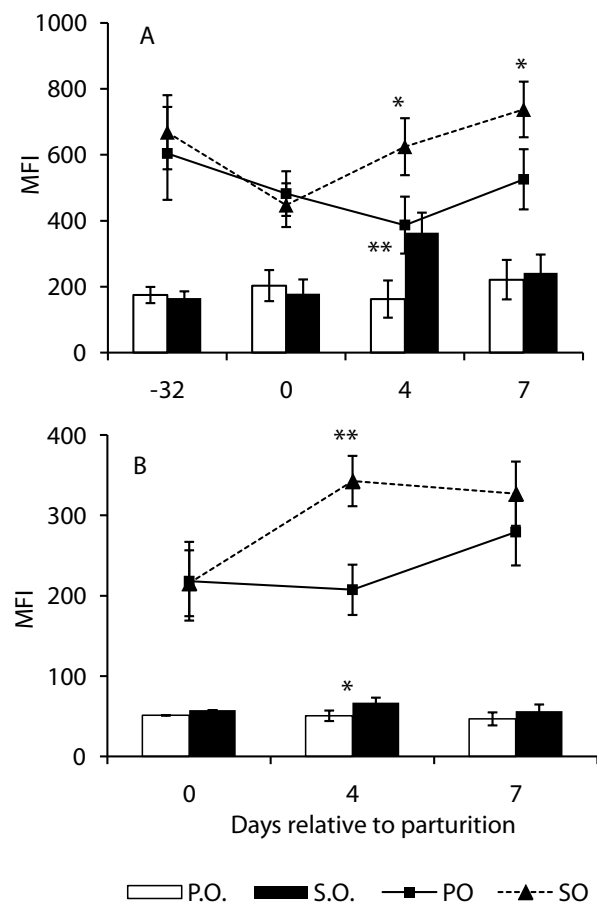
### Feeding of Supplemental Fatty Acids to Improve Reproductive Performance

The postpartum uterus undergoes dynamic changes associated with uterine regression, reabsorption of uterine tissues, and providing a localised immune response via the action of neutrophils to manage intrauterine populations of bacteria. Santos et al. (2008) reviewed the dynamics of dietary long chain fatty acids of the diet as factors influencing reproduction in cattle. Several of the eicosanoids exert pro-inflammatory actions (e.g.  $\text{PGE}_2$ ), and  $\text{PGF}_{2a}$  appears to be involved as well with neutrophil function as related to phagocytosis of bacteria. Feeding fatty acids (e.g. linoleic acid) during the periparturient period could act as a precursor for the biosynthesis of prostaglandins of the 2 series that may benefit postpartum health of the cow. Later on during the postpartum period (i.e. beginning at 30 d postpartum), it may be reasonable to feed fatty acids (e.g. eicosapentaenoic acid, EPA) that lead to the biosynthesis of fatty acids that are anti-inflammatory in nature (e.g. suppress neutrophil function and cytokine secretion). This would reduce possible residual inflammatory responses in the uterus associated with carryover effects of subclinical endometritis or as reported in repeat breeder cows, further enhance the immune-suppressive environmental state of pregnancy, and also reduce the potential luteolytic peaks at the time that the conceptus is suppressing  $\text{PGF}_{2a}$  secretion in order to maintain the corpus luteum for pregnancy maintenance.

In a recent Florida study, Silvestre et al. (2008a and b) randomly allocated cows ( $n = 1\ 582$ ) into two experimental transition diets beginning at approximately 30 d before the expected date of parturition and continued until approximately 30 d postpartum. After 30 d all cows within each transition diet were allocated randomly into the experimental breeding diets that were fed until approximately 160 d postpartum. Experimental transition and breeding diets differed only in the source of supplemental fatty acids. Transition diets consisted of calcium salts (CS) of palm oil (PO, 47% C16:0; EnerGII) or CS of safflower oil (SO, 64% C18:2n-6; Prequel 21) and breeding

diets consisted of CS of PO (EnerGII) or CS of fish oil (FO 11% of C20:5n-3 + C22:6n-3, StrataG). All CS of FAs were manufactured by Virtus Nutrition (Corcoran, CA, USA) and supplemented at 1.5% of dry matter. Diets were formulated to meet or exceed NRC (2001) nutrient requirements for net energy of lactation ( $\text{NE}_L$ ), crude protein (CP), fibre, mineral and vitamins and fed to obtain intakes of 200 and 400 g/d of CS of FAs, for pre- and postpartum cows, respectively. Diets were fed as a total mixed ration twice daily targeting 5% refusals. The experimental design encompassed four treatment groups to test the effects of feeding SO during the transition period and FO during the breeding period. Feeding PO as a saturated fatty acid control in both periods would not account for possible carry-over effects of the transition diets into the breeding period.

Blood samples were collected from sub-samples of cows at enrollment ( $n = 18$ ) and in the postpartum period ( $n = 47$ ) at parturition (i.e.  $2.8 \pm 1.8$  h after delivery), 4 d and 7 d postpartum for analyses of neutrophil activity and abundance of adhesion molecules using flow cytometry (Jain et al., 1991; Smits et al., 1997). Number of bacteria (*E. coli* and *S. aureus*) phagocytised/neutrophil was greater ( $P < 0.01$ ) for cows in the SO at 4 d postpartum associated with a greater ( $P < 0.05$ ) intensity of  $\text{H}_2\text{O}_2$  produced/neutrophil at 4 d and 7 d postpartum in cows fed SO fat supplement (Figure 3). Neutrophil abundance of



**Figure 3.** Least squares means ( $\pm$ S.E.) of neutrophil mean fluorescence intensity (MFI) for number of bacteria phagocytised per neutrophil (bars), and for intensity of  $\text{H}_2\text{O}_2$  produced per neutrophil (lines) in whole blood stimulated with *E. coli* (A) or *S. aureus* (B). Cows were supplemented with palm oil (PO;  $n=23$ ) or safflower oil (SO;  $n=24$ ) during the transition period. \* $P < 0.05$  and \*\* $P < 0.01$ .

**Table 2. First and second services pregnancies per AI at 32 d and 60 d after insemination and pregnancy loss [% (n=)]for experimental diets.**

	Diets				Diet contrasts <sup>1</sup> (P value)		
	PO-PO	SO-PO	PO-FO	SO-FO	C1	C2	C3
<b>1st service</b>							
D32	38.7 (107/276)	35.8 (96/268)	39.1 (103/263)	35.8 (89/248)	NS	NS	NS
D60	33.7 (92/273)	29.7 (79/266)	37.0 (97/262)	32.8 (81/247)	NS	NS	NS
Loss	11.5 (12/104)	15.9 (15/94)	4.9 (5/102)	7.9 (7/88)	NS	< 0.05	NS
<b>2nd service</b>							
D32	27.7 (43/155)	26.7 (41/154)	30.3 (44/154)	43.3 (65/150)	NS	< 0.05	= 0.10
D 60	21.0 (38/152)	22.5 (34/151)	27.3 (39/143)	41.3 (62/150)	NS	< 0.01	< 0.05
Loss	5.0 (2/40)	10.0 (4/38)	7.1 (3/42)	4.6 (3/65)	NS	NS	NS

<sup>1</sup>Contrast are C1 (transition diets [PO-PO + PO-FO vs. SO-PO + SO-FO]), C2 (breeding diets [PO-PO + SO-PO vs. PO-FO + SO-FO]) and C3 (interaction of diets [PO-PO + SO-FO vs. PO-FO + SO-PO]). PO (palm oil; EnerGII); SO (safflower oil; Prequel 21); FO (fish oil; StrataG). All fat supplements were manufactured as calcium salts by Virtus Nutrition, LLC (Corcoran, CA, USA) and supplemented at 1.5 percent of dry matter. NS = non-significant.

L-selectin (arbitrary units) was increased ( $P < 0.01$ ) after parturition (752.75) and was greater ( $P < 0.05$ ) at 4 d and 7 d postpartum for SO (1 205.3 and 1 134.2; S.E. = 96.2) compared with PO (862.5 and 892.8; S.E. = 95.8) supplemented cows, respectively. No effects of diet or day were observed in the abundance of  $\beta_2$ -integrin.

Neutrophil cytokine production and FAs profiles were measured in sub-samples of cows sampled at enrollment and at 35 d postpartum ( $n = 26$ ) which was the last day of PO and SO feeding for the sub-sample of cows. Neutrophils were isolated from whole blood and incubated in media, with or without lipopolysaccharide (LPS), at 37°C in a 5% CO<sub>2</sub> incubator for 18 h (Sohn et al., 2007). Mean concentrations of TNF- $\alpha$  and IL-1 $\beta$  in supernatants of isolated neutrophils stimulated with LPS were greater ( $P < 0.01$ ) for cows supplemented with SO (106.97 pg/ml and 1.45 ng/ml) compared with PO (63.25 pg/ml and 0.67 ng/ml), respectively. Concurrently, neutrophil LN content of the FAs, although numerically greater, was not significantly greater ( $P = 0.19$ ) in cows fed SO (23.23%) than with PO (20.61%). The predominant FAs in the neutrophils were linoleic, stearic, palmitic, oleic and erucic acids which comprised approximately 72% of all FAs. The ratio n-6 (C18:2 + C22:4): n-3 (C18:3 + C20:5 + C22:6) of FAs tended ( $P = 0.07$ ) to be greater for cows fed SO (9.16 $\pm$ 0.73) compared with PO (7.16 $\pm$ 0.73) supplements.

Blood samples were collected from PO ( $n = 15$ ) and SO ( $n = 17$ ) cows daily from parturition to 10 d postpartum and continued thrice weekly until 35 d postpartum for analyses of plasma concentrations of 13,14-dihydro-15-keto-PGF<sub>2 $\alpha$</sub>  (PGFM) and acute phase proteins (i.e. haptoglobin [HP] and fibrinogen [FB]), respectively. Plasma concentrations of PGFM were not affected by transition diets except for d 4 and d 7 postpartum, in which a greater ( $P < 0.05$ ) concentration was detected for the SO (2 809 $\pm$ 310 pg/mL and 2 667 $\pm$ 314 pg/mL) compared with PO (2 081 $\pm$ 325 pg/mL and 1 443 $\pm$ 325 pg/mL) diets, respectively. Plasma concentrations of HP and FB were higher ( $P < 0.05$ ) for SO (0.034 OD and 248.8 mg/dL,  $n = 17$ ) compared with PO (0.02 OD and 205.3 mg/dL,  $n = 15$ ).

Although feeding SO improved aspects of innate immunity (i.e. neutrophil function and acute phase response), SO ( $n = 562$ ) and PO ( $n = 554$ ) cows had similar frequency distributions of mucopurulent (10% and 14.4%) and purulent (30.4% and 28%) cervical discharges evaluated once between 8–10 d postpartum.

Collectively, feeding a linoleic fatty acid enriched diet, beginning in the close up ration pre-partum, changed fatty acid profiles of

tissues placing the cow in a 'pro-inflammatory state'. Such a state involves a lower threshold for initiation of an inflammatory response and increased sensitivity of cells upon stimuli. Inflammation is the first step for initiation of an immune response.

Cows at 43 d postpartum began a Presynch protocol with two injections of PGF<sub>2 $\alpha$</sub>  injected 14 d apart. The Ovsynch protocol was initiated 14 d after the second injection of PGF<sub>2 $\alpha$</sub> . of the Presynch with a GnRH injection (100  $\mu$ g) followed 7 d later by an injection of PGF<sub>2 $\alpha$</sub>  and a final injection of GnRH 56 h later. Timed AI for first service was performed 16 h after the second GnRH injection of the Ovsynch protocol. All cows received a controlled internal drug-releasing device (CIDR) containing 1.38 g of progesterone at 18 d after the first TAI followed 7 d later by removal of the CIDR device and an 100  $\mu$ g injection of GnRH. At 32 d after first TAI, cows were examined for pregnancy by per-rectum ultrasonography to identify presence of an embryo and an embryonic heart beat. Non-pregnant cows were injected with 25 mg of PGF<sub>2 $\alpha$</sub>  and then injected with 100  $\mu$ g of GnRH 56 h later. A TAI was performed 16 h after the last GnRH for the second service. Cows were examined for pregnancy by per-rectum ultrasonography at 32 d after second service. All cows diagnosed pregnant after first and second services were re-examined by per-rectum ultrasonography at 60 d after insemination to determine pregnancy losses.

Pregnancy per AI, pregnancy losses, and cumulative proportion of pregnant cows after two services were analysed using predetermined statistical contrasts to test the effects of the transition diets (PO-PO + PO-FO vs. SO-PO + SO-FO), breeding diets (PO-PO + SO-PO vs. PO-FO + SO-FO) and the interaction of transition and breeding diets (PO-PO + SO-FO vs. PO-FO + SO-PO) accordingly with the experimental feeding design described above.

Transition, breeding and interaction of diets did not affect pregnancy per AI at 32 d and 60 d after TAI for first service (Table 2). However, pregnancy loss from d 32 – d 60 after the first TAI was less ( $P < 0.05$ ) in FO compared with PO supplemented cows during the breeding period. For second service, breeding diet (i.e. PO or FO) altered ( $P < 0.05$ ) the 32 d estimates of pregnancy per AI and a tendency ( $P < 0.10$ ) for an interaction was detected between transition and breeding diets. The increase in d 32 pregnancy/AI caused by FO was greater in cows fed the SO transition diet, whereas there was no increase in pregnancy/AI in cows fed the PO breeding diet regardless of transition diet. Both breeding diet and a transition by breeding



diet interaction ( $P < 0.05$ ) were detected for the 60 d pregnancy/AI response in which FO stimulated pregnancy rate/AI but the response to FO was greater in cows fed the SO transition diet (**Table 2**).

Neutrophil cytokine production and profiles of FAs were measured in a sub-sample of cows ( $n = 28$ ) at 85 d postpartum at a time when cows were fed the breeding diets (i.e. PO or FO) for approximately 55 d. Mean concentration of TNF- $\alpha$ , but not IL-1 $\beta$ , in supernatants of isolated neutrophils was less ( $P < 0.01$ ) for cows supplemented with FO (42.55 pg/mL and 0.6 ng/mL) compared with PO (82.68 pg/mL and 0.78 ng/mL) in response to LPS, respectively. Concurrently, The neutrophil content of EPA (1.5% and 0.30%), DPA (C22:5n-3; 3.48% and 2.33%) and DHA (1.65% and 0.11%) FAs were greater ( $P < 0.01$ ) in cows fed FO compared with PO diets, respectively. Consequently, the ratio of n-6 (C18:2 + C22:4): n-3 (C18:3 + C20:5 + C22:6) FAs was less ( $P < 0.01$ ) in cows fed FO (3.75) compared with PO (8.48). These responses indicate that at the time of conducting inseminations, neutrophils available to reproductive tissues were under a greater anti-inflammatory response, which may complement the immune-suppressive effects of the conceptus in early pregnancy.

### Feeding of Organic Selenium to Improve Reproductive Performance

During the immediate postpartum period, the cow's immune system is challenged severely (Goff, 2006), and the innate and humoral defence systems are reduced. Selenium (Se) has long been associated with immunity. Cattle supplemented with Se-yeast had an 18% increase of Se in plasma in comparison to supplementation with inorganic sodium selenite (Weiss, 2003). The state of Florida, USA, as well as many areas of the world, is Se deficient in a subtropical environment, and lactating dairy cows are exposed to a seasonal period of heat stress that impacts reproductive performance and health. Furthermore, heat stress increases oxidative free radicals, and Se in the form of selenoproteins can function as an antioxidant that may benefit reproductive function.

Yeast converts Se to selenoamino acids, particularly selenomethionine, which are not destroyed by ruminal microbes and can be incorporated by the cow into a variety of selenoproteins. An experiment was conducted that fed organic selenium (Se; Se yeast [SY; Sel-Plex®, Alltech]) during the prepartum to postpartum periods (Silvestre et al., 2006a and b; 2007). Objectives were to evaluate effects of organic Se on P/TAI at the first and second postpartum services, uterine health, immune status and milk yield during the summer heat stress period. Cows were assigned ( $23 \pm 8$  d prepartum) to diets of organic Se (Se-yeast [SY; Sel-Plex®, Alltech;  $n = 289$ ]) or inorganic sodium Se [SS;  $n = 285$ ]) fed at 0.3 ppm (DM basis) for  $> 81$  d postpartum. Rectal temperature was recorded each morning for 10 d postpartum. Vaginoscopies were performed at 5 d and 10 d postpartum. Cows were programmed for TAI to first and second service using presynchronisation-Ovsynch programmes followed by a resynchronisation comprised of an Ovsynch beginning at 20d - 23 d after first service. An ultrasound pregnancy diagnosis was conducted at 27d - 30 d after first TAI. Strategic blood sampling determined anovulatory status at Ovsynch and ovulatory response after TAI to first service. The PR at second service was determined by rectal palpation at  $\sim 42$  d postpartum. Blood was sampled for Se ( $n = 20$  cows/diet) at -25, 0, 7, 14, 21, and 37d postpartum.

Plasma Se increased in SY compared to SS-fed cows ( $0.087 > 0.069 \pm 0.004$   $\mu\text{g/mL}$ ;  $P < 0.01$ ). Milk yield (35.6 kg/d for 81 d), and frequencies of retained foetal membrane (9.7%), mastitis (14.4%), anovulation (17.7%), and synchronised ovulation after TAI (82.5%) were not affected by diets or reproductive programme. Diet failed to alter first service PR at  $\sim 30$  d post AI (SY, 24.9% [62/249] and SS,

23.6% [62/262]) or pregnancy losses between  $\sim 30$  d and  $\sim 55$  d post AI (SY, 39.3% and SS, 37.1%). These low pregnancy rates and high embryonic losses are typical of cows managed during the summer heat stress period of Florida. Diet did indeed alter second service PR (SY, 17% [34/199] vs SS, 11.3% [24/211];  $P < 0.03$ ). The benefit of SY on second service pregnancy rate is very interesting. We hypothesise that cows of the SY group were better able to reestablish an embryo-trophic environment at second service following either early or late embryonic losses. For example, cows presented for second service may not have been pregnant to the first service by 30 d at the ultrasound diagnosis or were pregnant and underwent embryonic loss and required a second service. Indeed P/TAI to the second service for cows that had lost an embryo was 22.7% (5/22) for the SY versus 4.2% (1/24) in the control or SS group ( $P < 0.09$ ); P/TAI to the second service for cows that were not diagnosed pregnant at first service and were re-inseminated did not differ (16.3% [29/177] for SY versus 12.3% [23/187] for SS).

Diet altered frequency of multiparous cows detected with  $> 1$  event of fever (rectal temperature  $> 39.5$  °C; SY, 13.3% [25/188]  $<$  SS, 25.5% [46/181];  $P < 0.05$ ) but the SY effect was not observed in primiparous cows which had a much higher frequency of fever (40.5%). Vaginoscopy discharge scores, measured at 5 d and 10 d postpartum, were affected by SY and SS diets ( $P < 0.05$ ), respectively: clear (47.1% vs 35.0%), mucopurulent (43.4% vs 47.8%) and purulent (9.3% vs 17.1%). The frequency of cows with a purulent-fetid discharge was reduced and proportion of cows with a clean discharge was increased. This is additional support that feeding the organic selenium (i.e. SY) improved the uterine environment.

Innate immunity (i.e. neutrophil function) was determined by phagocytic and oxidative burst capacity of neutrophils in whole blood using a dual colour flow cytometric method. Samples were collected from a sub-sample of 36 cows at  $-26$  d postpartum and 40 cows at zero, 7, 14, 21 and 37 d postpartum and analysed for neutrophil function. Adaptive immunity (ability to induce an antibody response) was monitored with anti-IgG to ovalbumin (ovalb) following vaccination with ovalb antigen (1 mg [i.m.]) dissolved in an *E. coli* J5 endotoxaemia preventive vaccine at  $-60$  and  $-22 \pm 6$  d postpartum (day of initiating SY [ $n = 38$ ] and SS [ $n = 47$ ] diets) and again at parturition (d zero) with ovalb dissolved in PBS with Quil-A adjuvant. Serum samples were collected on d of immunization and at 21d and 42d thereafter.

Percentage of gated neutrophils that phagocytised *E. coli* and underwent oxidative burst did not differ between dietary groups at  $-26$  d postpartum ( $44.6 \pm 4.6\%$ ). For subsequent samples, a diet  $\times$  parity  $\times$  day interaction was detected ( $P < 0.05$ ); namely, SY improved neutrophil function at parturition in multiparous cows ( $42 \pm 6.14\%$  vs  $24.3 \pm 7.2\%$ ) and at seven, 14 and 37 d postpartum in primiparous cows (53.9 vs 30.7%, 58.6 vs 41.9%, and 53.4% vs 34.8%, respectively; pooled S.E. = 6.8%). Neutrophil function was suppressed in primiparous cows at the time of parturition and not restored until seven - 14 d postpartum. In contrast, the multiparous cows did not have a restoration in neutrophil function until 14 d - 21 d postpartum. Organic Se improved phagocytosis and killing activity of neutrophils in both multiparous and primiparous cows. However, the primiparous cows seemed to be more responsive in that SY stimulated neutrophil function throughout the first 21 d postpartum whereas, SY stimulation in multiparous cows was evident on only the d of parturition.

Anti-IgG to ovalb did not differ between dietary groups at  $-60$  and  $-22$  d postpartum ( $0.18 \pm 0.01$  and  $0.97 \pm 0.04$  OD). However, a diet  $\times$  parity interaction was detected; IgG concentration did not differ between diet groups in primiparous cows across all d of sample collection ( $1.37 \pm 0.08$ ,  $1.43 \pm 0.07$ ;  $P > 0.10$ ), but it was higher

in SY cows at 21 d and 42 d postpartum ( $1.91 \pm 0.1 > 1.24 \pm 0.07$ ,  $1.44 \pm 0.7 > 0.99 \pm 0.07$ ;  $P < 0.01$ ). Thus our measurement of adaptive immunity was improved in multiparous dairy cows in response to SY but not in primiparous cows. Our findings indicated that feeding Se as organic Se (Se-yeast, Sel-Plex®), beginning at 26 d prepartum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, and increased second service PR during summer in an environment that is Se deficient.

The ingredient  $\beta$ -carotene also has been fed twice daily as an antioxidant during the summer period of heat stress (Arechiga et al., 1998a). The  $\beta$ -carotene (Hoffmann-LaRoche Inc., Nutley, NJ) was added to the TMR as part of the vitamin premix at the morning feeding. The  $\beta$ -carotene premix was formulated to contain an additional 880 mg  $\beta$ -carotene/kg and was fed at a rate of approximately 0.45 kg/cow/d (400 mg  $\beta$ -carotene/d/cow). The premix was assayed and found to contain a mean of 1 113 mg  $\beta$ -carotene/kg (i.e. intake of approximately 500 mg  $\beta$ -carotene/d/cow). In cows that received supplemental  $\beta$ -carotene, beginning at 10–15 d postpartum and continued for at least a 90-d period postpartum, the percentage of cows pregnant at 120 d postpartum was greater ( $35.4\% > 21.1\%$ ;  $P < 0.05$ ). Furthermore,  $\beta$ -carotene feeding increased milk yield. Not only does  $\beta$ -carotene serve as an antioxidant but it is comprised of two retinol molecules. The PPAR transcription factor heterodimerises with retinol binding protein; retinol is a ligand for the retinol binding protein. Thus the molecule  $\beta$ -carotene interacts with fatty acid ligands to regulate various intracellular processes within the endometrium and other tissues.

## CONCLUSIONS

Multi-scenarios of management can improve reproductive performance of lactating dairy cows during seasonal periods of thermal stress. Implementation of various extensive to intensive heat abatement systems to improve productivity depends on the severity of the local environment. A system that sustains a skin temperature at or below 35°C avoids reductions in milk yield. Intensive cooling to completely eliminate heat stress results in close to normal fertility indicating that short periods of hyperthermia compromise fertility. Upgrading local *Bos indicus* breeds with *Bos taurus* dairy cattle benefits production with the optimal percent *Bos taurus* being dependent upon severity of the local environment. Genetic variance for heat tolerance exists among bulls of the Holstein breed such that more heat tolerant bulls have daughters with higher pregnancy rates. Indeed heat tolerant genes are being identified, such as the slick hair gene, which improves heat tolerance and productivity. *Bos indicus* embryos are more heat tolerant and offer the potential of introducing possible heat tolerant genes into *Bos taurus* breeds.

Various reproductive technologies can improve seasonal reproduction such as: timed embryo transfer of normal embryos or embryos treated with factors (e.g. IGF-1) that improve fertility; implementation of timed AI programmes in dairy heifers and lactating dairy cows to successfully implement a seasonal breeding programme to minimise heat stress, or to sustain an acceptable level of fertility in summer with dairy heifers, or to manage lactating dairy cows on pasture to coordinate seasonal availability of pastures with early lactation and inseminations prior to the heat stress period. A timed insemination reproductive management programme resulting in comparable fertility to the use of bulls in natural service during a seasonal period of heat stress, when excellent management and compliance were used in both systems.

Inclusion of nutraceuticals such as specific fatty acids (e.g., linoleic acid and eicosapentaenoic fatty acids), and organic selenium are able

to regulate postpartum immune function and improve subsequent health, production and fertility. The above technological approaches provide an array of alternatives for producers in different social-economic and environmental locations to improve productivity and fertility of dairy cattle.

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# Climate Change in Context: Implications for Livestock Production and Diversity

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## ABSTRACT<sup>2</sup>

Climate change is predicted increasingly to affect the livestock sector in the coming decades, with potentially harmful consequences for production and for livestock genetic diversity. This paper considers mostly the indirect effects of climate change. It is expected that livestock production systems will face more frequent disastrous events and that higher temperatures will, in the absence of adaptive measures, increase physiological stress in livestock — with negative consequences for production. Tropical breeds are often well adapted to high temperatures, but the wider diffusion of such breeds or their incorporation into breeding programmes is restricted by the limited extent to which they have been characterised and improved in structured breeding programmes, trade constraints and the difficulty involved in introducing a new breed if it possesses only one advantageous trait. It is, nonetheless, concluded that given the unpredictability of climate change and of the general development of the livestock sector, conservation of adapted breeds is important. The disruptive effects of climate change on breeds' agro-ecosystems of origin may mean that increasing attention has to be given to *ex situ* conservation. Exchange mechanisms are needed to ensure that if international transfers of genetic resources are required as part of adaptation strategies, they can take place efficiently and equitably.

**Keywords:** *climate change, animal genetic resources, eco-system.*

## INTRODUCTION

### Challenges for Food Security in the Next Decades

Agricultural development in the next 30 years will present unprecedented challenges. Globally, major increases in crop and livestock

production are needed to feed a population of around 9 billion people in 2050. Agricultural production would need to increase by 70% by 2050 to raise average food consumption and to cope with a 40% increase in world human population. Compared with 2005/07, this requires an additional production of one billion tonnes of cereals and 200 million tonnes of meat annually. Approximately half of the total increase in grain demand will be for animal feed. Despite the absolute increase, growth in overall agricultural production will decelerate as a consequence of the slowdown in population growth and because a growing share of population will reach medium to high levels of food consumption (Bruinsma, 2009).

Eighty percent of cattle, buffalo and small ruminants are located in developing countries (2001–2003); however, they produce only 41% of global milk, 51% of global beef, and 71% of small ruminant meat (Steinfeld et al., 2006). Between 1980 and 2007, global beef output per animal grew at 0.4%/year, milk at 0.3%, pork at 0.8% and poultry at 1.1% (FAO Statistical Database [FAO-STAT]). These general trends mask high variation in productivity between species and livestock production systems, both within and between regions. The differences are larger in ruminants than in monogastrics for which industrial systems prevail in both developed and developing regions, with 55% of pork, 68% of eggs and 74% of poultry meat globally coming from industrial systems (FAO, 2003; Steinfeld et al., 2006). The most revolutionary change in the meat sector is in poultry; its share in world meat production increased from 13% in the mid-1960s to 28% in 2003.

The most important supply drivers over recent decades were cheap grain and cheap energy, technological change, especially in genetics, feeding and transport, together with a policy environment, including incentives, favourable to intensive production (FAO, 2010a). The most important demand drivers were increasing incomes, urbanisation and changing consumption patterns. In developing countries, where almost all world population increases take place, consumption of milk and meat has been growing at about 4–5%/annum in the last few decades. *Per capita* consumption of poultry increased more than threefold between the mid 1960s and 2002 (FAO, 2003). This 'livestock revolution' (Delgado et al., 1999), however, is concentrated in a few countries, particularly China and Brazil, mainly because of lack of development and income growth in many countries. FAO (2010a) projects that given the consumption growth in the past by China and Brazil this push will not play the same role in the future. Therefore, growth in *per capita* meat consumption in developing countries is likely to slow down in the next decades.

Livestock currently use 30% of the earth's entire land surface. This is mostly permanent pasture; but 33% of global arable land is used to produce livestock feed. The sector also accounts for about 8% of global water use, mainly for irrigation of feed crops. However, in arid areas, water consumed directly by animals or for product processing

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can represent a considerable share of total water use. Furthermore, the sector is a large producer of greenhouse gases (GHG), accounting for eighteen percent of GHG emissions, as measured in CO<sub>2</sub> equivalent — via enteric fermentation, land use and land-use change (directly for grazing or indirectly through production of feed crops) and manure management (FAO, 2010a).

The sector is also changing in regard to its contribution to poverty alleviation and income growth. The dichotomy between large numbers of small-scale livestock keepers and pastoralists, and intensive large-scale commercial livestock production is growing. While traditional livestock systems contribute to the livelihoods of 70% of the world's rural poor, increasing numbers of large-scale operations with sophisticated technology based on internationally sourced feed and animal genetics, cater for the rapidly growing markets for meat, milk and eggs, and midsize family farms risk being squeezed out of expanding formal markets. Generally, this goes hand-in-hand with shifts from multifunctional to commodity-specific products, local to globally integrated markets and from scattered to clustered production. While livestock provide multiple roles and functions for the livelihoods of the poor, the same poor are especially vulnerable to environmental hazards and zoonotic diseases (FAO, 2010a).

At the same time, resource competition increases, for example through the decreasing availability of and competition for land and water (including from other land uses such as production of biofuels, urbanisation and industrial development). Poor soil fertility and reduced access to fertiliser, overgrazing and deforestation, and loss of wild and agricultural biodiversity are other challenges. Many countries, especially in Africa and small countries in Asia and Latin America are already struggling to adapt to current environmental degradation and climate variability. Climate change will exacerbate the existing challenges faced by the livestock sector. And while farmers have always adapted their production systems to changing climatic and environmental conditions, the speed and complexity of current changes are expected to outpace the adaptive capacity of a broad range of food production systems.

### Trends in Animal Genetic Diversity

Genetic diversity is a critical basis for food security and rural development. It enables stock to be selected in response to changing market conditions or societal needs, new knowledge of human nutritional requirements, changing environmental conditions, and new or resurgent disease threats. Only 37 mammalian and avian species used for food and agriculture are reported in FAO's Domestic Animal Diversity Information System (DAD-IS), and five species (cattle, sheep, goats, pigs and chickens) provide the majority of animal source food. Genetic improvement is estimated to contribute between 50% (Shook, 2006) and 80% (Havenstein et al., 2003) to overall productivity increase, and countries with structured commercial breeding programmes far exceed the production output/ animal of the rest of the world (FAO STAT).

Breeds can be categorised as local (reported by only one country) or transboundary (reported by several countries) (FAO, 2007a). The latest assessment identifies 7 040 local breeds and 1 051 transboundary breeds (FAO, 2009a). About two-thirds of reported breeds are currently found in developing countries. Local breeds are usually based in grassland-based pastoral and small-scale mixed crop-livestock systems with low to medium use of external inputs. Especially in developing countries, they deliver a wide range of products and services — each at a low level of output — supporting the livelihoods of their keepers. They are usually not phenotypically or genetically well characterised and their adaptedness to specific environments is not well understood. The improvement of a small proportion of

local breeds is targeted by structured breeding programmes and even fewer are included in conservation programmes. Some of these breeds are very localised (FAO, 2007a). In contrast, international transboundary breeds (defined as those breeds present in more than one region of the world) have spread globally for use in high external input, often large-scale, systems. They provide single products for the market at high levels of output. They are usually well characterised and their genetic improvement is supported by efficient, sometimes global, structured breeding programmes. Genebanks exist, often as back-ups to regular artificial insemination programmes.

While countries are interdependent with regard to animal genetic resources, due to the few centres of domestication and the subsequent global spread of species and breeds, trade flows in genetic material in the past 50 years were not evenly distributed (Valle Zárate et al., 2006). Gollin et al. (2008) estimated that more than 90% of exports originate from developed countries, with a 30% share of international trade from developed to developing countries in 2005. They found that the wealthier developing countries are importers of genetics, while the poorest countries are not engaged in any international trade in animal genetic resources. Most of the exported genetic material is from animals suited to high-input production systems, and the exporting countries are free of zoosanitary restrictions (Hiemstra et al., 2007).

These exchanges of animal genetic resources contribute to productivity increases in developing countries. Productivity gains are high in pigs and poultry, species for which the genetics, the husbandry and feeding technology of intensive production are easily transferable internationally, less so in more complex ruminant systems. Ludena et al. (2007) thus predict a degree of technological convergence in non-ruminant production, but divergence for ruminant production between developing and developed countries.

Such convergence may have implications for livestock diversity. Today, most of the livestock in developed countries (and increasingly so in developing countries) is kept in more or less controlled conditions; this is particularly true for pigs and poultry. As a consequence of the uniformity of environmental conditions, fewer breeds are needed. About 9% of all reported breeds are extinct and 20% are currently classified as being at risk; the loss of within-breed diversity is not known. High selection pressure within commercial breeds may lead to a narrowing genetic base. For another 36% of breeds the risk status is unknown (FAO, 2009a). FAO (2007a) indicates that breed loss and risk in the past century was highest in regions that have the most highly specialised livestock industries with fast structural change and in the species kept in such systems, with more than one third of cattle, pig and poultry breeds already extinct and currently at-risk (FAO, 2009a). Economic and market drivers also made up 26% of all responses on threats to animal genetic resources in FAO's questionnaire survey (FAO, 2009b).

### CLIMATE CHANGE

To assess climate change and food security, FAO (2008a) used a comprehensive definition of climate change that considers changes in long-term averages for all essential climate variables, e.g. changes in the climate system, including the drivers of change, the changes themselves and their effects. Climate change is already affecting all four dimensions of food security: food availability, food accessibility, food utilisation and food systems stability (FAO, 2009c). The consequences of climate change will be most acute in developing countries, where the increase in food demand is expected to be greatest, where demand for livestock products will continue to grow faster than production and where climate changes are projected to be greatest. As described above, climate change is, however,

just one of the many environmental, economic and social factors affecting the evolution of livestock production systems and related breed diversity. Many of the environmental changes that are already occurring as a result of human activities and those that are likely to occur in the future as a result of climate change are incremental, but they are cumulative and may eventually materialise in environmental crises. The IPCC has warned of 'tipping points' where damage due to climate change occurs irreversibly (Lenton et al., 2008). If societies are aware of the complex interrelated incremental changes, they may be able to adjust policies and production practices accordingly and minimise impacts. Within limits, some ecosystems will likely be able to adjust to incremental changes. Climate change will also expose inappropriate land use practices of farmers and livestock keepers that may have been hidden or tolerated by nature during periods of favourable rainfall. However, if the rates of change are too rapid for agricultural practices to adjust, then societies will face disruptions in the delivery of ecosystem services (MEA, 2005).

Livestock contributes to and will be affected by climate change; the sector is therefore crucial for mitigating, and adapting to, climate change. Producers will have to cope with both slow climatic changes and more frequent extreme weather events. Land-use changes for the production of feeds (mainly deforestation, 35%), as well as emissions from manure (31%) and enteric fermentation (25%) are the largest contributors to GHG emissions from the livestock sector (FAO, 2006a); they are therefore the primary targets for mitigation measures. As water cycles continue to change, the concept of 'virtual water' (water that is used to produce products that are exported to, or imported by, other countries) will become more relevant.

In addition to the Intergovernmental Panel on Climate Change (IPCC) reports describing the predicted impact of climate change on ecosystems and agriculture (IPCC, 2007; Parry et al., 2007; Easterling et al., 2007), several papers provide a general overview of the expected impact of climate change on livestock production (Adams et al., 1998; Feenstra et al., 1998; Smit and Skinner, 2002). Other papers model changes in production systems and species composition under climate change (Seo and Mendelsohn, 2007 and 2008), poverty impact (Thornton et al., 2007; Jones and Thornton, 2009) or projections of methane (CH<sub>4</sub>) emissions from African livestock (Herrero et al., 2008). De La Rocque et al. (2008) provide an overview of the effects of climate change on animal diseases. Hoffmann (2010b) provides a review of responses in animal genetic resources management and breeding.

Climate change will affect the products and services provided by agricultural biodiversity. However, the role of agricultural biodiversity in the resilience of food systems still needs to be assessed, and it needs to be properly integrated into strategies for adaptation to and mitigation of climate change (Gitay et al., 2002; FAO, 2008d). Assessments that estimate that 15–37% of terrestrial species are at risk of extinction due to climate change (Thomas et al., 2004; IPCC, 2007) do not take into account their value for food and agriculture (Lane and Jarvis, 2007). Jarvis et al. (2008) modelled 16–22% extinction risks for wild relatives of some food crops<sup>3</sup> and a reduction of over 50% in the range sizes of most of them. However, the low resolution of available data and the complexity of livestock production systems and weather patterns make it difficult to model the effects of climate change spatially or by production system. In addition, the domestication of several livestock species within one region implies that these species, and the breeds that were developed from them, have similar environmental envelopes. On the other hand, many breeds of the major livestock species are today globally distributed, and their geographic distribution is overlaid by different production systems. Data on most breeds' spatial distribution and adaptation traits are

scarce (FAO, 2008b). This makes breed-level modelling of climate change impacts very difficult. FAO is currently working on georeferencing the spatial distributions of breeds.

This paper aims to shed light on the likely sensitivity of breed diversity to climate change in combination with other drivers, the production and ecosystems breeds depend upon, and the goods and services they supply. First, the direct and indirect effects of climate change and secondly, their potential impact on livestock production and on breed diversity are discussed. Traditional local and commercial international transboundary breeds are taken as extremes on a continuum of breed management. Some policy implications are discussed.

## Ecosystem Changes

Ecosystem changes resulting from climate change are relevant to livestock production because of the land dependency of most production systems and the close interaction between livestock genetic resources and other agricultural biodiversity, including parasites and microbes (Mitchell, 2003; Hudson et al., 2006; Lafferty et al., 2008; Rosenzweig et al., 2008; Lafferty, 2009). Water, feed and forage are the most important inputs for livestock production. The overall and relative availability of these resources may be affected by ecosystem changes that are accelerated by climate change. However, at present the effects of direct human pressures such as non-sustainable practices, infrastructure development and fragmentation of rangeland ecosystems appear to be greater than those directly attributable to climate change. Anthropogenic landscape modification, in turn, has created obstacles to species migration in response to climate change. All those pressures combined, together with continued human population growth and concentration, are expected to result in larger areas being affected by disturbances such as fire, the effects of insects and diseases, and soil and vegetation degradation. The implications in the livestock sector are more complex due to, among others, the higher trophic level of animals as compared with plants.

Climate change will lead to evolutionary responses at species level. It will affect species distribution and interaction and lead to changes in communities and habitats. Shifts in species distribution are already being observed, with expansion at high altitudes and high latitudes and contraction at low altitudes and low latitudes. Tropical species (e.g. of insects) are expanding into temperate areas (Dukes and Mooney, 1999; Epstein, 2001) as a result of many drivers, one of which is climate change.

Evolutionary responses are faster in species with short generation intervals, large populations and high reproductive rates. Narrowly adapted and endemic species that rely on a particular environment are more likely to be disadvantaged by changes to their home environments than generalist species that can survive in a variety of different environments. Generalist or colonising species may take advantage of climate change to expand their ranges. This may be particularly significant for weed infestation or the spread of disease, because initially at least, invasive species are more likely to be generalists than specialists. One example is the highly pathogenic avian influenza (HPAI) virus, which changed its host range and infection pathways (Webster et al., 2006; Slingenbergh et al., 2010).

Marginal ecosystems (e.g. high altitude or arid systems) are often home to very endemic, specialised species that are considered ecologically valuable; these are also the systems particularly vulnerable

3 peanut (*Arachis*), potato (*Solanum*) and cowpea (*Vigna*).

to climate change (IPCC, 2007). In addition to the high number of adapted livestock breeds (FAO, 2006b), at least 30% of the world's cultivated plants and innumerable uncharacterised rangeland fodder species originated in drylands (UNCCD, 2005); these constitute a precious genetic stock for future agriculture. In the Near East, 90% of the region's breeds are kept in drylands. In Africa, this share is 56%, 42% in Asia and 19% in Latin America<sup>4</sup>. The distribution of some domesticated species is completely (camelids, yaks) or mainly restricted to drylands. More than 70% of breeds of ass, around 50% of sheep and goat breeds, and 30% of cattle and horse breeds reported are adapted to drylands (FAO, 2006b). It can be assumed that breeds adapted to drylands will be affected by natural resources degradation linked to climate change rather than temperature or precipitation change *per se*.

It is likely that ecosystem components will disintegrate and reassemble in a new ecological state (McCarty, 2001; Lovejoy, 2008). When usually interacting species have different phenological and evolutionary responses, differential range expansions may lead to new species relationships and trophic encounters (plant/herbivore, pathogen/host, predator/prey) and new communities. Such new encounters will lead to a reassessment of what is meant by 'invasive species' (Hoffmann, 2010a). Exchanges between cultivated and natural habitats, such as domestic and wild fowl systems, already occur and may further change with climate change (Gilbert et al., 2007 and 2008; Slingenbergh et al., 2010); this may include pathogen-host shifts as observed in HPAI (Webster et al., 2006).

The expected increases in rates of disturbance may facilitate successional change, providing a mechanism for ecosystem readjustment, but may also lead to a transient or permanent loss of biodiversity which in turn may adversely affect the function of the ecosystem. However, the loss of specific species does not necessarily lead to a loss of ecosystem function, as other species with the same function may occupy the vacant niche. Changes in plant community composition in response to climate change may be substantial in some areas and will probably lead to changes in water and nutrient cycling. In arid ecosystems particularly, the more than 20-year time lags complicate the attribution of multiple causes to vegetation changes (Valone et al., 2002). There are examples in which land-use practices including grazing had a bigger impact on arid ecosystems than climate (Wittig et al., 2007) and others in which the reorganisation of the ecosystem was triggered mostly by the effect of climate (Brown et al., 1997; Curtin, 2002).

Establishment of species in new locations depends not only on temperature and precipitation, but also on ecological parameters such as soil characteristics and vegetation composition in the target area, competition, disease pressure, reproductive behaviour, and the migratory capacity of the species. Current vegetation and disease vector models do not take into account the complex ecological relationships and non-linear processes that determine whether a species that migrates to remain in its ecological equivalent zone can actually establish itself where it arrives. Anthropogenic factors add another layer of complexity. Location-specific projections are therefore not easy, especially for agricultural ecosystems consisting of a combination of natural and managed biodiversity.

## Host-pathogen Interactions and Disease

About 75% of the new diseases that have affected humans over the past decade were caused by pathogens originating from animals or from products of animal origin. The link between disease risks from various pathogens and climate change has been increasingly recognised. Together with other factors such as land-use and habitat changes, the movement of human and animal populations and drug and pesticide resistance, climate affects vectors, pathogens, hosts and host-pathogen interactions from the level of cellular defence to that of the habitat (Epstein, 2001). The life cycles of parasites, such as seasonal presence, reproduction and transmission, overlap with the life cycles of their final and intermediate hosts. Hoberg et al. (2008) provide an overview of predicted responses of complex host-pathogen systems to climate change. Climate change may affect the spatial distribution of disease outbreaks, and their timing and intensity. In addition to long-term climate effects, short-term weather events affect the timing and intensity of disease outbreaks. Outbreaks of African horse sickness, peste des petits ruminants, Rift Valley fever, bluetongue virus and anthrax are triggered by specific weather conditions and changes in seasonal rainfall profiles (De La Rocque et al., 2008).

Extreme weather events or other short-term changes in the host-vector-pathogen interface may generate clusters of disease outbreaks. The predicted reduction in the availability and quality of water will increase the risk of water-borne diseases for humans and livestock. In addition to diseases affecting the animal itself, a new range of pests and diseases will impinge on crop and forage species, thus affecting the quantity and quality of livestock feeds.

Climatic effects on host-vector and host-parasite population dynamics are more pronounced in high latitudes and high altitudes, where rising minimum temperatures increase the growth potential of many parasite populations (Rogers and Randolph, 2006). Parasites may appear earlier in the season and go through more generations than they do today. The predicted temperature increase will further the geographic expansion of vector-borne infectious diseases (e.g. Rift Valley fever, bluetongue and tick-borne diseases) to higher elevations and higher latitudes and affect the transmission and course of the diseases. The bluetongue virus was able to establish itself successfully in new ecosystems by using a temperate vector instead of its traditional African-Asian *Culicoides* vector. The possible impact of bluetongue on endemic sheep breeds in the UK is raised by Carson et al. (2009).

Expansion of the range of a pathogen or vector does not necessarily result in wider disease transmission (De la Rocque et al., 2008). For some diseases, the effects of risk factors, such as the movement of animals and changes in production systems, habitats and ecosystems, will remain more important than climate change. Additionally, the risk of vector-borne diseases will display great local spatial variation and may be more affected by the abundance of competent hosts than climate change (Rogers and Randolph, 2006; Randolph, 2008 and 2009).

Rapid spread of pathogens or even small spatial or seasonal changes in disease distribution may expose livestock populations that lack resistance or acquired immunity to new diseases, resulting in more serious clinical disease. The expected increased and often novel disease pressure will favour genotypes that are resistant or tolerant to the diseases in question. FAO (2007a) lists 59 cattle, 33 sheep, 6 goat, 5 horse and 4 buffalo breeds, mainly from developing countries, that are reported to be resistant or tolerant to various pathogens, however, the underlying physiological and genetic mechanisms are not well understood.

4 The figures refer to camelids (Bactrian camel, dromedary, llama and alpaca), cattle and yaks, goats and sheep, asses and horses.



Beyond the individual-animal level, the contribution of genetic diversity in populations to the dynamics of pathogen transmission needs further investigation. Mathematical models (Springbett et al., 2003) and evidence from plants (Mitchell et al., 2002) indicate that high species diversity and high genetic diversity within populations affect both the probability of the occurrence of epidemics and their outcome. In the case of vector-borne diseases, highly diverse host communities show lower infection rates among vectors due to the presence of unsuitable hosts — a mechanism known as the ‘dilution effect’ (Morand and Guégan, 2008). This highlights the need to maintain biodiversity in agricultural production systems and landscapes (Slingenbergh et al., 2010).

### Feed and Fodder

There is a high probability that by the end of the century average growing-season temperatures will exceed the hottest seasons on record, and that heat stress will limit increases in crop production or cause output to fall. Without adaptation measures, food security will be threatened, especially in already food-insecure regions (Cline, 2007; Schmidhuber and Tubiello, 2007; Lobell et al., 2008; Jones and Thornton, 2009; Fischer, 2009).

In many livestock production systems, feed costs are the largest element of production costs. A key difference among livestock species is their differing ability to use feed resources that cannot be used directly as human food. While in extensive systems, forage may make up the entire diet of ruminants, it is supplemented by concentrates in intensive dairy, beef cattle and sheep fattening systems. Conversely, the diets of monogastric livestock consist largely of cereals and oilseed residues. Rowlinson (2008) suggests that it is unlikely that climate change will alter the current range of feed ingredients of commercial diets where rations for all species include high-quality by-products, many of which are imported from a wide range of countries and can be exchanged in least-cost ration formulation.

The effect of climate change on feed-grain supply is difficult to predict, as it depends on many factors in addition to climate. However, the non-food sector's demand for feed inputs, especially for biofuel and other industrial uses, is expected to increase, thereby potentially exacerbating the impact of climate change-induced reductions in feed supply for the livestock sector (FAO, 2008d; OECD-FAO, 2009). Afforestation for C-sequestration purposes reduces the land area available for grazing. While second-generation biofuels will increase competition with ruminants, by-products of first-generation biofuels are commonly used as supplemental protein feeds for ruminants. However, the feed rations of monogastric species may not be able to adjust as readily to absorb the increased supply of biofuel by-products. The mainly unknown impact of climate change on aquatic ecosystems, together with ongoing overfishing, and increasing use of fishmeal in aquaculture — thereby competing with its use as livestock feed - will also require changes in feeding rations.

Climate change is likely to affect the quality and quantity of the forage component of ruminant diets. Together with the predicted increase in fires and changing grazing regimes it may modify the plant species composition (grass, herbs, shrubs, trees) or give room to invasive species (Epstein et al., 2002; Easterling et al., 2007). In general, increases in temperature, CO<sub>2</sub>, precipitation and N deposition will result in increased primary production in pastures (Tubiello et al., 2007). As long as water requirements can be met, the projected increase in temperature in temperate regions may have benefits for early season growth and lead to shifts towards more productive forage species, allowing higher-output breeds to be kept. New feeds, browse species and improved rangeland management are already being introduced in developing countries to improve feed

supply, however, with mixed success in adoption. The reductions in precipitation expected in many already semi-arid areas, particularly if combined with increases in rainfall variability, will affect vegetation growth rates and may reduce rangeland production. This in turn will lower the stocking rates and productivity of grazing livestock (Hein et al., 2009), and increase the vulnerability of the system, including via land degradation that further exacerbates overgrazing of remaining areas.

Animal production in grassland-based systems is related to the availability of young and protein-rich plant material. High temperatures tend to increase lignification of plant tissues and thereby decreases forage digestibility. Also the climate change induced predicted shifts of plant species and communities may also affect forage production and quality. Examples include the predicted shift from C3 to C4 grasslands and the increase in shrub cover in grasslands (Christensen et al., 2004; Morgan et al., 2007). C4 plants are more efficient in terms of photosynthesis and water use than C3 plants. C3 and C4 plants coexist in the tropics, but react differently to increases in temperature and CO<sub>2</sub>, which may result in changes to the composition of rangeland vegetation. Besides various changes in ecosystem function (Easterling and Apps, 2005; Easterling et al., 2007; Tubiello et al., 2007), this has implications for forage supply. Firstly, because C4 perennial grasses tend to start growing later in the spring and to mature earlier than C3 perennials, seasonal forage quality becomes more variable and overall forage availability may decline (Liang et al., 2002). Secondly, C3 forage plants generally have higher nutritive value, but yield less, while tropical C4 plants contain large amounts of low-quality dry matter and have a higher C:N ratio. Ruminants fed subtropical C4 grasses emitted more CH<sub>4</sub> per unit of digested dry matter than those fed temperate C3 grasses (Ulyatt et al., 2002). Browse often contains higher protein concentration than grasses. The digestibility of natural browse can be lower than that of grasses due to secondary plant components (Illius, 1997), but higher in improved browse species (e.g. *Leucaena*). From range management and animal nutrition perspectives, it will therefore be important to maintain a balance between C3 and C4 grasses, legumes and shrubs. Well-managed rangelands also provide better soil C sequestration. FAO (2009d) provides estimates of the potential carbon storage and sequestration in pasture and rangelands in drylands and outlines the main land management measures for improving carbon cycling and grassland management.

Few ecosystem models predict the general impact of climate change on rangelands and livestock production (Hall et al., 1998; Christensen et al., 2004). Tietjen and Jeltsch (2007) found few models that aimed to understand rangeland system dynamics. They conclude that current models insufficiently reflect the impact of increased CO<sub>2</sub> levels and changes in intra-annual precipitation patterns on plant productivity — both crucial external drivers under climate change — and provide little guidance to livestock and land managers.

### Adaptation to Climate Change and Related Drivers

Commercial and small-scale livestock keepers choose the species best adapted to current climatic and agro-ecosystem conditions within their social, economic and technology contexts. As all these are constantly evolving, they have been selecting and introducing breeds for centuries. It can be expected that the portfolio of breeds demanded by society will continue to change. Projecting a continuation of trends prevailing over the past decades, this will imply a growing dichotomy between livestock kept by large numbers of smallholders and pastoralists, and those kept in intensive large-scale commercial production - independent of climate change. Many stakeholders

do not yet perceive climate change as a problem for the management and conservation of livestock biodiversity. In a recent survey on threats to livestock diversity (FAO, 2009b), climate change was mentioned by only 1% of 1 335 respondents; moreover, it was only mentioned in the context of extensive land-based production systems. It was not mentioned at all by respondents to a separate questionnaire which sought the reasons why breeds classified as being at-risk in DAD-IS got into this state (ibid.).

There is general agreement that the direct effects of climate change will be of a similar nature in low- and high-external input livestock production systems, both of which will be affected by increased frequency of catastrophic events, disease epidemics and water scarcity, which will lead to physiological stress and productivity losses in the animals. Loss of animals as a result of droughts and floods, or disease epidemics related to climate change may increase (FAO, 2008a). If breeds are geographically restricted in their distribution (endemic) – as is the case for some local and rare breeds – there is a risk that they will be lost in localised disasters (Heffernan and Goe, 2006; Carson et al., 2009). The direct effects of climate change depend very much on the production and housing system with intensively raised livestock kept in protected or controlled environments and fed with supplements being less likely to be directly affected by climate change (Adams et al., 1998; Freitas et al., 2006). As such intensive systems have more access to technologies and capital, the effect for the high-output breeds kept may be buffered.

Although the direct effects of climate change on the animal are likely to be small as long as temperature increases do not exceed 3°C (Easterling et al., 2007), projections suggest that further selection for breeds with effective thermoregulatory control and adaptive traits will be needed. The speed of selection depends on many genetic factors, but also on reproductive technology and biotechnology which can be differently applied among the main species (FAO, 2010c).

Adaptation traits are usually characterised by low heritability. In general, the genetic relationships between adaptive and productive traits and potential selection responses need further attention. While annual genetic gain in production traits has been 1–2% over the past 50 years, adaptation and disease resistance traits, most of them multi-locus, are more difficult to select for and often have antagonistic genetic relationships to production traits. However, many commercial breeding programmes today aim to improve production, longevity and functional traits simultaneously — for example in dairy cattle, pigs and layer chickens. Breeding for adaptation to climate change will not be fundamentally different from existing breeding programmes, but inclusion of traits associated with temperature tolerance in breeding indices, and more consideration of genotype x environment interactions to identify animals most adapted to specific conditions will become more important. Hoffmann (2010b) reviews breeding for improved heat tolerance, nutrition and disease stress.

The indirect effects of climate change such as ecosystem changes or the implementation of climate change mitigation measures will differ between production systems. According to models by Seo and Mendelsohn (2007 and 2008), livestock production in developing countries is sensitive to climate change. Their projections indicate that commercial production systems will be more vulnerable because their specialised nature makes it difficult for them to switch to other species. Conversely, they found net revenues of small farms will increase as temperature or rainfall levels rise due to their more diverse species portfolios, the ease with which they can shift between species and diversify, and their reliance on goats and sheep.

The indirect impacts of climate change may even exceed the direct impacts, they may lead to changes in the ranking of species and breeds and to regional shifts in the market. In low external input systems, changes in fodder quantity and quality and changing

host-pathogen interactions in local agro-ecosystems will come into play. In general, changes in the amount of land area devoted to cropping (including fodder) relative to rangeland and their relative productivity will influence the balance between non-ruminant and ruminant production.

In intensive landless systems, the indirect effects will be more related to changes in availability and price of inputs such as feed, energy and water. In this context, use of specific feeds and differences in feed conversion ratios (FCR) between breeds will influence the proportion of commercial *versus* local breeds.

In both types of system, the effects of climate change mitigation measures may play a role, but affect them and the breeds they harbour differently. The measures aimed at mitigating the effects of land-use change from/for? feed production and manure management are more relevant to intensive production systems where they may increase production costs.

The implications of climate change adaptation and mitigation strategies on breed diversity will depend on the public goods targeted and the trade-offs between them (FAO, 2009c): the effects of mitigation measures aimed at reducing GHG emissions from enteric fermentation may differ from the effects of those targeting emissions from manure or those aimed at improving soil C sequestration, and would certainly differ from the effects of adaptation measures that aim to increase social equity. In general, scenarios for ruminants and monogastrics differ because of differences in the feed base that they utilise and in the GHG emissions associated with the production process. Livestock keepers, particularly pastoralists, make use of the livestock species and breed differences in diet selection, walking ability, heat tolerance and water requirements to produce the products and services they need from their animals. For example, feed intake and digestibility in dromedaries do not decline under heat stress (Guerouali and Wardeh, 1998). Among cattle, taurine breeds have a better FCR with high quality feed while indicus breeds generally deal better with low quality forage than do taurine breeds. Taking into account the direct effects of climate change together with changes in agro-ecological conditions and production systems, the models by Herrero et al. (2008) and Seo and Mendelsohn (2008) indicate that farmers will switch from beef and dairy cattle and chickens towards goats and sheep as temperatures rise.

Livestock can compensate for the expected lower fodder quality and shrub encroachment to a certain extent if they are able to select high quality diets from different plant components or vegetation communities. There is some evidence for breed differences in diet selection behaviour and browsing ability linked to different metabolic profiles (Blench, 1999; Bester et al., 2002; Jauregui et al., 2008; Fraser et al., 2009). Also a number of plants and unconventional feed resources are adapted to harsh conditions, such as poor soil and drought. Many traditional livestock farmers use multi-species and multi-breed herds to maintain high diversity in on-farm niches and to buffer against climatic and economic adversities (Hoffmann et al., 2001; Hoffmann, 2003; FAO, 2009e). Traditional knowledge and practices are therefore very useful for adaptation to climate change; this includes species and breed substitution. For example in West Africa, dromedaries replaced cattle, and goats replaced sheep following the Sahelian droughts of the 1980s. This species mix made better use of available feed resources: While cattle and sheep feed largely on herbaceous vegetation, dromedaries browse on shrubs and trees and goats use both strata. The use of browsing species has several advantages: browse is increasing in some environments due to overuse of the herbaceous and layer; browse tends to offer green forage also during the dry season, and there is less competition by other domestic species. Replacing cattle by dromedaries in traditional manure contracts has opened a new vegetation stratum for recycling

nutrients to the soil (Hoffmann and Mohammed, 2004). In addition, camel milk is a reliable source of food during dry seasons, and interest in its special properties is rising. Disease resistance also plays a role, and while species and breed displacement from the arid and semi-arid to the sub-humid zones in West Africa has been observed, extension into the humid zones, where disease pressure (especially trypanosomiasis) is high, is still limited.

Degradation of the natural resource base for livestock production, especially overgrazing in grassland-based systems and pollution in intensive systems, are major future challenges (FAO, 2010a). Such environmental damage may exacerbate the impact of climate change and raise the costs of adaptation. For example, provinces of Western China recently imposed restrictions on grazing with the objective of reducing rangeland degradation (Zhang and Hong, 2009). Also reforestation measures aimed at C-sequestration tend to increase the value of land previously used for grazing, in addition to the already mentioned effects of increased demand for biofuel. This may further reduce the land area available for livestock production, thereby adding to the threats to local ruminant breeds and the livelihoods of their keepers.

In Africa already today most livestock are found in semi-arid environments. These systems are expected to expand at the expense of humid and temperate/tropical highlands systems. However, while Seo and Mendelsohn (2008) predict that ruminant numbers in rangelands will increase as long as there is sufficient precipitation to support vegetation growth, Herrero et al. (2008) model large shifts of livestock populations from rangeland-based grazing to mixed crop-livestock systems based on improved feeding of crop by-products. Jones and Thornton (2009) predict that livestock keeping will replace cropping in today's marginal mixed crop-livestock systems. The outcomes of those models imply different breed portfolios. Mixed crop-livestock systems, especially those close to markets, have increasingly used crossbreeding with high output breeds (e.g. dairying in East African highlands), but only local breeds are adapted to the harsh conditions in pastoral systems. It can be expected that local breeds kept by poor people will continue to play a role in marginal areas (Hoffmann, 2010c). Problems for their survival may occur if adapted higher performing breeds penetrate into the environments currently occupied by local breeds, or if climate change is faster than selection.

The availability and prices of high quality feedstuff and grain will affect the comparative advantage of different species and the distribution of high output breeds. If the present economic drivers including rising feed prices continue, superior FCR will grant monogastrics a comparative advantage over cereal-fed ruminants. High output breeds of all species selected for improved FCR and high yield will dominate the market production of milk, eggs and meat. These breeds will continue to out-compete local breeds, thereby increasing the threat of extinction for local breeds, especially of monogastric species (FAO, 2009a).

Increasing individual animal productivity, improving feed quality, optimisation of feed rations and feed additives, and reducing livestock numbers are the most immediate GHG mitigation options at the animal level. In general, CH<sub>4</sub> output from enteric fermentation in ruminants increases with the higher dry matter intake linked to high performance, but CH<sub>4</sub> emissions/unit product decrease as the proportion of concentrate in the diet increases. However, the production pathways of different animal products differ in their GHG emissions and this may influence the future emphasis given to different production systems, species and breeds. Intensive poultry production based on line hybrids has the lowest GHG emissions/kg meat, followed by pigs (Williams et al., 2006). Milk protein can be produced with less CH<sub>4</sub> emission than beef (Williams et al., 2006; FAO, 2010b). This may tend to disadvantage breeds kept in extensive grazing systems.

In an intermediate GHG reduction scenario, dairying might become the major focus of cattle production due to low CH<sub>4</sub> emissions/kg milk, and beef may become a by-product of dairying. Dual-purpose breeds and crossbreeding may gain importance.

Local ruminant breeds, which have low output and therefore a higher GHG emission/kg of single product, are considered unproductive. Ruminants use forages that cannot otherwise be used by humans; this enables human communities to inhabit harsh areas where production of crops is impossible (Gill and Smith, 2008). However, the productivity equation does not usually take into account the multiple products and services provided by livestock in most smallholder and pastoral production systems. Because of the above mentioned differences in feed quality, productivity improvements in ruminants grazing more digestible temperate pastures will result in lower relative CH<sub>4</sub> reductions than in pasture-fed ruminants in the tropics (McCrabb and Hunter, 1999). However, less investment in research and breeding is targeted towards the many local breeds and forages in extensive tropical systems.

Carbon sequestration in grasslands may partially offset GHG emissions from other components of the production process and offers co-benefits of improved grazing management and related positive environmental effects (soil C sequestration, biodiversity) and a favourable impact on livestock productivity (Smith et al., 2007). It is therefore proposed to include soil C sequestration, which has the highest GHG mitigation effect in agriculture and to which improved grazing could significantly contribute, in a post-Kyoto Protocol mechanism (Smith et al., 2007; FAO, 2009d). Payments for environmental services, be it C sequestration, landscape management or biodiversity conservation would increase the chances of local breeds being maintained, and may favour the return of herbivore livestock species to forage-based feeding and land-based production systems. However, this needs a favourable political and economic environment.

## Interdependence

Introducing new breeds into geographical areas or production systems is an adaptation strategy that livestock keepers have applied for millennia. If climate change exceeds the adaptive capacity of the currently used genetic portfolio, increased strategic crossbreeding with better adapted or higher performing breeds or insertion of specific genes through the use of biotechnology may occur. Climate change may thus increase the pressure to maintain wide access to animal genetic resources as countries will increasingly depend on better adapted or higher performing genetic resources from other countries to adapt their food and agricultural systems.

Such species or breed replacement processes may involve considerable costs and substantial investments in learning and gaining experience. Although in principle, a species or breed may replace the current one as single new components in a production system, more probably it will be introduced together with other components such as technology and knowledge (Ludena et al., 2007). FAO (2007a) provides a wealth of information on successful as well as failed introduction of breeds. In human-managed systems, 'establishment' of a new species or breed depends on how many components of the old production system can be transferred to the new system. This means that beyond the genetic material, the technologies and information supporting a new production system need to be available, accessible and affordable. In the USA, the introduction of new breeds has been successful when based on several production traits and when the private sector has been interested, while introductions aiming to take advantage of single traits have not proved sustainable (Blackburn and Gollin, 2008).

The heightened interdependence may increase the importance and value of specific genetic material and its associated knowledge, be it traditional or modern. The sharing of genetics and breeding-related knowledge generally declines the more specialised the knowledge associated with breeding and the more valuable the livestock species. This observation applies equally to commercial breeders of developed countries and pastoral breeders of developing countries. Knowledge about breeds and breeding in developed countries is increasingly privatised; commercial breeders keep their breeding lines and breeding programmes under tight control, including through the use of contracts, trade secrets or patents (Gura, 2007). The special knowledge needed for dromedary breeding and the maintenance of the breeds is still in the hands of the traditional pastoralists in the Sahel and in northern India where farmers buy dromedaries as draught animals (Hoffmann and Mohammed, 2004; FAO, 2009e). However, such specialist traditional knowledge in developing countries that may assist in targeted search for adaptive traits is increasingly under pressure due to the social changes linked with ongoing economic development and to climate change (FAO, 2009e).

The IPCC predicts large human population movements to adapt to climate change. The most widely accepted forecast for the number of 'environmentally induced migrants' by 2050 is 200 million (IOM, 2009). As humans migrate, they may for various social, economical and political reasons become disconnected from components of the agricultural ecosystems they currently manage. Livestock breeding and production systems are complex and knowledge intensive. They are often regionally stratified or integrated, with different human population groups having different roles and knowledge systems in the cycle — reproduction, growing, using the adults as dairy, meat or draught animals, etc. The vertical integration of developed-country production systems, for example the UK hill-lowland sheep system, is simple compared with that of systems in developing countries where different ethnic groups keep different breeds and have different roles in the cycle. As the climate changes, production systems will disintegrate and genetic resources will become uncoupled from traditional knowledge about their management. The HIV epidemic in Africa has demonstrated the detrimental effects of the loss of traditional knowledge in farming systems (Goe, 2005). Therefore, in order to increase the overall resilience of the global food and agricultural system in the advent of climate change, the associated knowledge for animal genetic resources, both commercial and local, should be documented and made widely accessible and transferable together with the genetics.

One can/may assume that specific traits of tropical adapted breeds may become important for climate change adaptation, leading to a reassessment of their values. In this case, it will be important to facilitate access to these resources and share the benefits from their use to ensure that adaptation efforts are not hindered by access restrictions by countries that happen to host such sought-after resources.

Hoffmann (2010b) argues that it is unlikely that climate change will reverse the current direction and trends of genetics exchange and make local breeds from developing countries more attractive to the international market. Reasons are developing countries' insufficient resources or capacity, resulting in a low level of characterisation or structured breeding programmes of the majority of local breeds. Additionally, the performance and technology focus on high output breeds indirectly reduce incentives for characterisation and selection within local breeds in developing countries for improved production or adaptation. Currently, only a few countries with well-developed research, extension and breeding and artificial insemination institutions have commercially relevant tropical cattle breeds and even

fewer countries have commercially significant breeding programmes for adapted breeds of the other species.

## Conservation

In nature conservation it is now argued that *in situ* strategies have to account for the fact that environmental conditions in species' historic ranges will change, and indeed are already changing (McCarty, 2001). Further to claims to facilitate species migration and maximising adaptation opportunities through the maintenance of intact ecosystems, this results in a review of *in situ* conservation areas, as protected habitats may be populated with new species, or rare species may migrate away from their protected habitats (*ibid.*). Thus, the role of *in situ* conservation areas as means to facilitate species migration and maximise adaptation opportunities by maintaining intact ecosystems needs to be reviewed. According to Jarvis et al. (2008), increased *in situ* and habitat conservation will be important for the survival of crop wild relatives whose range sizes are reduced by climate change. However, recognising that climate change may affect our food system quickly, they stress the urgent need to identify priority core species for collection and inclusion in genebanks.

Public germplasm collections such as for plant genetic resources do not exist for animal genetic resources where *ex situ* conservation is technically more challenging and ownership over the genetic resources is mostly private. Conservation measures for threatened breeds, which are a priority of the *Global Plan of Action for Animal Genetic Resources* (FAO, 2007b) have been established in some countries (FAO, 2007a). Data from 194 countries indicate that on average 7.5 breeds are at risk and 1.7 breeds are in conservation programmes (DAD-IS). Most conservation programmes are based in developed countries, with strong collaboration between genebanks and the animal breeding industry. In developing countries, the focus is currently on *in vivo* conservation with high variability in the quality of the programmes (*ibid.*), but in common with plant genetic resources, this may shift to *in vitro* conservation of animal genetic resources. Consequently, the sensitivity of *in situ* conservation programmes to the effects of climate change should be assessed and *ex situ* conservation measures taken if needed. As a first step in conservation, it is necessary to characterise animal genetic resources and subsequently to build inventories that include information on the spatial distribution of breeds and valuable breeding stocks. Then, conservation priorities have to be made (Boettcher et al., 2010). Cryo-conservation, from national to global, will depend on the infrastructure and resources available at each level and involve private genebanks held by breeding organisations or companies and public genebanks.

## CONSEQUENCES FOR ANIMAL GENETIC DIVERSITY

The livestock sector is highly dynamic, and the need for increasing production while reducing the environmental footprint of livestock will continue to be major future challenges (Pelletier, 2008; FAO, 2010a). Climate change, with its slow but long-term effects, will be an additional factor affecting this dynamic (FAO, 2010a); it will further the need for resource-efficient livestock production and may thus intensify current trends with a growing dichotomy between livestock kept in extensive and those in intensive systems. However, the livestock community is not yet fully aware of its possible impact.

The direct effects of climate change will depend very much on the livestock production and housing system, with high output breeds in confined systems being better protected from natural adversities than breeds in extensive grazing systems. In systems where the rate of technology adoption is generally low, or in regions that have a limited capacity to adapt (e.g. sub-Saharan Africa),

the risk of breed displacement or loss may increase. Local breeds under traditional management are generally more adapted to harsh conditions and resilient to environmental changes than are high output breeds, but their adaptation to complex stressors is not well described, understood and valued.

Although many existing technologies will be crucial for climate change adaptation and mitigation, research gaps exist, especially with regard to the interactions between adaptation and mitigation. The outcomes of different scenarios and models imply different breed or species portfolios, making it difficult for decision makers to make rational choices. With the current state of knowledge it is not possible to predict whether climate change will be faster than natural or artificial selection. It would be an interesting research question to assess and model the likelihood, speed and impact of the various aspects of climate against genetic evolution of livestock and use the results to guide the need for interventions. The variable degree of confidence of projections, and the uncertainties related to climate change vulnerabilities and impacts are often regarded as a hindrance for immediate and concrete action, and FAO (2009c) argues that considerable precaution must be taken in policy making for food security under climate change projections.

In view of the uncertainty for future developments and climate change, the adaptive traits harboured in all breeds should be assessed and the use and non-use values of animal genetic resources be maintained. This applies equally to transboundary commercial and local breeds which fulfil different societal and ecological functions. Immediate needs for research and method development include:

- developing simple methods to characterise, evaluate and document adaptive and performance traits in specific production environments (FAO, 2008b), including the associated knowledge. For example, feeding and water intake behaviour and relationships between energy reserves, endocrinological parameters and breeds' reproduction performance, and improved methods to assess the use and non-use values of animal genetic resources;
- making breed inventories that including relevant spatial information, and monitor threats to breeds, be they caused by climate change or other drivers. This may entail some basic predictive modelling of future breed distribution and early warning systems;
- exploring possible synergies between plant and animal breeding (FAO, 2008c);
- developing methods for life-cycle assessments and include delivery of ecosystem services in the analysis, recognising and rewarding C sequestration and sustainable use of biodiversity in well managed rangelands with local breeds;
- strengthening agro-ecosystem modelling as a tool to improve understanding about the complex interaction between livestock genetics and adaptation with changes in the biophysical and socio-economic environment.

Developed and developing countries differ with regard to their genetic resources portfolios and the management of these resources. Most high output breeds are selected for the requirements of developed country markets and production systems. Most tropically adapted breeds exist as key resource in the livelihoods of rural people in developing countries, but these breeds and their keepers are often neglected. This undervaluation of many local breeds results in their not being well characterised and with few structured breeding or conservation programmes. The recent adoption of the *Global Plan of Action for Animal Genetic Resources* (FAO, 2007b) provides for the first time an internationally agreed framework for the management of animal genetic resources, and also proposes measures to support developing countries and small-scale livestock keepers in their endeavours (FAO, 2009e). Without strengthening capacity for

breed characterisation, improvement and conservation in developing countries, climate change in combination with ever increasing demand for food may exacerbate current trends in the livestock sector and the divide between developed and developing countries (OECD-FAO, 2009). While *in situ* conservation is favourable due to its pro-poor effects, *ex situ* conservation programmes need to be developed to cater for habitat destruction and allow for emergency response. It will be necessary to improve adaptive traits in high output and performance traits in adapted breeds, and to facilitate wide access to genetic resources and enable transparent exchange of the associated knowledge in local and commercial breeds.

Developed and developing countries also differ in their adaptive capacity and in the expected interactions between climate change adaptation and mitigation. Due to their low capacity, developing countries will not implement adaptation measures solely as a response to climate change, but will promote closer relationships between climate change adaptation, pro-poor investment and development policies, allowing for co-benefits. Equally, they will exploit the other potential benefits of many measures aimed at reducing GHG emissions for improving the productivity and environmental integrity of agricultural ecosystems (Smith et al., 2007). Special care should be taken to ensure that climate change mitigation measures enhance systems' resilience; they should not reduce future adaptation options or undermine food security and rural livelihoods. The livestock sector, being a contributor to and affected by climate change on the one hand and a mainstay of the livelihoods of millions of rural poor on the other, has a role to play. Breeds, agricultural biodiversity and ecosystems must be an integrated part of general mitigation and adaptation efforts.

Pro-poor policies need to be developed that strengthen livestock keepers' adaptation strategies, their ecological knowledge and local institutions. Especially in marginal areas, land management for climate change mitigation and adaptation, biodiversity conservation and poverty alleviation should complement each other (UNCCD, 2005; FAO, 2007b, 2008c, and 2009d).

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# Indigenous Cattle in Sri Lanka: Production Systems and Genetic Diversity

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## ABSTRACT

Production status, farming systems and genetic diversity of indigenous cattle in Sri Lanka were evaluated using six geographically distinct populations. The indigenous cattle population of the country is considered as a nondescript mixture of genotypes, and represents more than half of the total cattle population of 1.2 million heads. Five distinct indigenous populations were investigated for morphological analysis, and four were included in evaluating genetic differences. Farming systems were analysed using a pre-tested structured questionnaire. The genetic variation was assessed within and between populations using 15 autosomal and two Y-specific microsatellite markers, and compared with two indigenous populations from the African region. Farming system analysis revealed that indigenous cattle rearing was based on traditional mixed-crop integration practices and operates under limited or no input basis. The contribution of indigenous cattle to total tangible income ranged from zero to 90% reflecting the high variation in the purpose of keeping. Morphometric measurements explained specific phenotypic characteristics arising from geographical isolation and selective breeding. Though varying according to the region, the compact body, narrow face, small horns and humps with shades of brown and black coat colour described the indigenous cattle phenotype in general. Genetic analysis indicated that indigenous cattle in Sri Lanka have high 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 ( $0.72 \pm 0.04$  to  $0.76 \pm 0.03$ ). Genetic distances between regions were low (0.085 and 0.066) suggesting a similar mixture of genotypes across regions. Y-specific analysis indicated a possible introgression of Taurine cattle in one of the cattle populations.

**Key words:** *indigenous cattle, farming systems, phenotype, genetic analysis, microsatellite markers, alleles.*

## INTRODUCTION

Sri Lanka is an island in the Indian Ocean between the latitudes of 5° 55' N and 9° 51' N and longitudes of 79° 41' and 81° 53' E, covering an area of about 65 610 km<sup>2</sup>, and lies in close proximity to the southeastern coast of India. The country's economy is considered as agricultural, but the contribution of the sector to the gross national product is only 20%. The main contributors to the agricultural sector are from the plantation sub-sector and from field crops

such as paddy rice. The contribution of livestock to the agriculture sector is only 8% (DCS, 2008). The livestock sector consists mainly of dairy cattle and poultry sub-sectors, the main emphasis of the former being to increase the availability of liquid milk within the country because around ten million Rupees is spent annually in importing milk products of which 96% is milk powder (DCS, 2008).

The total cattle population of the country has fluctuated during the past few decades but at present it is estimated at 1.2 million heads (DCS, 2008). Around 0.2 million heads are slaughtered annually, the majority of which are indigenous cattle (Silva et al., 2004). Indigenous livestock species constitute a major proportion of the total value of the animal genetic resources (AnGR) of Sri Lanka, but the value of indigenous cattle as a component of rural agriculture is diminishing for a variety of reasons which are common to many other countries in the region. Generally, indigenous cattle are evaluated only in terms of milk production as this is the main economic product. Heat tolerance and resistance to several endemic diseases make these indigenous breeds thrive better in local rural environments and production systems than other breeds of cattle.

These characteristics become prominent in the context of small-holder production systems, which are the predominant cattle rearing systems in the country and where resource levels are low (Ranaweera, 2007). In most instances, traditional cattle farmers, who follow set farming practices acquired from their ancestors, manage low input systems. It has been estimated that there are around 400 000 dairy farmers in Sri Lanka and 2.45 million people (70% of the estimated 3.5 million livestock dependent people) sustain their livelihoods from the dairy sector (Ibrahim et al., 1999; Ranaweera, 2007). Although estimates are not available, a considerable proportion of these dairy sector dependents rely on indigenous cattle. Based on the proportional geographic distribution of cattle, indigenous cattle and their crosses represent 60% of the total cattle population in the country (Ibrahim et al., 1999) but their contribution to milk production is marginal.

Indigenous cattle have long been identified as a separate category of cattle and have been used for several genetic improvement programmes in the past (Wijeratne, 1970; Tilakaratne et al., 1974). However, no systematic approach has been taken to identify and describe their phenotypic or genotypic differences, and as a result, the total indigenous population remains as a nondescript type of animal except for few breeds/types. The objective of this research was therefore to identify the production status, farming systems and genetic diversity of indigenous cattle in Sri Lanka.

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## MATERIALS AND METHODS

### Selection of Locations and Sampling Process

Sampling locations were selected to represent the general indigenous population of the country. Five geographically distinct indigenous cattle populations (NC – North Central, So – Southern, No – Northern, Tk – Thamakaduwa and Th – Thawalam) were investigated for morphological traits (Figure 1), and four (all except Th) were included in genetic diversity analysis. While the NC, So and No sampling locations represent the general indigenous cattle populations, the Tk and Th locations signify two cattle populations subjected to isolated breeding and selection for specific purposes over a long period. The respective farming systems were also evaluated to complete the requirement in developing conservation and utilisation strategies. Sampling was carried out based on the non-existence of artificial insemination facilities to assure the target populations were indigenous. The six populations were assumed to be genetically isolated from each other in the absence of a nomadic pattern of rearing and regular cattle migration. The farming systems were analysed by single visits to each location using a pre-tested structured questionnaire. Single visits were practised as there is no variation in farming system according to the period of the year. Morphometric measurements were taken during the visit. Blood samples were collected from the jugular vein for molecular genetic analysis.

### Data Analysis

Farming system and morphometric data were compiled and analysed using Microsoft Access and Microsoft Excel software packages.

### Molecular Analysis

DNA was purified from blood samples following a standard protocol (Sambrook and Russel, 2001) and all samples belonging to each population, were genotyped using 15 autosomal microsatellite DNA markers (AGLA293, ILSTS023, ETH225, HEL01, ILSTS006, INRA032, MGTG4B, TGLA122, ETH152, INRA035, ILSTS050, BM2113, ILSTS005, CSSM66 and INRA005) and a Y-specific microsatellite marker (INRA 132), at the Biotechnology Laboratory of the International Livestock Research Institute (ILRI), Nairobi, Kenya. Molecular data were analysed using Microstat and DISPAN software packages.

## RESULTS AND DISCUSSION

Farming system analysis revealed that indigenous cattle are reared as a traditional practice in all regions of the country under limited or no input environments. Since their low productivity masks their real contribution to rural livelihoods, the level of utilisation needs to be assessed taking the systems approach into consideration. This is an important aspect since the utilisation of cattle always is confounded by the attributes of respective farming systems (Silva et al., 2008). The contribution of indigenous cattle to total tangible income ranged from zero to 90% in different regions reflecting the high variation in the purpose of keeping indigenous cattle (Figure 2). Integration with crops, especially paddy rice, was the most common feature of the systems across the regions. Feeding and other management practices are not expensive and hence their contributions to total production costs are marginal. Feeding practices within indigenous cattle production systems varied depending on the resource availability within the system (Figure 3). Expenses connected with keeping the animals are limited to veterinary care and health management. However, in most cases, money is not spent on medicines unless these are essential, and use of indigenous medicine is common.

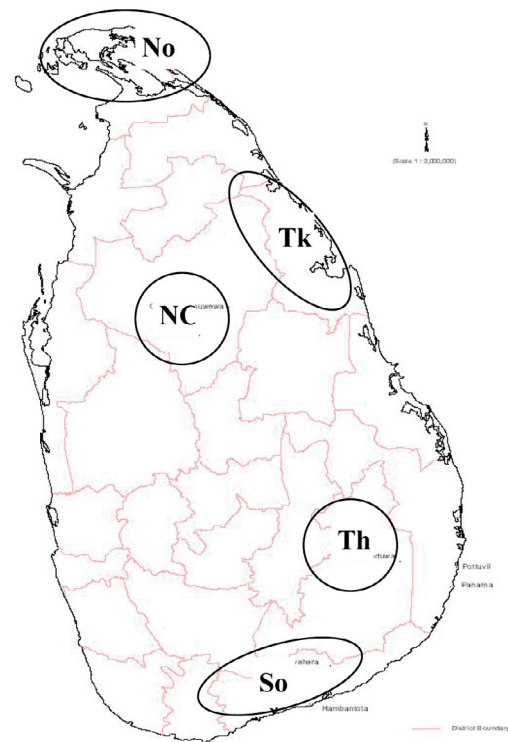


Figure 1. Sampling locations of indigenous cattle populations from Sri Lanka.

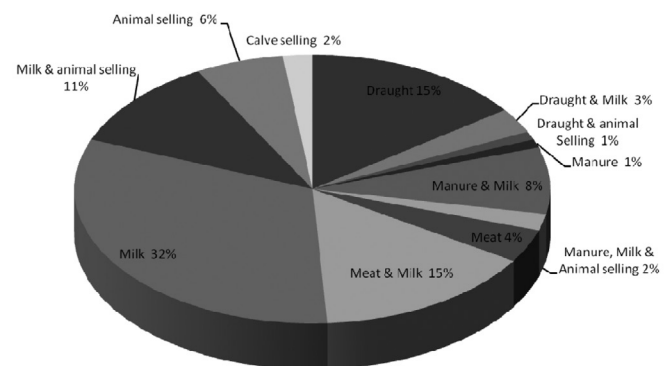


Figure 2. Purpose of rearing of indigenous cattle in Sri Lanka.

In this context, the present study confirms previous findings that the rearing of indigenous cattle is a profitable enterprise (Silva et al., 2008).

Morphometric measurements identified specific phenotypic characteristics resulting from geographical isolation and selective breeding. Table 1 shows the morphometric measurements of indigenous cattle in general and in different populations. The comparison among different populations revealed a significantly higher ( $P < 0.05$ ) heart girth, height at withers and body length in Tk and Th populations. These two populations have been selected and bred for the purpose of providing draught power. Morphological measurements showed that the indigenous cattle are small and compact, mostly similar to the Zebu type cattle found in the tropics (Table 2). These observations are comparable with earlier results obtained concerning the phe-

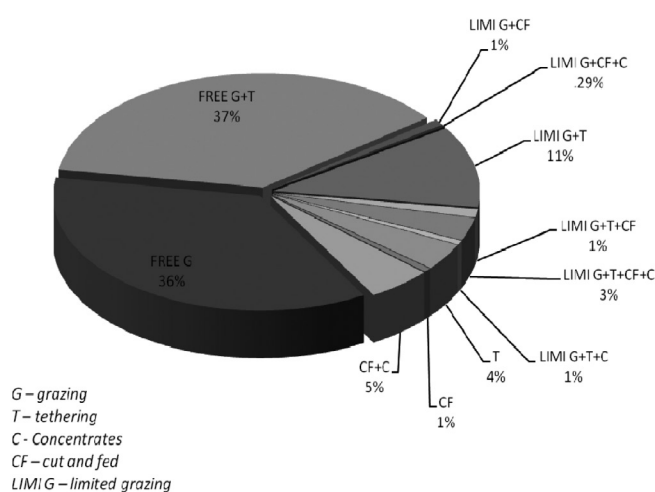


Figure 3. Variation of feeding practices of indigenous cattle in Sri Lanka.

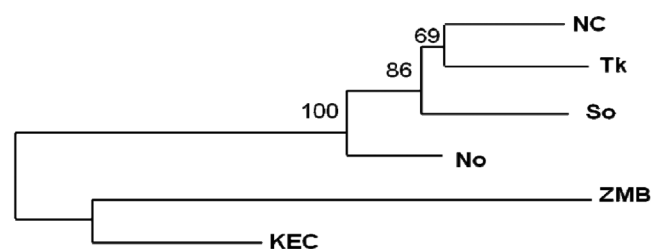


Figure 4. Phylogenetic tree showing the genetic distance among four indigenous cattle populations from Sri Lanka (NC, So, No and Tk) and two African populations (ZMB and KEC) (1 000 bootstrap).

Table 1. Morphometric measurements (cm) of different indigenous cattle populations from Sri Lanka.

Population <sup>1</sup>		Heart Girth	Height at withers	Hip width	Body length	Head length	Head width
NC	M	132 ± 09.7	101 ± 06.5	32 ± 3.2	103 ± 03.8	41 ± 3.1	16 ± 1.4
	F	132 ± 09.7	101 ± 06.4	32 ± 3.3	106 ± 08.8	41 ± 3.1	16 ± 1.6
So	M	125 ± 13.7	96 ± 09.7	30 ± 5.6	97 ± 12.2	38 ± 5.1	16 ± 2.7
	F	125 ± 13.2	96 ± 09.2	30 ± 5.5	97 ± 11.0	38 ± 4.9	16 ± 2.6
No	M	110 ± 18.9	93 ± 08.7	29 ± 4.2	97 ± 09.4	35 ± 3.7	12 ± 1.5
	F	111 ± 18.6	94 ± 08.5	30 ± 4.1	97 ± 09.2	35 ± 3.7	12 ± 1.5
Tk	M	150 ± 15.8	123 ± 16.0	33 ± 3.8	128 ± 33.2	41 ± 2.6	16 ± 1.7
	F	131 ± 13.0	118 ± 20.1	38 ± 5.0	109 ± 05.0	41 ± 3.0	14 ± 2.0
Th <sup>2</sup>	M	140 ± 12.3	112 ± 09.6	31 ± 4.6	116 ± 10.2	44 ± 4.1	14 ± 2.7
Overall	M	131 ± 19.1	100 ± 12.0	32 ± 5.6	105 ± 15.1	40 ± 4.4	15 ± 2.8
	F	131 ± 19.1	100 ± 11.9	32 ± 5.5	104 ± 15.1	40 ± 4.2	15 ± 2.5

<sup>1</sup> NC — North Central population; So — Southern population; No — Northern population; Tk — Thamakaduwa population; Th — Thawalam population; M — male; F — female.

<sup>2</sup> Only male population was available for measurements in pack animals.

Table 2. Morphological and production characteristics of indigenous cattle from Sri Lanka.

Morphological/production characteristics	Measurement/ Description
Coat colour	Black, brown (dark, light), gray, white and mixtures
Ear	Small / medium erect in horizontal orientation
Hump and top line	Small hump with straight top line
Head shape	Long and narrow with flat forehead
Hair type	Short and glossy
Gestation period (d)	250–290 d
Calving interval (months)	18 (10–27)
Age at first calving (months)	36 (18–60)
Milk yield (L/cow/d)	1.05 (0.5–1.2)
Lactation length (months)	3.5 (0.5–12)
Dry period (months)	3.0 (2–12)

**Table 3. Number of alleles and allelic range from 15 autosomal microsatellite DNA markers in indigenous cattle from Sri Lanka.**

Locus	No. individuals	No. alleles	Allelic range
AGLA293	207	16	216–244
ILSTS023	207	12	170–202
ETH225	207	11	137–159
HEL01	207	9	101–117
ILSTS006	207	11	276–302
INRA032	207	13	162–204
MGTG4B	207	13	134–162
TGLA122	207	15	137–171
ETH152	207	7	189–203
INRA035	207	10	105–129
ILSTS050	207	11	140–162
BM2113	207	9	126–144
ILSTS005	207	7	180–194
CSSM66	207	15	178–206
INRA005	207	7	138–148

**Table 4. Between population DA distance of indigenous cattle from Sri Lanka.**

	NC	So	No	Tk
NC	1			
So	0.0723	1		
No	0.0772	0.0854	1	
Tk	0.0662	0.0773	0.0820	1

notypic characteristics of indigenous cattle (Tilakaratne, 1980 and 1984).

Genetic diversity analysis, based on genotyping of individuals using 15 microsatellite markers (Table 3), indicated that indigenous cattle in Sri Lanka have a high genetic diversity with the average number of alleles per locus ranging from 7.9 to 8.5. The average heterozygosity of different regions varied within a narrow range ( $0.72 \pm 0.04$  to  $0.76 \pm 0.03$ ). As shown in Table 4, the genetic distances (Da distance) between regions were low (ranging 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 was found between their distribution in different regions. The comparison of indigenous cattle in Sri Lanka with two other indigenous cattle types (KEC and ZMB) revealed that the indigenous cattle in Sri Lanka are genetically distinct (Figure 4). Introgression of Taurine cattle was

evidenced in one of the cattle populations (NC) as suggested by the Y-specific microsatellite DNA analysis.

## CONCLUSIONS

Indigenous cattle in Sri Lanka are reared in low input mixed crop farming systems and serve a variety of purposes. Based on the phenotypic evaluations, the Sri Lankan indigenous cattle could be described as a distinct population consisting of small compact non-specialised animals with little regional variation. Molecular investigations revealed high levels of diversity within populations and a predominant Zebu origin with subsequent introgression of Taurine cattle.

## ACKNOWLEDGEMENTS

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# Potential of Tropical Plants to Exert Defaunating Effects on the Rumen and to Reduce Methane Production

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## ABSTRACT

This paper summarises the principal results obtained in Cuba concerning the potential of different tropical plants to exert defaunating effects in the rumen and to reduce methane (CH<sub>4</sub>) production. The plants studied were *Sapindus saponaria*, *Morus alba*, *Trichanthera gigantea*, *Tithonia diversifolia*, *Gliricidia sepium*, *Leucaena leucocephala*, *Stizolobium aterrimum* and *Arachis pintoi*. Grasses used as forage in the assays to obtain grass:foliage mixtures were *Pennisetum purpureum* Cuba CT-115 or Star grass (*Cynodon nlemfuensis*). The experiments were conducted using an *in vitro* system. Gases produced in the fermentation process were collected at intervals of 4, 8, 12 and 24 h and CH<sub>4</sub> production was determined by gas chromatography. Phytochemical analyses indicated the presence of tannins, saponins and others secondary compounds. *Enterolobium* and *Leucaena* had a high content of tannins and moderate levels of saponins. *Morus* contained moderate amounts of saponins. The inclusion of 15% *Leucaena* and *Gliricidia*, 20% *Sapindus* and *Arachis* as well as 40% *S. aterrimum*, negatively affected protozoal populations. The inclusion of 25% *Sapindus*, *Morus* and *Trichanthera* foliages using *P. purpureum* as the pasture base reduced CH<sub>4</sub> production significantly. The results suggest that the use of trees and shrubs to supplement low quality forages seems appropriate for reducing CH<sub>4</sub> production and improving animal nutrition in tropical areas.

**Key words:** *tropical plants, defaunating effects, methane, phytochemical analysis, tannins, saponins.*

## INTRODUCTION

The tropical zone includes 37% of plant lands. Many domestic ruminants live in this environment and their principal sources of feed are low quality pastures and forages with little or no supplementation. The efficiency of digestion in the rumen requires a diet that contains essential nutrients for fermentative micro-organisms. Lack of or deficiencies in these nutrients decreases animal productivity and raises CH<sub>4</sub> emissions/unit of product.

Methane emitted from livestock not only represents a loss of between two and 12% of the gross energy consumed by the animal (Johnson and Johnson, 1995), but contributes to global warming because it is a greenhouse gas (Bauchemin et al., 2008). Thus, miti-

gating CH<sub>4</sub> losses from cattle has both long-term environmental and short-term economic benefits.

Numerous strategies have been developed to reduce ruminal methanogenesis by means of chemical (Itabashi et al., 2000), and biotechnological methods (McAllister et al., 1996; Attwood and McSweeney, 2008) as well as by nutritional management, including through supplementation. It is well established that inclusion of concentrates in the diet reduces the proportion of dietary energy converted to CH<sub>4</sub> and improves animal performance (Bauchemin et al., 2008). However, the use of concentrates to mitigate CH<sub>4</sub> emissions from livestock is impossible to implement in many areas of the world due to the high price of cereals on the market and its competition with human feed.

There are naturally occurring compounds in some forages that appear to have anti-methanogenic properties, specially tannins and saponins. The use of trees and shrubs as ruminal manipulators, mainly to reduce protozoal population has been reported (Navas-Camacho et al., 1994; Hess et al., 2003; Galindo et al., 2005).

The enormous biological diversity within tropical trees and shrubs provides a bank of materials that can be used in animal feeding to improve productive efficiency and also as future strategies for CH<sub>4</sub> mitigation. The use of these plants in agro forestry or sylvopastoral systems could be an economically viable strategy in Cuba. Here, trees and shrubs have been evaluated with the objective of determining their nutritive value and effects on ruminal microbial fermentation and CH<sub>4</sub> mitigation, and other new plants are now being evaluated for these purposes under *in vitro* and *in vivo* conditions.

This paper summarises the principal results obtained in Cuba concerning the potential of different tropical plants for use as animal feeds, and regarding their possibilities for exerting defaunating effects on the rumen and reducing CH<sub>4</sub> production.

## MATERIALS AND METHODS

### Locations, Plants and their Preparation

The studies were carried out in areas around the Instituto de Ciencia Animal which is located at a northern latitude of 22° 53' and a western longitude of 82° 02', and at 92 m above sea level. The plants studied were *Sapindus saponaria*, *Morus alba*, *Trichanthera gigantea*, *Tithonia diversifolia*, *Gliricidia sepium*, *Leucaena leucocephala*, *Stizolobium aterrimum* and *Arachis pintoi*. For the preparation of plant foliages, leaves with petioles and young stems were collected in a manner simulating animal selection according to the methodology of Paterson et al. (1983). The grasses used as forage to obtain mixes of grass:foliages were *Pennisetum purpureum* Cuba CT-115 and four-

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month old established or mature Star grass (*Cynodon nlemfuensis*). The foliage and grass forage were sun dried on a plate for 2–3 d until their humidity was reduced to between 20% and 25%; they were then ground to obtain a particle size of 1 mm.

### Screening for Secondary Metabolites

Phytochemical screening was carried out and the presence of secondary metabolites detected as described by Rondina (1969). The experimental procedure was as follows: dry and milled material was macerated for 48 h in ethanol and refluxed for 4 h., which enabled separation into a part (Fraction A) with the remainder being evaporated to dryness. The dry extract was then diluted with HCl and the insoluble fraction diluted with dichloromethane; this constituted Fraction B. The acid solution was then neutralised with NH<sub>4</sub>OH and extracted with dichloromethane, the organic phase constituting Fraction C. The liquid phase was extracted with a mixture of dichloromethane-ethanol, the solid phase constituting Fraction D and the liquid phase Fraction E. Starting with the raw material, Fraction F was obtained by extraction with boiling water.

The assays used to detect the secondary metabolites were:

- *Tannins*: in Fraction A by means of the Jello test
- *Triterpenoides and steroids*: in Fractions B, C and D by means of the Lieberman Bourchard reaction
- *Alkaloids*: in Fractions C and D using the Mayer and Dragendorff tests
- *Flavinoids*: in Fractions D and E, detected by the Shinoda reaction
- *Leucoantocianidins and catequins*: in Fractions D and E by means of the Rosenheim test
- *Saponins*: in Fraction F, by the foam test
- *Total polyphenols and condensed tannins (CT)*: condensed tannins were quantified according Terrill et al. (1992), and total polyphenols by the methodology of Makkar and Goodchild (1996).

### Microbiological Studies

The effects of the most promising trees and shrubs on ruminal microbial fermentation were conducted by the technique of submerged tissue culture with mechanical agitation at 39°C. Fermentation tubes with a capacity of 250 mL were placed in incubation baths. In each tube, 1.5 g of previously dried material was added containing Star grass (*Cynodon nlemfuensis*), the selected plant and 150 mL of filtered rumen liquid from two Holstein bulls fed Star grass and fitted with a simple rumen cannula.

The effect of condensed tannins on the ruminal protozoa population was similarly studied. The treatments consisted of: i) Star grass + ground maize and ii) Star grass + ground maize and condensed tannins (CT). Star grass was included at a rate of 0.75 g DM/tube and the maize at 0.25 g DM/tube. The amount of CT was equivalent to 5 mg/100 g DM. The condensed tannins were isolated from *L. leucocephala* foliage. Sampling of ruminal liquid were carried out before and at two and four h and the experiment was replicated eight times.

### Effects on Methane Production

Seven treatments were studied: T1: grass forage (*Pennisetum purpureum* clon Cuba CT-115; T2: *Trichanthera gigantea* foliage, T3: *Sapindus saponaria* foliage; T4: *Morus alba* foliage; T5, T6 and T7 were foliage:grass mixes at ratios of 25:75 of *Trichanthera:Pennisetum*, *Sapindus:Pennisetum* and *Morus:Pennisetum*, respectively.

The technique of *in vitro* gas production (Theodorou et al., 1994), used Menke media (Menke and Steingass, 1988). Samples (500 mg ground feed) were added to 30 mL buffer/rumen fluid mixture (10

mL rumen/20 mL buffer) bottles of 100 mL (four replicates per treatment), sealed in anaerobic conditions and incubated in a water bath at 39 °C. Rumen fluid from two rumen-fistulated crossbreed Zebu steers fed on low quality Star grass and one kg of concentrate was strained before the morning feed through polyester cloth. Ruminal fluid was maintained under O<sub>2</sub>-free CO<sub>2</sub>.

The mixture of gases from the fermentation process was collected by displacing the volume in 20 mL syringes at intervals of 4, 8, 12 and 24 h. The gaseous content of the syringes was injected into a gas chromatograph, and the percentage CH<sub>4</sub> calculated by reference to a standard sample (purity 100%). Methane concentrations were then calculated by the general gases equation.

### Microbial Analysis

Total viable and cellulolytic bacteria and cellulolytic fungi were counted according to the anaerobic technique of Hungate (1950). The culture medium of Caldwell and Bryant (1966) modified by Elias (1971) was used for total viable and cellulolytic bacteria. The culture medium for fungi included 100 000 IU penicillin and 0.1 g of streptomycin /100 mL of culture media. Protozoa were counted using a microscope after being preserved in 10% formaldehyde.

### Statistical Analysis

Data were analysed using the SPSS system for Windows by a simple classification model for a completely random design. Differences between means were detected by the Duncan test (1955). In the studies of the effect of foliage on microbial populations, a factorial design was used to evaluate treatments.

## RESULTS

The chemical composition of the plants evaluated is shown in **Table 1**. Protein levels of foliage ranged between 10.5% and 28.5%, the average being 20.8% which is higher than those observed in most tropical grasses. *Arachis* was the foliage with the lowest crude protein (CP) and highest neutral detergent fibre (NDF) content.

The phytochemical analysis (**Table 2**) indicated the presence of secondary compounds. All plants had variable amounts of tannins, saponins and other metabolites. *Enterolobium*, *Leucaena* and *Stizolobium* showed high content of tannins and medium levels of saponins. *Morus alba* showed high levels of saponins and triterpenes, while the content of secondary metabolites in *Tithonia* and *Gliricidia* was not very high.

The content of total polyphenols and condensed tannins in foliage (**Table 3**) shows the higher values of these compounds (5.84% and 1.80%, respectively) in *Leucaena*, while *Sapindus* was the plant with lowest values of condensed tannins.

In other assays, information from previous studies on these plants was used to establish the relationship between the concentration of condensed tannins and total polyphenols and the population of ruminal protozoa. It was found that there was a quadratic relationship between these variables:

Total polyphenols/protozoa:  $y = 7.1915 - 171.72 X + 2137.8 X^2$ ,  $R^2 = 0.99$

Condensed tannins/protozoa:  $y = 45.523 - 1004 X + 5921.3 X^2$ ,  $R^2 = 0.99$ .

**Table 1. Chemical composition plants (% DM).**

Plant	OM	CP	NDF	ADF	Lignin
<i>L. leucocephala</i>	90.5	27.69	47.37	33.86	8.30
<i>G. sepium</i>	93.7	28.5	40.40	20.10	4.40
<i>S. saponaria</i>	83.78	16.3	50.90	33.2	9.40
<i>E. cyclocarpum</i>	88.20	14.8	55.80	35.80	8.30
<i>S. aterrimum</i>	92.42	25.69	46.33	29.18	—
<i>A. pinto</i>	87.47	10.57	58.75	43.32	8.27
<i>T. gigantea</i>	78.15	19.83	45.34	28.37	6.11
<i>M. alba</i>	88.80	19.51	31.76	27.15	7.65
<i>T. diversifolia</i>	94.06	23.95	33.43	29.54	—
Mean	88.56	20.76	45.51	31.27	7.36
S.D.	5.09	5.81	9.16	6.05	1.56

**Table 2. The phytochemical screening of plants.**

Plants foliage	Tannins	Flavonoids	Saponins	Triterpenes	Esteroids	Antho-cianidins	Alcaloids
<i>L. leucocephala</i>	+++*	+	++	++	++	+	+++
<i>Arachis pinto</i>	+	+	++	+	+	+	+++
<i>S. aterrimum</i>	+++	+	+	+	+	+	+++
<i>E. cyclocarpum</i>	+++	+	++	+	+	+	++
<i>S. saponaria</i>	++	—	+	+++	—	—	++
<i>Gliricidia sepium</i>	+	+	++	+	+	+	—
<i>T. gigantea</i>	++	—	—	++	ND	—	+
<i>Morus alba</i>	++	—	+++	+++	ND	—	+
<i>Tithonia diversifolia</i>	++	++	+	+	++	+	++

ND — not detected.

\*+ indicates qualitatively the level of metabolite in the sample.

**Table 3. Content of total polyphenols and condensed tannins (% DM).**

Plants	Total polyphenols (%)	Condensed tannins (%)
<i>L. leucocephala</i>	5.84	1.80
<i>G. sepium</i>	1.92	0.30
<i>S. saponaria</i>	0.46	0.18
<i>E. cyclocarpum</i>	1.37	1.49
<i>S. aterrimum</i>	2.14	2.30
<i>A. pinto</i>	1.18	1.19

### Effect of Some Legume Trees and Shrubs on Ruminal Microbial Populations

Supplementation of low quality grasses (Star grass or *Pennisetum*) with levels ranging from 2.5% to 60% of DM of different tropical foliages produced a marked defaunating effect, as well as promoting a greater population of bacteria and ruminal cellulolytic fungi, which may increase ruminal cellulolysis, the degradation of the fibrous fraction and consequently feed intake (Table 4). Unexpectedly, *Enterolobium* did not exert defaunating effects at least under the experimen-

tal conditions in which the studies were conducted, but increased the ruminal cellulolytic fungi population.

Supplementing the diet with 20% *Tithonia diversifolia* reduced the methanogenic bacteria and protozoa populations. In addition, *S. saponaria* at a rate of 2.5, 5 and 10% of the DM in forage diets, did not increase cellulolytic bacteria populations, but activated viable cellulolytic fungi, leading to a significant reduction in protozoa.

Levels of substituting *L. leucocephala* for grass at up to 60% increased populations of ruminal cellulolytic bacteria from  $2.92 \times 10^7$  to  $6.83 \times 10^7$  cfu/mL. Likewise, this increased the population of total

**Table 4. Effect of different levels of tropical foliage on ruminal microbial population.**

Foliage/Star grass rate, % DM	Cellulolytic bacteria	Protozoa	Cellulolytic fungi	Methanogenic bacteria
<i>L. leucocephala</i>				
	10 <sup>6</sup> cfu/mL	10 <sup>6</sup> n/mL	10 <sup>6</sup> cfu/mL	
0/100	0.46 (2.92) <sup>a</sup>	1.67 (46.4) <sup>a</sup>	1.54 (34.5) <sup>a</sup>	—
20/80	0.56 (3.67) <sup>b</sup>	1.66 (45.7) <sup>b</sup>	1.67 (47.3) <sup>a</sup>	—
60/40	0.83 (6.83) <sup>c</sup>	1.73 (53.4) <sup>b</sup>	1.99 (99.1) <sup>b</sup>	—
SE ±	0.28***	1.4***	4.7*	—
<i>T. diversifolia</i> <sup>1</sup>				
	10 <sup>6</sup> cfu/mL	10 <sup>5</sup> n/mL	10 <sup>6</sup> cfu/mL	10 <sup>9</sup> cfu/mL
0/100	1.40 (24.9) <sup>a</sup>	0.57 (3.75) <sup>a</sup>	1.42 (26.1)	1.65 (45.2) <sup>a</sup>
10/90	1.75 (55.8) <sup>b</sup>	0.51 (3.25) <sup>a</sup>	1.46 (28.9)	1.44 (27.5) <sup>b</sup>
20/80	1.46 (29.2) <sup>a</sup>	0.18 (1.5) <sup>b</sup>	1.40 (25.2)	1.22 (16.8) <sup>c</sup>
SE ±	0.12*	0.02*	0.15	0.45**
<i>G. sepium</i> <sup>1</sup>				
	10 <sup>6</sup> cfu/mL	10 <sup>5</sup> n/mL	10 <sup>5</sup> cfu/mL	—
0/100	0.8 <sup>a</sup> (6.3)	1.70 <sup>c</sup> (45.7)	0.90 <sup>a</sup> (7.4)	—
15/85	0.9 <sup>a</sup> (7.9)	1.05 <sup>b</sup> (11.2)	0.92 <sup>a</sup> (8.3)	—
30/70	1.14 <sup>b</sup> (13.9)	0.41 <sup>a</sup> (2.6)	1.19 <sup>b</sup> (15.5)	—
SE ±	0.14*	0.09***	0.3*	—
<i>E. cyclocarpum</i> <sup>1</sup>				
	10 <sup>5</sup> cfu/mL	10 <sup>5</sup> n/mL	10 <sup>6</sup> cfu/mL	—
0/100	0.41(4.49)	0.59(3.95)	0.24 <sup>a</sup> (5.26)	—
15/85	0.58 (9.13)	0.56(3.62)	0.89 <sup>b</sup> (12.30)	—
20/80	0.67 (7.88)	0.69(4.99)	0.69 <sup>a</sup> (4.99)	—
SE ±	0.04	0.04	0.19*	—
<i>S. saponaria</i> <sup>1</sup>				
	10 <sup>6</sup> cfu/mL	10 <sup>6</sup> n/mL	10 <sup>6</sup> ftu/mL	—
0/100	0.74 (5.50)	0.86 <sup>a</sup> (7.24)	0.69 <sup>b</sup> (4.90)	—
2.5/97.5	0.66 (4.58)	0.73 <sup>b</sup> (5.31)	0.76 <sup>ab</sup> (5.75)	—
5/95	0.47 (2.95)	0.65 <sup>bc</sup> (4.47)	1.05 <sup>a</sup> (11.22)	—
10/90	0.69 (4.90)	0.58 <sup>c</sup> (3.80)	1.07 <sup>a</sup> (11.95)	—
SE ±	0.14	0.04***	0.11*	—
<i>S. aterrimum</i> <sup>1</sup>				
	10 <sup>7</sup> cfu/mL	10 <sup>6</sup> n/mL	10 <sup>7</sup> cfu/mL	—
0/100	1.55 (35.5)	1.52 (33.4)	0.76 (5.8)	—
20/80	1.39 (24.6)	1.19 (15.7)	0.97(9.3)	—
40/60	1.01 (10.2)	1.05 (11.2)	1.024 (18.6)	—
<i>A. pinto</i> <sup>1</sup>				
	10 <sup>7</sup> cfu/mL	10 <sup>6</sup> n/mL	10 <sup>7</sup> cfu/mL	—
0/100	0.64 (4.4)	0.90 (8.0)	0.92 (8.3)	—
20/80	0.62 (4.2)	0.97 (9.3)	0.58 (3.8)	—
40/60	0.66 (4.6)	1.00 (10.1)	0.99 (9.8)	—

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

<sup>1</sup> Data transformed according to log<sub>10</sub>x where x = total colony count. Original values between parentheses.

\* (P < 0.05); \*\* P < 0.01); (\*\*\*)P < 0.001).

viable bacteria and cellulolytic fungi in the rumen. The presence of this plant in the feed reduced (P < 0.001) the protozoal population.

*S. aterrimum* is another plant that negatively affects the population of protozoa in the rumen. The inclusion of this legume reduced the population of ruminal protozoa in proportion to its level of inclusion in the diet. Also, cellulolytic bacteria were affected negatively. *Arachis pinto*, on the other hand, did not modify ruminal bacteria populations, but it also affected the populations of total protozoa.

### Effect of Condensed Tannins from *L. leucocephala* on Populations of Ruminal Protozoa

A study was conducted to evaluate the effect of condensed tannins extracted from leaves and petioles of *L. leucocephala* on the population of ruminal protozoa. The results (Table 5) suggest that tannins exert a reducing effect on total populations and that both *Entodiniomorphid* and *Holotrichia* protozoa are modified in the presence of these metabolites.



**Table 5. Effect of the condensed tannins extracted from *L. leucocephala* on the population of ruminal protozoa (10<sup>6</sup> cells/mL).**

Treatments	Control (time in h)			Condensed tannins (time in h)		
	0 h	2 h	4 h	0 h	2 h	4 h
Total protozoa	64.75	81.0	126.5	62.5	31.0	24.5
<i>Entodiniomorphid</i>	62.63	77.38	120.0	60.50	29.63	23.63
<i>Holotrichia</i>	2.38	4.13	6.5	2.00	1.38	1.63

**Table 6. Methane emissions and dry matter degradability in the experimental diets with tropical plant foliage and *Pennisetum purpureum* forage.**

Treatments	Methane production		
	mL	mL/kg DM	g/kg DM
<i>Pennisetum</i>	13.48 <sup>a</sup>	26.18 <sup>a</sup>	16.96 <sup>a</sup>
<i>Sapindus</i>	7.32 <sup>c</sup>	14.01 <sup>cd</sup>	9.10 <sup>de</sup>
<i>Trichanthera</i>	5.62 <sup>c</sup>	10.82 <sup>d</sup>	7.01 <sup>e</sup>
<i>Morera</i>	7.52 <sup>c</sup>	13.76 <sup>cd</sup>	8.93 <sup>e</sup>
<i>Sapindus</i> :forage 25:75	9.13 <sup>c</sup>	17.02 <sup>bc</sup>	11.37 <sup>d</sup>
<i>Morus</i> :forage 25:75	10.30 <sup>ab</sup>	19.10 <sup>b</sup>	12.40 <sup>b</sup>
<i>Trich.</i> :forage 25:75	8.02 <sup>c</sup>	18.02 <sup>bc</sup>	11.60 <sup>d</sup>
ES ± Sign	1.20 <sup>*</sup>	1.52 <sup>**</sup>	0.94 <sup>**</sup>

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

\* (P<0.05); \*\* (P<0.01).

When the effect of *Sapindus Saponaria*, *Morus alba* and *Trichanthera gigantea* on CH<sub>4</sub> production in *in vitro* conditions was evaluated, CH<sub>4</sub> production (mL/kg DM) produced by the grass was higher than that obtained with foliages or its mixes with *Pennisetum* (Table 6). Methane values for *Trichanthera*, *Sapindus*, *Morus* were, however, similar. Foliage/*Pennisetum* mixtures produced more CH<sub>4</sub> than foliages alone, but values were lower than for grass, showing the anti-methanogenic effects of these plants.

## DISCUSSION

To develop new feeding strategies with the aim of mitigating CH<sub>4</sub> emissions to environment and to improve the productivity of ruminants, the most promising approaches are those which suppress the microbes involved in methanogenesis without affecting fibre-degrading bacteria (Soliva et al., 2003).

In tropical conditions, there are many possibilities available for manipulating the rumen microbial ecosystem to achieve CH<sub>4</sub> reductions using trees and shrubs with high nutritive value and naturally secondary compounds, principally tannins and saponins that appear to have defaunants and antimethanogenic properties. The use of these plants in feeding strategies could be advantageous and cheaper if appropriate technologies are developed.

In general, tannins and saponins were found in all the foliages studied. These suppress CH<sub>4</sub> emissions, reduce rumen protozoa counts, and change rumen fermentation patterns (Hristov et al., 1999; Hess et al., 2003; Galindo, 2004; Hu et al., 2005).

When the correlation between polyphenols and condensed tannins present in tropical plants and protozoal population were studied, it was found that there was a quadratic relationship between them. Galindo (unpublished data) found inverse relationships between the methanogenic population of ruminal bacteria and protozoal populations. The presence of these compounds in most foliages eval-

uated could explain the effects of reducing rumen protozoa counts and suppressing the CH<sub>4</sub> emissions recorded in the plants studied.

The polyphenolic fraction in *Leucaena* was greater than in other foliages (Table 3). Garcia et al. (2009) characterised the chemical composition of the foliage of 53 Cuban accessions from the *Leucaena* genus and found that the polyphenolic fraction ranged between 3.4% and 5.02% DM, i.e. lower than the lowest measured in this study (5.82%). However, the condensed tannins fraction reported by the same authors was higher than that obtained here (3.78% vs 1.80%, respectively). Different experimental conditions, plant species, chemical structures of the compounds, their biological activity, analytical methods used and other factors could all explain these differences, and clearly further work is required to explain such variations.

Supplementing low quality forage with *Gliricidia*, *Leucaena*, *Thitonia* and *Stizolobium* produced a marked defaunating effect as well as encouraging a larger population of bacteria and ruminal cellulolytic fungi. Such effects may increase ruminal cellulolysis and fibre degradation, and consequently could reduce CH<sub>4</sub> emissions. It is interesting to note that most plants reduced protozoal counts, increasing the cellulolytic microorganisms responsible for fibre degradation.

*Leucaena* is possibly one of most used plants for animal feed in Cuba. It is used as a protein biomass bank and in sylvopastoral and agroforestry systems with good results (Alvarez et al., 2006). In the present studies, *Leucaena* foliage had the highest levels of polyphenols and tannins. The condensed tannins extract exerts a decreasing effect on total rumen protozoa populations. Both protozoa *Entodiniomorphid* and the *Holotrichia* were modified in presence of condensed tannins, which proves, once more, that these plant metabolites act as defaunating agents though death of these microorganisms. Recent studies carried out in grassland of *L. leu-*

*cocephala* in association with natural grass mixtures indicated that ruminal protozoa were reduced in cattle when this tree was included in the system, independently of inclusion level (30% or 100% of the area, Galindo et al., 2007).

Methane production decreased with the inclusion of foliages in the diets. There is some experience with the use of plant foliages as defaunants, but results concerning effects on CH<sub>4</sub> production are scarce, although Woodward et al. (2001) found a depression of CH<sub>4</sub> emissions in sheep and dairy cows fed with the condensed tannin-containing legume *Lotus corniculatus*. Also, Soliva et al. (2003) reported that *S. saponaria* (fruits) reduced CH<sub>4</sub> emissions and counts of ciliate protozoa and that all three foliages studied (*Trichanthera*, *Sapindus* and *Morus alba*) reduced such emissions relative to grass forage when included in the diet at the rate of 25% DM.

## CONCLUSIONS

The results suggest that as strategies of supplementation of low quality forage, the use of trees and shrubs seems to be an adequate option to reduce CH<sub>4</sub> production and improve animal nutrition in tropical areas. With the exception of *Arachis* and *Enterolobium*, the plants examined here showed defaunating properties and had the potential to mitigate CH<sub>4</sub> emissions.

The contribution of such plants to improve the efficiency of the rumen and reduce CH<sub>4</sub> emissions is an attractive area of study and while their potential has been demonstrated in the laboratory, it is necessary to verify this in livestock production systems.

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# Evaluation of Spineless Cactus (*Opuntia ficus-indicus*) as an Alternative Feed And Water Source for Animals during the Dry Season in Eritrea

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## ABSTRACT

Throughout East Africa, animal feed resources fluctuate seasonally and are often of limited availability. Finding alternative feed resources that can sustain animal production during the long dry season is an essential need. Cactus is a drought-tolerant and succulent feed resource available throughout the year in Eritrea. This study was conducted to evaluate the effect of including increasing levels of spineless cactus in the diet of sheep fed urea-treated barley straw. Twenty four fat tailed Highland male sheep with mean live weight of 21.1 kg were randomly assigned to four treatments (T1-T4). Animals in T1 received urea (5%) -treated barley straw (UTBS) alone *ad libitum*, while those in T, T3 and T4 received *ad libitum* UTBS supplemented with 175 g, 350 g and 525 g of spineless cactus (dry matter [DM] basis), respectively. With increasing level of cactus, there were significant increases in DM intake ( $P < 0.001$ ) and bodyweight (BWt) gain ( $P < 0.05$ ), while water consumption decreased ( $P < 0.001$ ). The highest DMI was found in the last two treatments (101.8 g/kg BWt<sup>0.75d</sup> and 96.5 g/kg BWt<sup>0.75d</sup>, respectively) as compared with the first two treatments (94.4 g/kg BWt<sup>0.75d</sup> and 87.6 g/kg BWt<sup>0.75d</sup>). Water intake was significantly decreased with the progressive increase in cactus intake. The highest BWt gain (51.9 g/d) was found when sheep received 350 g DM of cactus (T3), while the lowest was in the control diet (26.8 g/d). The metabolism data demonstrated that available energy intake (TDNI) was directly related to animal performance. In conclusion, feeding cactus with UTBS can significantly increase animal performance and feed intake, and reduced water intake.

**Key words:** spineless cactus, urea-treated barley straw, feed intake, water intake, body weight gain.

## INTRODUCTION

Animal feed and water shortages are among the main constraints for the livestock sector in arid and semi-arid regions of East Africa. The major feed resources come from the rangeland pasture and crop residue, the quality and availability of which 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, utilisation of multipurpose trees and shrubs 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. Spineless cactus (*Opuntia ficus-indica*) possesses important characteristics for animal feed in drought-prone regions. This includes high DM yields, drought tolerance, nutritive value and palatability for animals (Tegegne, 2001). Spineless cactus is a fast growing xerophytic plant. Cactus has high water use efficiency due to its crassulacean acid metabolism (CAM) photosynthetic pathway (Nobel, 1995). This makes cacti an extremely important fodder in water-scarce semi-arid regions (Felker and Inglese, 2003). Cactus is a naturalised plant in Eritrea being well adapted to marginal land with poor soil fertility and the low, erratic rainfall conditions. Cactus remains succulent during the long dry season and can serve the animal as a source of feed and water during this period. Furthermore, cactus is suitable as human food, as fuel, for medical uses, as bee forage, and in rangeland rehabilitation projects (Barbera et al., 1995). Cactus pear plays a key role as a lifesaving feed both for human and animals especially in time of drought.

In Eritrea, the use of cactus for animal feed is currently limited to grazing and during the dry season. Cut and carry of cactus is practised during drought periods, but is not common. The cactus peels waste and surplus fruit contribute a substantial amount of feed to ruminants, especially to peri-urban dairy cattle

Although cactus pears have great potential in promoting a sustainable animal production system, knowledge in Eritrea is limited concerning its nutritive value, its utilisation as animal feed, and its role in animal performance. Farmers usually report that their animals get diarrhoea when fed high level of cactus during the dry season. Therefore the aim of this research was to assess the potential of spineless cactus as an alternative source of feed and water for ruminant animals fed poor quality roughage during the dry season.

## MATERIAL AND METHODS

The experiment was carried out in the highlands of Eritrea, which have a semi-arid climate. A randomised complete block design was used to allocate 24 fat tailed Highland male sheep with initial mean live weight of 21.1 kg into one of two replications for four feed treatment groups (T1-T4), consisting of six animals per group. Animals in T1 received *ad libitum* amounts of urea (5% by weight) -treated bar-

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ley straw alone, while those in T, T3 and T4 received *ad libitum* urea-treated barley straw supplemented daily with 175 g, 350 g and 525 g of spineless cactus (DM basis), respectively. Diets were offered twice daily, aiming at 20% refusals. At the end of the feeding trial, four sheep were transferred to metabolic crates where digestibility trials were conducted for each diet over seven days of feeding and collecting. Data were analysed using standard analysis of variance (ANOVA) and general linear models (GLM) with GENSTAT statistical software.

## RESULTS

### Chemical Composition of Spineless Cactus Cladode

The chemical composition (proximate analysis) of the spineless cactus and urea-treated barley straw is presented in **Table 1**. Spineless cactus cladodes were high in water and ash, but low in crude protein and crude fibre content. In this analysis, cactus had 65% more digestible energy than urea-treated straw.

### Effect of Supplementation on Feed, Nutrient and Water Intakes

The performance characteristics of sheep fed urea-treated barley straw supplemented with increasing level of spineless cactus are presented in **Table 2**. The amount of cactus in this study was

restricted; however, it was a highly palatable feed. Except for T, there was a highly significant ( $P < 0.001$ ) increase in DMI with increasing spineless cactus levels in the diet (94.35, 87.57, 101.81 and 96.48 g/kg BWt<sup>0.75d</sup>, for T1, T, T3 and T4, respectively), and as expected a comparable reduction in straw DMI. The highest DMI expressed on a metabolic weight basis (102g/kg BWt<sup>0.75d</sup>) was found in sheep that received 350 g spineless cactus, and this was highly significantly higher ( $P < 0.001$ ) than with the other treatments.

The crude protein intake of the animals was lower for T2 than for the either T1 or T3. When compared with the estimated NRC (1985) requirements (**Table 3**), these animals may have lacked an adequate protein intake.

Increasing the level (or proportion) of spineless cactus in the diet increased the energy density of the diet. Therefore, supplementation of cactus tended to increase energy intake (total digestible nutrient intake or digestible organic matter intake). In contrast, increasing the level (or proportion) of cactus decreased the CP density in the diet. Thus, supplementation of cactus tended to decrease CP intake. The DCPI was highest on diets T1 and T3, and lowest on diet T4.

Water consumption was significantly reduced ( $P < 0.001$ ) with increasing intake of cactus (**Table 2**). Sheep in T1 consumed more water (2 L/d) than the other treatments (0.85, 0.51, 0.15 L/d for T, T3 and T4, respectively). Compared with only urea-treated straw (T1),

**Table 1. Chemical analysis composition of experimental feed ingredients.**

Nutrients (%)	Diets	
	Spineless Cactus	Treated Straw
Dry matter (DM)	12.9	69.42.5
<i>Analysis on DM basis</i>		
Crude protein (CP)	4.75	10.2
Ash	16.77	7.3
Crude Fibre (CF)	15.85	46.5
Ether extract (EE)	0.88	1.1
Nitrogen free extract (NFE)	61.6	34.8
Gross energy (MJ/kg)	13.22	8.0

**Table 2. Performance of sheep supplemented with increasing level of cactus.**

Parameters	T1	T2	T3	T4	LSD	SE
Initial BWt (kg)	21.25	21.25	21.08	21.25	NS	-
Final BWt (kg)	23.67b	24.25b	25.67a	25.50a	0.83	0.26
Weight gain (g/d)	26.80b	33.30b	51.90a	47.20a	12.26	
Cactus DMI (g/d)	0	175	350	525	-	
Total DMI (g/day)	987.2c	905.2d	1080.1a	1062.2b	3.65	8.94
Total DMI (g/kg BWt <sup>0.75d</sup> )	94.35b	87.57c	101.81a	96.48b	3.42	4.05
Water Intake (L/d)	1.98a	0.78b	0.57c	0.18d	0.03	0.04
DOMI (g)	541.80	504.80	667.80	656.30	-	
DCPI (g)	61.20	51.80	61.50	42.60	-	
TDNI (g)	542.00	588.20	672.00	663.10	-	

LSD — least significance difference; DOMI — digestible organic matter intake; DCPI — digestible crude protein intake; TDNI — total digestible nutrient intake; SE — standard error.

Means with different superscripts (a – d) in the same row differ significantly ( $P < 0.001$ ).

**Table 3. Crude protein intakes (CPI in g/d) of sheep (20–25 kg weight) compared with estimated NRC (1985) requirements.**

CPI determined in this study	Treatment	T1	T2	T3	T4
	Observed		97.80	82.79	98.28
CPI estimated from NRC (1985)	25g ADG	92.5	92.5	92.5	92.5
	50g ADG	100.5	100.5	100.5	100.5

ADG — average daily gain; CPI — crude protein intake.

sheep in T2 drank of 50% less water/kg feed intake and sheep in T4 approached no water consumption.

### Effect of Supplementation on Animal Performance

The BWt gain was significantly ( $P < 0.05$ ) higher for sheep on T3 and T4 compared with the control diet (T1). The highest gain (51.9 g/d) was found when sheep received 350 g DM of cactus (T3), while the lowest was in the control T1 diet (26.8 g/d). Although DMI of the sheep in the control was higher than that of sheep in T, sheep in the latter group performed better.

### DISCUSSION

The high water and low CP content of spineless cactus cladodes found in this trial are similar to values reported by other authors (Nefzaoui and Ben Salem, 2001; Flachowsky and Yami, 1985) for cactus pear grown on poor soils. The protein content of cactus was below the general minimum of 7% CP required for normal microbial activity in the rumen. Therefore, animals fed with cactus-based diets need appropriate protein supplementation. This study confirms that urea treatment of low quality forage can be a suitable protein source. In semi-arid regions, where water is very scarce, the high water content of cactus can be considered as a benefit. To develop a feeding system using locally available feed resources, an abundant and cheap source of carbohydrate is very important (Preston and Leng, 1987). This makes spineless cactus highly valuable for its energy content (Felker, 1995).

Cactus is a highly palatable feedstuff and the higher total DM intake in T2 could be associated with the higher consumption and digestibility of cactus. In agreement with this result, Tegegne et al. (2005) found that total DM intake in sheep increased progressively from 77 kg BWt<sup>0.75</sup>/d to 100g/kg BWt<sup>0.75</sup>/d when cactus supplementation increases from zero to 60% in pasture and hay based diets. In the current study, the gradual decrease in DMI of urea-treated straw can be explained by the substitutive or associative effect of feed when replaced with a highly soluble source of carbohydrate. Such an effect was also reported by Njwe and Olubajo (1989) when they supplemented West African Dwarf goats fed fresh Guatemala grass (*Tripsacum laxum*) with increasing levels of cassava flower and groundnut cake. In this study, no digestive disturbances or ill health effects were observed even at the highest cactus inclusion rate which constituted about 50% of DMI. Previously, Ben Salem and coworkers (1996) observed that spineless cactus could be fed without any digestive disturbance to a level of up to 55% of the total DMI. This would help farmers to economise on their straw budget. The absence of a negative effect coupled with higher digestibility and water content would facilitate a rapid disappearance of cactus dry matter from the rumen (Nefzaoui and Ben Salem, 2001).

Water intake was high when sheep were fed urea-treated barley straw alone and this is in agreement with King (1983), who found that 2.2 L/d was consumed by sheep in East Africa. However, water intake decreased significantly with the progressive increase in cactus intake. This is consistent with previous work (Ben Salem et al.,

1996; Tegenge et al., 2005), reporting that water intake decreased significantly as the level of cactus intake increased in diets of low quality roughages. Sheep drank negligible amounts of water when cactus supplementation reached 525 g/d. In line with this finding, Ben Salem and coworkers (1996) indicated that sheep stop drinking water when cactus intake reached 600 g/d. In the tropics, the dry season is characterised by higher temperatures, decreased supply of water and higher herbage DM. Therefore, animals that are sustained on poor quality dry roughages require high amounts of water to facilitate digestion. Also, animals travel long distances to reach water points, spending more energy and losing BWt. During the drought season in East Africa, the distance traveled by small ruminants to watering points increased by between 43% and 52% (Ndikumana et al., 2002). Several authors have shown that spineless cactus can supply considerable water to the animals (Le Houerou, 1996; Sirohi et al., 1997). Also, De Kock (1980) reported that sheep could survive for up to 500 d without drinking water when allowed to consume unrestricted amounts of cactus. In countries where water is a vital resource during the dry season, the high water content of cactus could therefore play a significant role in mitigating drinking water shortage.

There was a significant improvement in BWt gain when urea-treated barley straw was supplemented with spineless cactus — a result in accordance with previous work in sheep (Tegegne et al., 2005) on supplementing urea-treated wheat straw fed with cactus. The higher performance of sheep in T3 and T4 as compared with those fed the control (T1) diet can be ascribed to the combined effects of the higher DMI of the sheep on the supplemented diets and the higher readily digestible carbohydrate content of spineless cactus. This result is in accordance with previous works (Tegegne et al., 2005; Tikabo et al., 2006) on supplementation of cactus to urea-treated crop residue fed sheep.

Although DMI in the control group (T1) was higher than in T, sheep in the latter group performed better. This could be attributed to the more readily fermentable carbohydrate intake associated with the inclusion of cactus in the latter group. The DM digestibility of urea-treated straw was higher when supplemented with cactus than when fed alone. Therefore, the value of cactus as a cheap source of energy for efficient utilisation of non-protein nitrogen is also important in improving the nutritive value of poor quality roughage. A 22% improvement in BWt was achieved in this study, which is quite significant since animals normally lose weight during the dry season although cactus is abundant and succulent in this season. However, a much greater improvement (72%) in BWt was reported when cactus is supplemented with by-pass protein source (Shoop et al., 1977), highlighting the importance of dealing with the deficiencies in nitrogen or CP content of cactus when developing feeding strategies.

The results of this experiment support previous work on using cactus as a cheap source of energy for efficient utilisation of non-protein nitrogen. Earlier, Shoop et al. (1977) suggested that the high level of soluble carbohydrates in cactus could be combined with ammonia- or urea-treated straw, as it could provide a readily available source of energy necessary for the efficient utilisation of the non-

protein nitrogen in the rumen. Tegenge et al. (2005) clearly showed that cactus pear could substitute for wheat bran at up to 40%, as long as it was combined with straw treated with urea.

The digestible nutrient intake data from the metabolism trial are in agreement with the feeding trial. Increasing spineless cactus supplementation of the urea-treated straw based diet increased the energy density of the diet (higher percentage TDN). Body weight gain was highly correlated with DM intake and its energy concentration; these results are consistent with the findings of others (Solaiman, et al., 1980; Moore et al., 1999;). The higher performance of sheep in T3 and T4 compared with those in T1 and T2 may be explained by the increased dietary energy made available through an associative effect i.e. the addition of cactus improves the total digestibility of the diet, including the digestibility of the straw. With increasing level of cactus in the diet, there was an increase in the energy concentration the diet, while the protein concentration decreased. Besides the slight improvement in performance of sheep in T, cactus supplementation at the lower level (175 g DM) did not result in a significant difference relative to the control diet (T1). This might be because, although T2 shows an improvement in energy, there is both a decreased percent dietary protein and lower daily digestible protein intake. In this case, the higher energy may not properly be utilised for better growth, because protein becomes limiting to animal performance.

Supplementation of 350 g DM spineless cactus (T3) seems to be the optimum supplementation rate. At this level, the animals are able to maintain protein intake, so that the animals can benefit from the extra energy and optimise growth. Therefore animals in T3 had a higher TDNI as compares with the other groups, illustrating a more optimal balance of protein and energy. Inclusion of cactus at a higher rate (T4) was still better than the first two treatments, indicating benefits from additional energy, but it may have begun to decrease due to the low protein intake.

## CONCLUSIONS

Feeding cactus to sheep in combination with urea-treated barley straw can significantly increase feed intake and animal performance and reduce water intake. For diets based on urea-treated straw, the optimal inclusion rate for cactus is about a one third of the diet, or 350 g/d of cactus DM. Below this level, cactus reduced the protein content of the diet, so only a slight improvement was seen over urea-treated straw alone. Above this level, cactus could contribute a laxative effect to the diet, so that no further improvement was seen over the 350 g/d level. Therefore, utilisation of spineless cactus as an animal feed could play a vital role in promoting sustainable livestock production by providing an alternative feed as well as water for animals in dry areas.

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# Effects of Supplementing Urine Treated Rice Straw with Concentrates on Productivity and Methane Emissions of Ongole Crossbred Cattle

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## ABSTRACT

Cattle were fed rice straw treated with urine (1 kg urine/1 kg DM rice straw, and kept in the container for a week) *ad libitum* and a concentrate mixture (50:50) of wheat bran and beer cake. One group of cattle (C25, four animals) was fed the concentrate at 25% of estimated dry matter intake (DMI) at 3% of body weight, while the other four cattle (C75) received concentrate at 75% of estimated DMI for a period of six weeks. Daily DMI, methane (CH<sub>4</sub>) production and live weight gain (LWG) were measured. Dry matter intakes were similar in both groups (6.72 kg/d and 7.59 kg/d), but LWG of C75 (1.07 kg/d) was higher ( $P < 0.05$ ) than that of C25 (0.47 kg/d). Methane production was similar in both groups (219.3 L/d vs 240.4 L/d and 29.29L/kg DMI vs 35.15 L/kg DMI for C25 and C75 respectively). However, when calculated per kg LWG, CH<sub>4</sub> production was significantly lower ( $P < 0.05$ ) than that of C25 (205.8 L/kg LWG and 967.2 L/kg LWG, respectively). The results suggest that better feeding not only increases productivity but also leads to significant mitigation of CH<sub>4</sub> emissions. Feeding management should therefore be considered for controlling CH<sub>4</sub> emissions in the animal industry.

**Key words:** *Ongole crossbreds, rice straw, supplementation, methane emissions.*

## INTRODUCTION

Methane is a potent greenhouse gas, and therefore reducing emissions from livestock and enhancing animal production efficiency are effective strategies for mitigating global warming (IPCC, 1995). The production of ruminal CH<sub>4</sub> represents a pathway of carbon loss that reduces energetic efficiency. If the gross energy intake that is lost in generating CH<sub>4</sub> could be channeled into weight gain or milk production, the efficiency of production would improve (Howden and Reyenga, 1999). Manipulation of diets to enhance animal production (milk or meat) can reduce total CH<sub>4</sub> emissions into the atmosphere/unit of milk or meat produced (Johnson and Johnson, 1995; Kurihara et al., 1999). Therefore, the simplest strategy to improve animal production is improving feed quality (Leng, 1993).

Rice straw is the most abundant feedstuff in tropical countries but has low quality. It therefore needs to be improved and this can be

achieved in several ways e.g. by ammonia treatment, through the fermentation process or by supplementation with better quality feeds. The first two methods need skillful farmers and are less amenable to adoption by small holder farmers than the last one. The easiest method to improve rice straw quality, especially its protein content is by urine treatment (Purnomoadi et al., 2005; unpublished). In that study conducted on buffalo, rice straw treated with urine increased feed intake but decreased chewing activity, suggesting that it would contribute to a better efficiency of energy utilisation for production. Supplementation with concentrates was reported to increase productivity (Dillon et al., 1997; Kennedy et al., 2003) as well as to reduce enteric CH<sub>4</sub> production when conserved forages formed the basal ration (Moss and Givens 1995; Ferris et al., 1999; Purnomoadi et al., 2003). This showed that CH<sub>4</sub> emissions from low quality tropical feedstuffs could be controlled by modified feeding management (Devendra, 1992).

Since most livestock in the world are raised by small holder farmers, efforts to improve feeding management should be based on local feed resources, including by-products of agricultural industries. Examples of such feedstuffs include beer cake that contains highly digestible fractions (Amari and Purnomoadi, 1996), and wheat bran which is the residue from wheat flour industries that contains highly soluble carbohydrates fraction. These feedstuffs could decrease CH<sub>4</sub> production (Zinn, 1994; Moe and Tyrell, 1979). This study investigated the effectiveness of by-product concentrate feedstuffs (a mixture of beer cake and wheat bran) in improving productivity and reducing CH<sub>4</sub> production in cattle.

## MATERIALS AND METHODS

### Experimental Animals

Eight cattle (average age 1.5 y, bodyweight [BWt] 240 kg) were fed rice straw treated with urine (1 kg urine/1 kg DM rice straw, and kept in the container for a week) and a 50:50 concentrate mixture of wheat bran and beer cake. The chemical composition of the feeds are presented in **Table 1**. The cattle were divided into two groups for feeding treatments. The first group (four animals) was allowed concentrate feed at 25% (C25 group) of estimated DMI at 3% BWt, while the other group was given concentrate feed at 75% (C75 group) of estimated DMI. Both groups were given rice straw treated with urine *ad libitum* and allowed free access to water. The cattle were fed the diets for six weeks, followed by a further two weeks during which time samples were collected for digestion studies and CH<sub>4</sub> measurements.

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**Table 1. Chemical composition of feeds (%DM).**

	OM	CP	EE	CF	NFE	GE, kJ/g
Rice straw	79.9	7.9	2.3	27.5	42.2	16.86
Concentrate*	94.3	20.6	6.3	14.7	50.2	20.68

OM — organic matter; CP — crude protein; EE — ether extract; CF — crude fibre; NFE — nitrogen free extract; GE — gross energy.

\*Concentrate = 50:50 mixture of beer cake and wheat bran.

## Experimental Parameters, Measurements and Data Analysis

Parameters measured were daily DMI, total digestible nutrients (TDN), daily CH<sub>4</sub> production and live weight gain (LWG). Dry matter intake was measured daily for six weeks by subtracting weights of feed offered and refused and multiplying by their DM contents which were determined by oven-drying at 135°C for 2 h. Crude protein intake was calculated by multiplying DMI with CP content in feed, while the TDN was determined from 7 d total collections. Liveweight gain was measured before and after six weeks of feeding the cattle on their respective diets

Methane measurement was done using the facemask method which was performed for 10 min at 3-h intervals over 2 d, following the description by Purnomoadi et al. (2003). In this method, a mask is connected to a CH<sub>4</sub> analyser (infra-red gas analyser, VIA-510, Horiba Ltd., Japan) equipped with an airflow meter (STEC SEF-6470, Horiba Ltd., Japan) for measuring total air volume (L/min). Data on CH<sub>4</sub> concentration and airflow were averaged and recorded automatically every 3 sec using a computer. The 2 d CH<sub>4</sub> production (L/d) measured was averaged to obtain daily CH<sub>4</sub> production.

Data were analysed using the *t*-test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

### Feeding and Animal Production

Daily DMI, nutrient intake and LWG are presented in **Table 2**. Feeding the higher level of concentrate (C75) resulted in higher LWG ( $P = 0.0145$ ), although DMI and DM digestibility were similar in both treatments. The better LWG of C75 than C25 resulted from the bet-

**Table 2. Daily dry matter intake, nutrients intake, and live weight gain.**

	C25	C75	P
Initial LWt (kg)	281.1	288.4	
LWG (kg/d)	0.31	1.11	0.0145 *
DMI (kg/d)	6.72	7.59	0.1933
Rice straw (kg/d)	3.54	1.95	0.0003 **
Concentrate (kg/d)	3.18	5.64	0.00003 **
Nutrients intake			
CP (kg/d)	0.90	1.28	0.0006 **
Digestible CP (kg/d)	0.63	0.96	0.0002 **
TDN (kg/d)	2.20	3.39	0.0008 **
DM digestibility (%)	46.54	54.01	0.1112
TDN (%)	32.8	44.7	0.0083 **

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

**Table 3. Methane Production.**

	C25	C75	P
Methane production			
L/d	240.42	219.34	0.6537
L/kg DMI	35.15	29.29	0.3524
L/kg LWG	967.17	205.77	0.0186 *
MJ/ kg LWG	13.72	6.21	0.0324 *

\*  $P < 0.05$ .

**Table 4. Methane production and the predicted value by Shibata's equation (1993) and Kurihara's prediction (1995) for hot conditions.**

Treatments	Measured (L/d)	Predicted value (L/d)	
		Shibata	Kurihara
C25	240	237	243
C75	219	265	266

ter nutrient intake in C75, especially protein and TDN (which fulfilled energy requirements). Protein intake in C75 was higher ( $P = 0.0006$ ) than in C25 (1.28 kg/d and 0.90 kg/d, respectively). Similarly, TDN intake in C75 was higher ( $P = 0.0008$ ) than that in C25 (3.39 kg/d and 2.20 kg/d, respectively). The high LWG in C75 (1.11 kg/d) was higher than that mostly reported for Ongole crossbred cattle (range 0.4 kg/d – 0.7 kg/d) under various feeding regimes with CP content up to 15% (Arifin et al., 1998; Amini, 1998; Purnomoadi et al., 2007).

### Methane Production

Daily total CH<sub>4</sub> production (L/d) was similar in C75 and C25 (**Table 3**). This was an unexpected result. Generally, highly digestible diets yield lower emissions than poor quality diets (Johnson and Johnson, 1995). This phenomenon might be explained by the effect of rice straw which is a highly fibrous and poorly digested feed, resulting in a longer retention time in gastrointestinal tract, and hence greater CH<sub>4</sub> production arising from fermentation. At higher levels of concentrate supplementation, microbial fermentation of straw might be activated resulting in a shorter retention time. However, this shorter retention time would not result in lower CH<sub>4</sub> production, since rumen distension lower it will stimulate animal to eat. This agrees with the statement of Shibata et al. (1993) that CH<sub>4</sub> production is highly correlated with DMI. In this experiment, the DMI was also correlated with DMI. The higher LWG in C75 was associated with lower ( $P = 0.0186$ ) CH<sub>4</sub> production/kg LWG than in C25 (205.8 L/kg LWG vs 967.2 L/kg LWG respectively).

This study showed that supplementing a low quality diet such as rice straw with a high quality concentrate did not lower CH<sub>4</sub> production quantitatively, but if animal productivity such as LWG was taken into account, the level of mitigation achieved was significant. These results are similar to those obtained in our previous study (Purnomoadi et al., 2003) using soybean pulp to supplement Napier grass hay.

Measurement of CH<sub>4</sub> production in developing countries is lacking due to the high cost of equipment. Thus, prediction equations have been established such as those by Shibata et al. (1993) and by Kurihara et al. (1995) which were developed using mainly dairy cattle in Japan. Shibata's equation is  $Y = -17.766 + 42.793X - 0.849X^2$ ;



where Y is CH<sub>4</sub> production (L/d), and x is DMI (kg/d) was established for thermoneutral conditions, while Kurihara's ( $Y=63.27+0.02678X$ ; where Y is CH<sub>4</sub> production (L/d), and x is DMI (g/d)) was established for hot conditions (30°C–32°C) and would presumably be more applicable to tropical areas like Indonesia. These equations were applied to predict CH<sub>4</sub> production in this study (Table 4). In comparison with the values measured, CH<sub>4</sub> production predicted by Shibata's differed by –3 L/d and +46 L/d for C25 and C75, while Kurihara's differed by +3 L/d and +47 L/d for C25 and C75, respectively. These results show that both equations more accurately predicted the C25 emissions than those associated with C75, suggesting that feed quality has an important influence on their predictive value.

## CONCLUSION

A combination of rice straw treated with urine and wheat bran and beer cake can lead to significant productivity increases while mitigating CH<sub>4</sub> emissions from cattle in tropical climates.

## ACKNOWLEDGEMENTS

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# Effect of Supplementing Urea-Treated Sorghum Stover with Sesame Cake or Fishmeal on the Body Weight of Sheep and Cattle

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## ABSTRACT

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 (BWt) of sheep and cattle. Twenty one male sheep and nine Barka cattle were divided into three groups of seven sheep and three cattle in each trial. The trials were conducted at the same time, but on two different farms. The control diet consisted of UTSS fed *ad libitum* to both species of animals. The second and third diets were UTSS supplemented daily with SC or FM. The experimental period lasted for 90 d. Feed intakes and BWt 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/d followed by the FM supplemented and control groups at 826 and 821 g/head/d., respectively. Sheep supplemented with SC had the highest ( $P < 0.05$ ) body weight gain (BWtG) (134 g/head/d) followed by the group supplemented with FM (115 g/head/d). The controls had the lowest BWtG (66 g/head/d). In cattle, the group supplemented with SC had the highest ( $P < 0.05$ ) total DMI (6.13 kg/head/d) followed by animals supplemented with FM (5.81 kg/head/d) and the controls (5.78 kg/head/day), which were not significantly different ( $P > 0.05$ ) from each other. The BWtG of cattle fed urea-treated sorghum stover alone was 559 g/head/d. This increased to 650 g/head/d with FM and to 741 g/head/d with SC supplementation. In cattle, BWtG was not significantly different ( $P > 0.05$ ) between the treatments. Feed conversion was best on SC followed by FM supplementation in both species (6.92 and 7.70 for sheep and 8.28 and 8.93 for cattle respectively). It can be concluded that feeding UTSS alone or supplemented with small amounts of SC or FM can increase the live weights of cattle and sheep at a reasonable cost.

**Key words:** urea treatment, sorghum stover, fishmeal, sesame cake, weight gain, feed conversion.

## INTRODUCTION

Feed is the most important input in livestock production and its adequate supply in terms of quantity and quality throughout the year

is a pre-requisite for any substantial and sustained expansion in livestock output. Among the major constraints limiting the potential development of livestock production in Eritrea, inadequate feed has been identified as the crucial bottleneck. In most areas, especially during the dry period, livestock fed only on crop residues or the native pasture cannot even meet their maintenance requirements or they lose BWt. Most ruminants are consequently subjected to chronic under nutrition.

Low quality feeds such as sorghum and barley straws and stalks are staple feeds for ruminant livestock in the traditional subsistence farming systems of Eritrea. These feed sources contain insufficient nitrogen to provide the ammonia needed by rumen microorganisms for the efficient fermentative digestion of such feeds. However, there are appropriate technologies to improve the nutritive value of these residues. Urea treatment and correct supplementation with locally available supplements have been successfully used in many developing countries (Dolberg, 1992; Hector, et al., 1990; Chenost and Kayouli, 1997). Nevertheless, until recently sorghum stover was not properly collected and stored by farmers in the lowlands of Eritrea, instead being grazed while in the field.

The potential nutritive value of straw and stover or low quality forage cannot be exploited if the microbes in the animal's rumen do not receive the correct balance of nutritive elements for their efficient digestion. The objective of nitrogenous and energy supplements is therefore to ensure additional supply of nutritional elements to the animal to achieve a targeted performance level. Several positive results have been achieved from supplementing urea-treated sorghum stover with oilcakes and fishmeal (McDonald, et al., 1973; Maglad et al., 1984; Williams, 1984; Preston and Leng, 1987; Little et al., 1991). However, this approach has not yet been studied in Eritrea. As a result there is no reliable information on the application of supplementation methods to livestock in Eritrea, in particular to intensive and semi-intensive livestock production. The objectives of this study were therefore to examine the effect of feeding urea-treated sorghum stover supplemented with locally available protein sources (fishmeal or sesame cake) on the performance of local sheep, and to estimate the economic value of the treatments.

## MATERIALS AND METHODS

Two trials involving sheep and cattle were conducted on-farm, the former within the Adi-Omer Mixed Farming Project and the latter at the Gash Setit Agro-Industry and Trade farm.

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**Table 1. Feeds provided to the experimental animals.**

Species	Feed 1 (Control)	Feed 2	Feed 3
Cattle	UTSS <i>ad lib</i>	UTSS <i>ad lib</i> + 576 g SC	UTSS <i>ad lib</i> + 432 g FM
Sheep	UTSS <i>ad lib</i>	UTSS <i>ad lib</i> + 80 g SC	UTSS <i>ad lib</i> + 60 g FM

UTSS — urea treated sorghum stover; SC — sesame cake (g DM); FM — fishmeal (g DM).

**Table 2. Chemical composition of the feed ingredients (% DM).**

Feed ingredient	DM (%)	Ash	CP	EE	CF	Energy (MJ/kg)
NTSS	87.00	12.60	6.25	0.72	32.50	9.35
UTSS	60.00	12.70	11.12**	0.88	29.67	9.83
SC	89.81	11.87	44.47	13.93	9.24	16.12
FM	89.87	27.95	59.15	5.44	1.93	12.90

NTSS — non-treated sorghum stover; DM — dry matter; UTSS — urea-treated sorghum stover; CP — crude protein; SC — sesame cake; EE — ether extract; FM — fishmeal; CF — crude fibre.

\*\* — Estimated value (Chenost and Kayouli, 1997).

**Table 3. Mean values for live weight change, feed intake, feed conversion and cost of feed by sheep fed urea-treated sorghum stover supplemented with sesame cake or fishmeal.**

Parameters	Feed		
	1 (Control)	2	3
No. of animals	7	7	7
Initial BWt (kg)	20.643	20.243	20.343
Final BWt (kg)	26.557	32.286	30.671
Experimental period (d)	90	90	90
Daily gain (kg)	0.066 <sup>a</sup>	0.134 <sup>b</sup>	0.115 <sup>c</sup>
Daily DMI (kg)	0.821 <sup>a</sup>	0.927 <sup>b</sup>	0.886 <sup>ab</sup>
Daily treated sorghum stover intake (kg)	0.821	0.847	0.826
Daily supplements (FM or SC) intake (kg)	0.000	0.080	0.060
Feed conversion (kg feed/kg gain)	12.439	6.918	7.704
Cost of feed (Nfa/kg gain)	25.72	18.94	25.80

<sup>a b c</sup> Means in the same row not having common letters differ significantly ( $P < 0.05$ ); Least significant difference (LSD) for LWG = 0.01758; LSD for DMI = 0.0833. Feed 2 — control + sesame cake; Feed 3 — control + fishmeal.

## Animals and Feeds

The feeding trial was conducted on 21 growing male sheep from local breeds aged between six and seven months, and nine male Barka male cattle aged between three and four years. The experimental period lasted for three months and was preceded by two weeks of adaptation. The animals were divided into three treatment groups (three cattle and seven sheep in each treatment) after balancing for initial live weight and age. Each group was then randomly allocated into one of the three dietary treatments by drawing lots (Table 1). All the animals were fed on a DM basis. Five kg urea were dissolved in 60 L water and evenly sprinkled on layers of 100 kg of chopped sorghum stover. The stover was then stacked into a concrete pit and covered with a plastic sheet to maintain an air-tight condition for a period of at least two weeks. The diets in the two treatments with supplements of SC or FM were formulated to be iso-nitrogenous and were thoroughly mixed before being offered to the animals.

## Measurements and Analysis of Data

Samples of the feeds were collected at regular intervals and analysed according to standard procedures (AOAC, 1984). The chemical composition of the diets is given in Table 2.

After 15 d of adaptation, BWt was measured before feeding and watering at intervals of 15 d. Dry matter intake was determined for each group from the difference between the daily weight of feed offered and feed refused.

The feeding trials were designed according to a completely randomised design (CRD). Mean LWtG and daily DMI were analysed using GENSTAT Release 12.2 (2003) Windows software.

## RESULTS AND DISCUSSION

### Effects of Urea Treatment on Composition of Sorghum Stover

Urea treatment of sorghum stover was effective in upgrading its nutritional value, particularly the CP content of straw which increased

by 77.9% from 6.25% to 11.12% following treatment (Table 2). This was similar to the results of Saadullah et al. (1981) where the CP content of sorghum stover increased from 2.2% to 11.9% when treated with five percent urea.

### Sheep Trial

Results from the sheep trial are given in Table 3 and in Figures 1 and 2. Urea-treated stover displayed all the expected characteristics. Animals were highly attracted to treated stover and readily consumed it. The increase in DMI following urea treatment (Table 3 and Figure 1) can be attributed in part to the softer texture, odour of ammonia and addition of nitrogen (N), which made it more palatable. These changes are known to produce a faster rate of digestion (Mbanya et al., 2005). The reason for the increased DMI in the supplemented group could be due to the N added by SC or FM which facilitates the cellulolytic process and increases the rate of digestion in the rumen. Chenost and Kayouli (1997) pointed out that the ultimate objective of adding supplements to low quality forages is to increase digestive processes in the rumen and thus increase intake. This can only be achieved by increasing fibre breakdown in the rumen.

The straw intake and LWtG obtained in the control group in this study were higher than those reported by others. Hadjipanayiotou et

al. (1993) found that Awassi sheep fed untreated straw gained 73 g/d and this increased to 88 g/d with urea treatment, but the straw intake remained constant at 744 g/d. However, Jurgens (1997), showed that a 21 kg sheep consumed a total of 1 kg of feed daily, a value similar to the DMI recorded in this study.

Feed conversion was higher in the SC supplemented group than in the control and FM supplemented group.

### Cattle Trial

Dry matter intake of treated straw by cattle in this study was 5.78 kg/d (Table 4 and Figure 3). This value is similar to the daily average straw intake of 5.6 kg/d found in cattle in China (Tengyun, 2000).

The higher DMI of the SC supplemented group was due to the added SC and not to a higher consumption of the treated stover since intake of stover (5.56 kg/d) was less than that of the control group (5.78 kg/d). In fact, the added supplements reduced stover intake in both supplemented groups as there was no significant difference ( $P > 0.05$ ) in stover intakes between the three groups. The results of DMI of cattle given in Table 4 are comparable with the figures given by Jurgens (1997), who showed that mature cattle weighing around 290 kg consume 2.5% of their BWt (i.e. 7.25 kg/d).

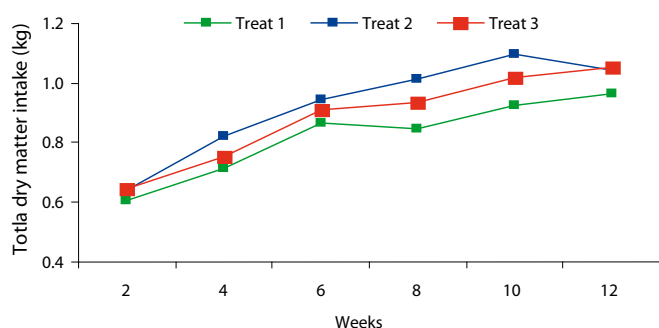


Figure 1. Dry matter intake of control and supplemented sheep (SED = 0.0419); Treat 1 — control; Treat 2 — control + sesame cake; Treat 3 — control + fishmeal.

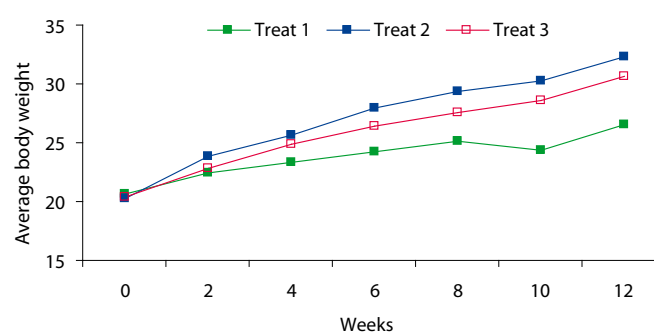
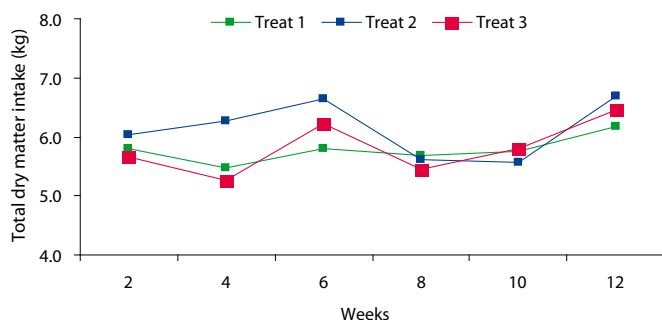


Figure 2. Changes in body weight of control and supplemented sheep (SED = 0.00807).

Table 4. Mean values for live weight change, feed intake, feed conversion and cost of feed by cattle fed urea-treated sorghum stover supplemented with SC or FM.

Parameters	Feed		
	1 (Control)	2	3
No. of animals	3	3	3
Initial body weight (kg)	287.3	287.0	284.0
Final body weight (kg)	337.7	353.7	342.5
Experimental period (days)	90	90	90
Daily gain (kg)	0.559 <sup>a</sup>	0.741 <sup>a</sup>	0.650 <sup>a</sup>
Daily total DMI (kg)	5.780 <sup>a</sup>	6.133 <sup>b</sup>	5.807 <sup>a</sup>
Daily treated sorghum stover intake (kg)	5.780 <sup>a</sup>	5.557 <sup>a</sup>	5.375 <sup>a</sup>
Daily supplement (FM or SC) intake (kg)	0.000	0.576	0.432
Feed conversion (kg feed/kg gain)	10.34	8.28	8.93
Cost of feed (Nfa/kg gain)	19.92	23.43	29.54

<sup>a</sup> <sup>b</sup> Means in the same row not having common letters differ significantly ( $P < 0.05$ ); LSD for LWtG = 0.3814; LSD for DMI = 0.3105. Treatment 2 — control + sesame cake; Treatment 3 — control + fishmeal.



**Figure 3. Weekly dry matter intake of cattle fed the control and supplemented diets (SED = 0.1562).**

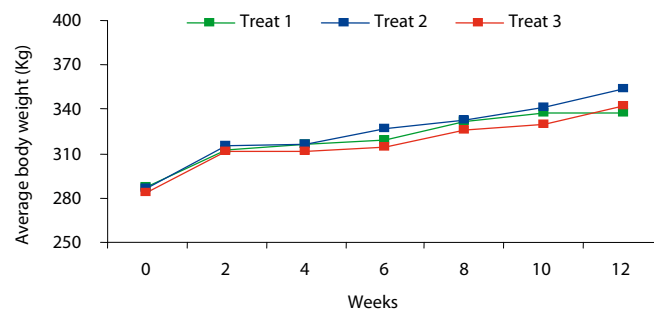
In the control group, as a result of urea treatment which improved the nutritive value of the diet, animals not only maintained their weights but also gained more weight (559 g/head/d) than recorded in some other studies (Figure 4).

The literature reviewed by Chenost and Kayouli (1997) showed the improvement of DMI and weight gain of cattle fed 5% urea-treated straw. They found that mature cattle with an average BWt of 285 kg consumed 6.82 kg/d and gained 564 g/d. The figures obtained in this experiment (5.78 kg/d DMI and a gain of 559 g/d) were similar to the results of Chenost and Kayouli (1997), but higher than those obtained by Wongsrikeao and Wanapat (1985) on buffalo, which had a DMI of 4.75 kg/d and a BWtG of 261g/d. The values in this experiment were also higher than those obtained by Little et al. (1991), who recorded an average daily gain of 169 g in controls and 272 g and 271 g respectively when SC and cottonseed meal were used as supplements.

Fishmeal supplementation in cattle (432 g/head/d) did not result in any significant difference in BWt gain and DMI compared with the control group.

The daily intake of treated sorghum stover intake means the amount of treated sorghum stover consumed daily without SC or FM by the animals in all the groups. In this case the amount of feed consumed by the control group is the same as the daily total DMI. However, in feeding regimes two and three, the daily total DMI included SC and FM in addition to the daily treated sorghum stover intake. Therefore, the difference in the daily intake of treated sorghum stover was the result of the additional supplements (SC and FM in diets two and three respectively).

Although feed conversion was higher in the group supplemented with SC, (Table 4), both supplemented groups had higher feed con-



**Figure 4. Weekly body weight change of cattle fed the control and supplemented diets (SED = 0.1374); Treat 1 — control; Treat 2 — control + sesame cake, Treat 3 — control + fishmeal.**

versions than the control. The feed conversion obtained in the control group was higher than that obtained by Khan and Davis (1981) who found conversion ranges of 13.5–28 kg feed /kg LWtG.

#### Economic Data from the Feeding Trials

There has to be a good economic reason for a farmer to feed treated straw or stover and /or to add supplements. The effects have to be evident. In this study, treating sorghum stover with urea for feeding cattle was as, and in the case of cattle, more economically advantageous for increasing daily LWtG and there was no economic reason to supplement cattle beyond urea treatment (Table 5). Also evident is that the cost of feed/kg LWtG was also lower for the SC supplemented groups than for the FM groups, the higher cost of FM supplementation being due to a combination of the relatively higher high cost of FM and the lower weight gain attained by the animals.

Preston and Leng (1987) indicated that the extent and rate of digestion of fibrous feeds are increased by a N supplement, which results in a greater DMI. This is reflected in the LWt changes recorded here.

#### DISCUSSION

There are different possible explanations for why SC supplemented animals had higher LWt gains than animals supplemented with FM. The increased total DMI recorded in SC supplemented animals could account for their higher LWt gains. Lindsay et al. (1982) indicated that the greater weight gain of supplemented animals was due to the increased intake of the basal diet. Another reason for the superiority of the SC could be related to its chemical composition. The SC used in this trial had lower ash, higher ether extract (crude fat)

**Table 5. Cost summary of the sheep and cattle feeding trials.**

	Cattle: cost of each treatment group			Sheep: cost of each treatment group		
	T1(Control)	Feed 2	Feed 3	T1(Control)	Feed 2	Feed 3
Total cost (Nkfa)	5 007.10	6 687.10	7 184.70	2 069.50	2 599.10	2 869.50
TCost (Nkfa/head/d)	18.55	24.77	26.61	3.285	4.13	4.55
BWtG (g/head/d)	0.559	0.741	0.650	0.066	0.134	0.115
TCost (Nkfa/kg gain)	33.18	33.43	40.92	49.77	30.82	39.57

BWtG — body weight gain; TCost — total cost; total number of d — 90; Nkfa= Eritrean currency (US\$ 1— 15 Nkfa).

T1 — control; Feed 2 — control + sesame cake, Feed 3 — control + fishmeal.

and higher energy content than the FM. These differences may be important in affecting DMI, and consequently LWtG. Meals that contain high amounts of ash are generally considered to be lower in protein quality and have a lower amino acid digestibility (Parsons, 2006; Shah and Muller, 1983). McDonald et al. (1995) suggested that a high ash content is one reason for the low energy content of a feed ingredient.

Depending on the diet, fat contributes approximately 7–10% of the digestible energy of rations (Preston and Leng, 1987). Dietary fat is converted to depot fat in the animal and stored in different parts of the body. The higher fat (EE) content of SC, which contains dietary long chain fatty acids (LCFAs), could therefore have contributed to the increased gain of the groups supplemented with SC. On the other hand, Sanderson et al. (2001) reported that FM supplementation increased rates of ash and crude protein gain, but had a small effect on fat gain.

Cattle supplemented with FM gained more weight than unsupplemented animals. This could be due to the fact that FM is well balanced in amino acids and has a high mineral and vitamin content (McDonald et al., 1973). Generally, FM has 20–30% of rumen degradable protein and about 70–80% of by-pass protein (Preston and Leng, 1987).

## CONCLUSIONS

Urea treatment is a simple, cheap and applicable method to improve low quality roughages in Eritrea. Urea treatment improved the CP content of sorghum stover and increased the intake of animals. Animals fed urea-treated sorghum stover not only maintained their BWt, but also gained an appreciable and economically worthwhile amount of weight which was comparable with supplemented animals, particularly in cattle. The result of the experiment was similar for both species (sheep and cattle). Small supplementation with SC (8% of ration DM) as a protein source resulted in an increase in weight gain of cattle and sheep. The potential availability of SC is high in Eritrea and inclusion of this supplement in the diets of ruminant livestock significantly increased LWtG and reduced the cost of feed per unit gain of weight. Therefore, it can be concluded that feeding cattle only with urea-treated sorghum stover or supplementing it with small amounts of SC can lead to cost-effective weight gains. Nevertheless, further research with different levels of SC supplementation is required to determine the most economic level as is continuous evaluation of the quality of available FM. Also, long term on-farm trials on feeding urea-treated straw on milk production should be carried out to determine the practical advantages and disadvantages, as well as the economic returns from feed treatment and supplementation, including on crop residues like finger millet, maize stover and dried grasses. Strong extension linkages have to be developed to popularise urea treatment of straw, particularly in farming systems where there is wastage of crop residues.

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# Effect of Replacing Dietary Soybean Meal with Tropical Legumes on the Performance of Lambs

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## ABSTRACT

This study determined how supplementing bahiagrass hay (*Paspalum notatum* Flügge cv. 'Pensacola') with soybean (*Glycine max*) meal or warm-season legume hays affected intake, digestibility, and N utilisation by lambs. Dorper × Katadhin crossbred lambs (30.6 ± 5.5 kg; n = 42) were fed bahiagrass hay *ad libitum* and supplemented with nothing (control), soybean meal, or hays of annual peanut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), perennial peanut (*Arachis glabrata*), pigeonpea (*Cajanus cajan*), or soybean. Legume hays were supplemented at 50% of diet dry matter (DM); soybean meal was supplemented at 4.25% of diet DM to match the average crude protein (CP) content (10.8%) of the legume hay supplemented diets. Cowpea, pigeonpea, and soybean were harvested at maturities that maximised DM yield and nutritive value, and peanuts were first cuttings. Diets were fed to six lambs per treatment for two consecutive 21-d periods. Supplementation with annual and perennial peanut, cowpea, and soybean hay increased (P < 0.01) DM intake versus control, but apparent DM digestibility was only increased (P = 0.03) by supplementation with either peanut. Nitrogen intake, digestibility, and retention were increased (P < 0.01) by supplementation particularly with annual or perennial peanut hay. Ruminal ammonia concentration was increased (P < 0.01) by all legume hay supplements versus the control. Microbial N synthesis and ruminally degraded organic matter (OM) were increased (P = 0.03) by perennial and annual peanut hay supplementation, but the efficiency of microbial synthesis was not different (P = 0.52) among diets. Annual and perennial peanut hays were the best supplements for the bahiagrass hay in this study.

**Key words:** bahiagrass, digestibility, intake, nitrogen retention, supplementation, tropical legume.

## INTRODUCTION

Bahiagrass (*Paspalum notatum* Flügge) and bermudagrass [*Cynodon dactylon* (L.) Pers.] are the main forage grasses in Florida and much of the southern United States. The yield of these grasses is normally sufficient to meet intake requirements of most ruminant livestock during the grazing season; however, their quality is insufficient for growing or lactating ruminants due to low DM digestibility and CP

concentration (Duble et al., 1971; Johnson et al., 2001). Because of rapidly escalating fertilizer and feedstuff commodity prices, ruminant feeding strategies that are less dependent on these inputs merit evaluation. Supplementing poor quality basal grass diets with legume forage has increased feed intake and diet digestibility by ruminant livestock (Minson and Milford, 1967; Getachew et al., 1994;). Legume supplementation improves N retention by the ruminant when grass diets that do not meet ruminant energy and N requirements are fed (Mosi and Butterworth, 1985; Matizha et al., 1997).

Alfalfa (*Medicago sativa* L.), the legume fed most commonly to livestock in the USA does not persist in the warm, humid climate of the Gulf Coast region (Prine et al., 1981). Perennial peanut (*Arachis glabrata* Benth.) is the main warm-season forage legume in Florida; however, it is sprig-planted and it takes 1–2 y to establish; it is therefore costlier to establish than seeded, warm-season legumes like cowpea [*Vigna unguiculata* (L.) Walp.], soybean [*Glycine max* (L.) Merr.], or annual peanut [*Arachis hypogaea* (L.)] (French et al., 2006). Effects of supplementing bahiagrass hay with these legume hays on animal performance are unknown.

This study determined the feed intake, digestibility, and N retention of lambs fed bahiagrass (cv. 'Pensacola') hay supplemented with soybean meal, or hays of perennial peanut (cv. 'Florigraze'), annual peanut (cv. 'Florida MDR 98'), soybean (cv. 'Pioneer 97B52'), cowpea (cv. 'Iron clay'), or pigeonpea [*Cajanus cajan* (L.) Millsp. cv. 'GA-2'].

## MATERIALS AND METHODS

### Forage Production

Legume hays were produced at the North Florida Research and Education Center in Marianna, FL, (31° N) and fed at the Department of Animal Sciences, University of Florida, Gainesville, FL. The legumes were harvested at the recommended maturity stage for maximising both DM yield and nutritive value i.e. when pods began to turn yellow for cowpea (NDA, 1997), pod setting for pigeonpea (Le Houérou, 2004), and stage R6 (pod with full size seed at one of the four uppermost nodes and completely unrolled leaves) for soybean (Coffey et al., 1995; Sheaffer et al., 2001). Established stands of perennial (4-y-old) and annual peanut (6-y-old; self reseeding) were harvested as first cuttings. Perennial and annual peanut, and cowpea were harvested to a stubble height of 10 cm, whereas soybean and pigeonpea were harvested to stubble heights of 20 and 40 cm based on previous recommendations for the respective forages (Romero et al., 1987; Le Houérou, 2004; Misleve et al., 2005;). Cowpea, soybean, and pigeonpea were rolled into 200 kg round bales using a Vermeer 504 L baler (Vermeer Manufacturing Inc., Pella, IA). Annual and perennial peanut were stored as square bales (50 kg). An established stand of bahiagrass (11-y-old) was harvested as a 6-week regrowth to a stubble height of 8 cm and rolled into round bales. After at least 5

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months, each hay bale was chopped to approximately 8-cm particle length in a tub grinder (Roto Grind, model 760, Burrows Enterprises, Greeley, CO) to limit refusals.

### Animals, Feeding, and Housing

Dorper × Katadhin cross ram lambs ( $n = 42$ ) weighing  $30.6 \pm 5.5$  kg were stratified by weight and assigned randomly to seven treatments (six lambs per treatment per period) within each weight stratum. The experiment was a completely randomised block design with two periods ( $n = 12$ ), each consisting of 14 d of adaptation and 7 d of measurement, and each lamb received a different diet in each period. Lambs were fitted with canvas faecal collection bags and housed in individual metabolism crates ( $0.5 \times 1.5$  m). Thirty-eight of the crates were also adapted for collection of urine. Water was provided *ad libitum* and 20 g of a mineral premix (Ranch House Trace Mineralized Salt, United Salt Corp., Houston, TX) was added to the diet of each lamb daily. Lambs were fed *ad libitum* (110% of previous day's intake) diets consisting of bahiagrass hay supplemented with nothing (control), soybean meal, or hays of perennial peanut, annual peanut, cowpea, pigeonpea, or soybean. Legume hays were fed at 50% of total diet DM, and soybean meal was fed at 4.25% of diet DM to match the average CP concentration (10.8% DM basis) of the legume hay diets. The bahiagrass and respective legume supplements were offered in the same feed trough at 0800 and 1500 h daily; the soybean meal was top dressed on the bahiagrass hay and fed at the same times.

### Sample Collection

Samples (1 kg) of each hay and soybean meal were taken daily during the 7-d collection period, and daily orts were weighed and stored. Total faecal output was collected daily from each lamb, weighed, and a 10% subsample was frozen ( $-20^{\circ}\text{C}$ ) for subsequent analysis. The weight and volume of daily urine output was recorded. Sulphuric acid was added to subsamples of urine to ensure that the pH remained below 3.0, and the urine was stored ( $-20^{\circ}\text{C}$ ) for further analysis. Lambs were weighed and blood sampled by jugular venipuncture on d 0, 21, and 42. A Vacutainer tube (BD, Franklin Lakes, NJ) containing sodium heparin anticoagulant was used to collect 10 mL of whole blood from each lamb and the tubes were stored on ice and processed within 2 h. The blood was centrifuged at 1 920 g for 20 min at  $4^{\circ}\text{C}$  to separate the plasma, which was stored at  $-20^{\circ}\text{C}$  until analysed. Ruminal fluid (100 mL) was collected from 28 lambs (four selected randomly per treatment) on the last day of period two by aspiration from orally-inserted stomach tubes at 0, 2.5, 5, 7.5 and 10 h after the morning feeding. A representative (100 mL) sample was analysed immediately for pH (Accumet, model HP-71, Fischer Scientific, Pittsburg, PA), acidified with concentrated  $\text{H}_2\text{SO}_4$ , centrifuged for 30 min at  $4^{\circ}\text{C}$  and 2 795 g, and frozen ( $-20^{\circ}\text{C}$ ) for subsequent analysis.

### Chemical Analyses

Samples of hays, soybean meal, orts, and feces were composited by period and analysed for DM by oven drying at  $105^{\circ}\text{C}$  overnight, and for ash by combustion in a muffle furnace at  $600^{\circ}\text{C}$  overnight. Samples reserved for additional analyses were dried at  $60^{\circ}\text{C}$  for 48 h in a forced air oven and ground to pass through a 1 mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Total N was determined by rapid combustion using a macro elemental N analyser (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ) and used to calculate CP ( $\text{CP} = \text{N} \times 6.25$ ). Neutral detergent fibre

was analysed using the method of Van Soest et al. (1991). Amylase and sodium sulfite were used for NDF analysis and the results were expressed on a DM basis. Apparent digestibility of DM, OM, N, and neutral detergent fibre (NDF) were calculated. Feed samples were analysed for acid detergent fibre (ADF) and acid detergent lignin (ADL) with the method of AOAC (1990). The ADIN concentration of the hays was measured with the elemental N analyser on ADF residues. The Van Soest et al. (1966) method and an ANKOM Daisy<sup>II</sup> Incubator (ANKOM Technologies, Macedon, NY) were used to measure *in vitro* true DM digestibility (IVTD) of hays. Condensed tannin (CT) concentration of hays was analysed with the method of Terrill et al. (1992). Quebracho tannin (Unitán ATO, Buenos Aires, Argentina) was purified with Sephadex LH-20 (GE Healthcare Life Sciences, Piscataway, NJ) according to Asquith and Butler (1985) as modified by Hagerman (1994). Condensed tannin results were expressed as quebracho tannin equivalents.

Urine was analysed for N by rapid combustion with the elemental N analyser and for total purine derivatives (PD) as allantoin (Bochers, 1977). Xanthine, hypoxanthine, and uric acid were converted to allantoin using the procedure of Fujihara et al. (1987). Microbial protein supply to the small intestine was calculated from the urinary output of PD using the equation of Chen et al. (1992). Microbial efficiency was calculated as g of microbial N/kg of OM apparently digested in the total tract.

Ruminal fluid  $\text{NH}_3\text{-N}$  concentration was determined by an ALPKEM auto analyser (ALPKEM Corporation, Clackamas, OR) with an adaptation of the Noel and Hambleton (1976) procedure that involved colorimetric quantification of N.

### Statistical Analyses

Data were analysed with PROC MIXED (SAS Inst. Inc., Cary, NC). The model for intake, digestibility, N excretion and retention, microbial protein parameters included dietary treatment, period, dietary treatment × period, and lamb (random variable). The model for ruminal fluid pH and  $\text{NH}_3\text{-N}$  included dietary treatment, time of collection (repeated measure), dietary treatment × time of collection, and lamb (random variable). Means were separated with a PDIFF statement. Significance was declared at  $P \leq 0.05$ .

## RESULTS

### Forage Chemical Composition

Dry matter concentrations were not different among hays, but OM concentration was less ( $P = 0.04$ ) in perennial peanut than in all other hays (Table 1). As expected, CP concentration was least ( $P < 0.01$ ) in bahiagrass hay. Among legume hays, CP concentrations were greater in annual and perennial peanut hays than in cowpea and pigeonpea hays. Concentration of NDF was greatest ( $P < 0.01$ ) in pigeonpea hay followed by bahiagrass hay, and least in annual and perennial peanut hays. The greatest ( $P < 0.01$ ) ADF concentration was in pigeonpea hay, and the least ( $P = 0.02$ ) in perennial peanut hay. Lignin ( $P = 0.10$ ) and ADIN ( $P < 0.01$ ) concentrations were greater in pigeonpea hay than the other hays. *In vitro* true digestibility was greatest ( $P < 0.01$ ) in perennial peanut hay followed by annual peanut hay. Bahiagrass hay contained less ( $P < 0.01$ ) IVTD than all legume hays except pigeonpea hay which contained the least ( $P < 0.01$ ) IVTD. Condensed tannin concentrations were low in all hays. Extractable CT concentration was greatest ( $P < 0.01$ ) in perennial peanut hay followed by cowpea hay. Bound CT concentration was greatest ( $P < 0.01$ ) in perennial and annual peanut hays followed by pigeonpea hay.

**Table 1. Chemical composition and *in vitro* true digestibility of hays fed to lambs.**

Item	Bahiagrass	Annual peanut	Perennial peanut	Cowpea	Pigeonpea	Soybean	SEM <sup>1</sup>
DM, %	91.1	91.0	90.8	91.5	91.8	91.6	1.8
OM, % DM	94.5 <sup>a</sup>	92.4 <sup>b</sup>	90.8 <sup>c</sup>	92.6 <sup>b</sup>	94.7 <sup>a</sup>	93.8 <sup>ab</sup>	0.53
CP, % DM	8.1 <sup>d</sup>	14.7 <sup>ab</sup>	15.2 <sup>a</sup>	11.7 <sup>c</sup>	12.2 <sup>c</sup>	13.5 <sup>b</sup>	0.40
NDF, % DM	73.8 <sup>b</sup>	46.2 <sup>e</sup>	43.3 <sup>f</sup>	62.2 <sup>c</sup>	78.6 <sup>a</sup>	59.0 <sup>d</sup>	1.0
ADF, % DM	39.8 <sup>cd</sup>	37.8 <sup>d</sup>	32.1 <sup>e</sup>	48.7 <sup>b</sup>	60.2 <sup>a</sup>	42.8 <sup>c</sup>	1.3
ADIN, % N	15.1 <sup>b</sup>	7.1 <sup>e</sup>	6.5 <sup>e</sup>	13.4 <sup>c</sup>	25.4 <sup>a</sup>	9.1 <sup>d</sup>	0.43
ADL, % DM	6.2 <sup>b</sup>	7.9 <sup>b</sup>	6.7 <sup>b</sup>	9.5 <sup>b</sup>	17.1 <sup>a</sup>	9.6 <sup>b</sup>	1.1
IVTD <sup>2</sup> , % DM	50.7 <sup>d</sup>	71.4 <sup>b</sup>	77.2 <sup>a</sup>	57.9 <sup>c</sup>	35.1 <sup>e</sup>	57.4 <sup>c</sup>	1.1
Total CT <sup>3</sup> , % DM	0.46 <sup>d</sup>	2.68 <sup>b</sup>	3.82 <sup>a</sup>	1.03 <sup>c</sup>	1.13 <sup>c</sup>	0.20 <sup>d</sup>	0.14
Extractable CT <sup>3</sup> , % DM	0.12 <sup>cd</sup>	0.16 <sup>cd</sup>	1.56 <sup>a</sup>	0.56 <sup>b</sup>	0.26 <sup>c</sup>	0.05 <sup>d</sup>	0.07
Bound CT <sup>3</sup> , % DM	0.34 <sup>cd</sup>	2.52 <sup>a</sup>	2.26 <sup>a</sup>	0.57 <sup>c</sup>	0.87 <sup>b</sup>	0.15 <sup>d</sup>	0.11

<sup>a,b,c,d,e,f</sup> Within a row means without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup> SEM values reflect the variation of samples collected daily and composited within each of two periods ( $n = 2$ ).

<sup>2</sup> *In vitro* true DM digestibility (IVTD); <sup>3</sup> Condensed tannin.

**Table 2. Performance indices (DM basis) of lambs fed bahiagrass hay diets supplemented at 50% of DM with warm-season legume hays or at 4.25% of DM with soybean meal (SBM).**

Item	Bahia-grass	SBM	Annual peanut	Perennial peanut	Cowpea	Pigeonpea	Soybean	SEM
Total intake, g/d								
Dry matter	665 <sup>ef</sup>	726 <sup>de</sup>	975 <sup>b</sup>	1105 <sup>a</sup>	803 <sup>cd</sup>	612 <sup>f</sup>	864 <sup>c</sup>	29
Organic matter	629 <sup>ef</sup>	685 <sup>de</sup>	911 <sup>b</sup>	1034 <sup>a</sup>	752 <sup>cd</sup>	579 <sup>f</sup>	811 <sup>c</sup>	28
Neutral detergent fibre	500 <sup>e</sup>	522 <sup>de</sup>	594 <sup>b</sup>	654 <sup>a</sup>	558 <sup>cd</sup>	468 <sup>e</sup>	583 <sup>c</sup>	19
Digestibility, %								
Dry matter	58.5 <sup>cd</sup>	60.3 <sup>c</sup>	64.3 <sup>b</sup>	67.8 <sup>a</sup>	58.8 <sup>cd</sup>	56.3 <sup>d</sup>	60.7 <sup>c</sup>	0.9
Organic matter	60.6 <sup>c</sup>	61.4 <sup>c</sup>	65.4 <sup>b</sup>	68.7 <sup>a</sup>	59.7 <sup>cd</sup>	57.5 <sup>d</sup>	61.7 <sup>c</sup>	1.0
Neutral detergent fibre	60.8 <sup>abc</sup>	60.8 <sup>abc</sup>	57.9 <sup>cd</sup>	62.2 <sup>a</sup>	56.6 <sup>d</sup>	58.7 <sup>bcd</sup>	58.9 <sup>bcd</sup>	1.0
N utilisation indices								
N intake, g/d	8.8 <sup>e</sup>	12.1 <sup>d</sup>	17.7 <sup>b</sup>	21.3 <sup>a</sup>	12.9 <sup>d</sup>	11.8 <sup>d</sup>	15.6 <sup>c</sup>	0.54
Fecal N output, g/d	4.8 <sup>d</sup>	5.1 <sup>d</sup>	6.5 <sup>b</sup>	7.3 <sup>a</sup>	5.8 <sup>c</sup>	5.2 <sup>d</sup>	6.4 <sup>b</sup>	0.20
Urinary N output, g/d	2.1 <sup>c</sup>	2.7 <sup>b</sup>	4.3 <sup>a</sup>	3.6 <sup>ab</sup>	2.5 <sup>c</sup>	2.6 <sup>c</sup>	4.0 <sup>a</sup>	0.35
Retained N, g/d	2.0 <sup>d</sup>	4.2 <sup>c</sup>	7.0 <sup>b</sup>	10.5 <sup>a</sup>	4.6 <sup>c</sup>	4.1 <sup>c</sup>	5.1 <sup>c</sup>	0.54
N digestibility, %	46.5 <sup>e</sup>	56.8 <sup>cd</sup>	62.4 <sup>b</sup>	66.8 <sup>a</sup>	54.0 <sup>d</sup>	55.6 <sup>cd</sup>	58.1 <sup>c</sup>	1.1
PD <sup>1</sup> output, mmol/d	7.4 <sup>bc</sup>	6.3 <sup>c</sup>	10.1 <sup>a</sup>	11.0 <sup>a</sup>	6.2 <sup>c</sup>	7.2 <sup>c</sup>	9.7 <sup>ab</sup>	1.0
Microbial N, g N/d	6.4 <sup>bc</sup>	5.5 <sup>c</sup>	8.7 <sup>a</sup>	9.5 <sup>a</sup>	5.4 <sup>c</sup>	6.2 <sup>c</sup>	8.4 <sup>ab</sup>	0.91
DOM <sup>2</sup> , g/d	385 <sup>e</sup>	437 <sup>de</sup>	587 <sup>b</sup>	681 <sup>a</sup>	469 <sup>cd</sup>	336 <sup>f</sup>	500 <sup>c</sup>	21
Microbial efficiency, g microbial N/kg of DOM	16.5	12.8	15.1	13.2	11.9	18.4	17.0	2.30
Rumen NH <sub>3</sub> -N, mg/dL	2.5 <sup>c</sup>	3.7 <sup>bc</sup>	6.6 <sup>a</sup>	7.0 <sup>a</sup>	6.1 <sup>a</sup>	5.5 <sup>ab</sup>	5.5 <sup>ab</sup>	0.70

<sup>1</sup> Urinary purine derivatives; <sup>2</sup> Apparently digested organic matter.

Within a row means without a common superscript letter differ ( $P < 0.05$ ).

## Intake and Digestibility

With the exception of pigeonpea hay, legume hay supplementation increased intake of DM, OM, and NDF (Table 2). Intakes of DM, OM, and NDF were greatest ( $P = 0.04$ ) in lambs supplemented with perennial peanut hay, followed by annual peanut hay, and they were least ( $P = 0.04$ ) in lambs consuming bahiagrass hay alone or pigeonpea hay compared with those consuming other legume hays. Intakes of DM, OM, and NDF were not improved by addition of soybean meal. Digestibilities of DM and OM were greatest ( $P = 0.03$ ) when diets were supplemented with perennial peanut hay, followed by annual peanut hay. Addition of the other supplements did not affect digestibility of DM or OM except that pigeonpea hay supplementation reduced ( $P = 0.04$ ) OM digestibility. Digestibility of NDF was greater ( $P < 0.01$ ) in lambs fed bahiagrass hay alone, soybean meal or perennial peanut hay than in lambs supplemented with cowpea hay.

## Nitrogen Utilisation

Nitrogen intake was increased ( $P < 0.01$ ) by supplementation regardless of supplement type and it was greatest ( $P < 0.01$ ) in lambs fed perennial peanut hay, followed by ( $P < 0.01$ ) annual peanut hay. Faecal N output was greatest ( $P < 0.01$ ) in lambs fed perennial peanut hay, followed by annual peanut and soybean hays, and it was least in lambs fed bahiagrass hay alone, soybean meal, or pigeonpea hay. Urinary N excretion was greater ( $P = 0.04$ ) in lambs fed perennial and annual peanut or soybean hays than in those fed bahiagrass alone, bahiagrass plus cowpea hay, or bahiagrass plus pigeonpea hay. Nitrogen retention and digestibility were increased by supplementation and the greatest ( $P < 0.01$ ) values occurred in lambs fed perennial peanut, followed by annual peanut. Purine derivative excretion and microbial N production were greater ( $P = 0.03$ ) in lambs fed perennial and annual peanut hays than those fed all other diets except soybean hay. Apparent digestible OM intake was greatest ( $P < 0.01$ ) in lambs fed perennial peanut hay, followed by annual peanut hay and it was least in those fed pigeonpea hay. Microbial efficiency was not affected by supplementation.

## Ruminal Fluid pH and $\text{NH}_3\text{-N}$

No interactions between treatment and time occurred. Ruminal pH was not different among dietary treatments. Ruminal ammonia-N concentration was greater ( $P < 0.01$ ) in lambs fed annual and perennial peanut and cowpea hays, than those fed bahiagrass alone or soybean meal.

## DISCUSSION

The nutritive value of the bahiagrass hay was similar to that reported by Kostenbauder et al. (2007) and the NDF, ADF, and CP concentrations of perennial peanut were similar to those reported for the Florigraze cultivar (Romero et al., 1987). The CP concentration of cowpea was similar to that reported for Iron clay cowpea grown in Florida (Higuera et al., 2001), but the NDF, ADF and ADL concentrations were almost twice as great as those of Iron clay cowpea harvested at an earlier maturity stage (canopy close) in Texas (Muir et al., 2001). The CP concentration and IVTD of pigeonpea were much less than those reported (20% and 49–55% IVDMD, respectively) for similar early-maturing cultivars that were harvested earlier (50% flowering) and cut at a greater stubble height (0.6 m; Alexander et al., 2007). The CP concentration of soybean was similar to those reported for other cultivars (Seiter et al., 2004) but the NDF and ADF concentrations were greater.

Perennial and annual peanut hays had greater IVTD than other legume hays because they contained less NDF and ADF. Although the values were not identical, the ranking of forages by IVTD was the same as that by *in vivo* apparent DM digestibility, indicating that the IVTD method is suitable for comparing these warm-season legume forages. All forages contained low concentrations of CT. Condensed tannins reduce forage quality at concentrations of 6% of DM or greater (Waghorn et al., 1994), but concentrations of 2–4% of DM usually result in improved forage nutrient utilisation by ruminants (Min et al., 2003 and 2005). Condensed tannin concentrations were consistent with those reported previously for soybean (Reddy et al., 1985), annual peanut (Karchesy and Hemingway, 1986), cowpea (Baloyi et al., 2001), pigeonpea (Alexander et al., 2007), and perennial peanut (Valencia et al., 2007) forages.

Legume hays contained less CP than anticipated based on concentrations of CP in the standing plants (Foster, 2008) primarily because of leaf shatter during harvest and chopping, indicating that harvest management practices that minimise such losses are critical for preserving the quality of the hays. Nevertheless, supplementation with all legume hays, except pigeonpea, increased DM and OM intake, though only supplementation with annual and perennial peanut hay also increased DM and OM digestibility. Moore et al. (1999) reported that supplements decreased voluntary forage intake when the forage TDN:CP ratio was  $< 7$  with a few exceptions such as when basal forage intake was  $> 1.75\%$  of bodyweight (BWt) as for our bahiagrass diet.

The intake responses in this study typify effects of legume forage supplementation to poor quality basal grass diets (Said and Tolera, 1993). The reticulate venation of legume leaves confers less resistance to ruminal degradation than the parallel venation of grass leaves (Frame, 2005). Consequently, legumes are degraded more easily and rapidly by ruminal microbes than grass leaves. In addition, lesser structural carbohydrate concentrations in legumes versus grasses contribute to the faster degradation and passage rates of legumes (Waghorn et al., 1989; Wilson, 1994; Jung and Allen, 1995; Dewhurst et al., 2003). Collectively, these factors increase feed intake due to the decreased rumen fill resulting from faster degradation and passage rates (Mertens, 1973; Reid et al., 1988). Relative differences in DMI and digestibility among legume hay supplemented diets reflect partly the structural fibre concentrations and morphological characteristics of the legumes. Annual and perennial peanut had less NDF and ADF than the other legume hays; consequently, they were more digestible. Pigeonpea hay had greater NDF, ADF, and ADL concentrations because of its thick, woody stems, which probably decreased DM and OM intake and OM digestibility. As in other studies (Mir and Mir, 1993; Haddad, 2000; Mupwanga et al., 2000), legume supplementation did not increase NDF digestibility partly because legumes had more lignin than grasses (Wilson, 1994).

Legume hay supplementation increased N intake because of the greater CP concentrations of the legumes versus bahiagrass, as well as the greater DMI of most of the legume-grass diets by lambs. Nitrogen retention increased accordingly because all supplements increased N digestibility and most decreased the proportion of intake N lost as urine. Legume supplementation increased ruminal  $\text{NH}_3\text{-N}$  concentrations because it increased N intake relative to the control diet and most of the protein in legumes is in the form of soluble protein or RDP (Broderick, 1995). Legume supplementation ensured that ruminal  $\text{NH}_3\text{-N}$  concentrations exceeded the recommended concentration (5.0 mg/dL; Satter and Slyter, 1974) for maximising microbial N synthesis. Microbial N synthesis was only increased by supplementation with annual or perennial peanut diets, partly because they provided more energy (apparently digested OM) for microbial growth (Clark et al., 1992) than other supplements.

Soybean meal supplementation increased N intake and retention, reflecting the greater N concentration of soybean meal; however, the small amount of soybean meal that was fed was not sufficient to significantly improve other measures of digestion. Supplementation with N from legume hays or soybean meal increased N intake, digestion and retention, indicating that supplementation may improve the performance of lambs on bahiagrass diets. As a consequence of the relatively small amount of soybean meal supplemented, annual and perennial peanut hay supplements were more effective than soybean meal supplementation at improving N intake, digestion, retention, and microbial N production. Due to these positive effects on N metabolism, perennial peanut and annual peanut hays were the best legume hay supplements for the lambs although soybean and cowpea hays also showed some promise. Pigeonpea hay was the least desirable supplement because it did not improve DMI and it reduced OM digestibility. Pigeonpea should be harvested at greater stubble heights for use as a legume supplement, though this would reduce biomass yields. Future research should determine the optimal inclusion rates of perennial peanut, annual peanut, cowpea, and soybean hays in the diets of growing lambs and beef calves.

## CONCLUSION

Annual and perennial peanuts were the best supplements and pigeon pea was the worst supplement for the lambs in this study.

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# Methane Emissions by Livestock in India and Mitigation Strategies

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## ABSTRACT

In India 92% enteric methane (CH<sub>4</sub>) is emitted by cattle and buffalo, about 7% by sheep and goats and only a very small fraction (less than 1%) is attributed to other ruminants and non-ruminant herbivores like yak, mithun, pig, horse, mules, ass, etc. Therefore the strategies to be adopted to mitigate CH<sub>4</sub> emissions by livestock are primarily centred around cattle and buffalo. Keeping in mind the agricultural practices in India, the major mitigation techniques which might have some scope for practical application include replacement of non-productive or low productive animals with superior livestock, improving the quality of feeds offered to animals and the use of plant secondary metabolites which are present in many tropical plants. After screening more than 150 plant extracts, it was found that *Terminalia chebula*, *Sapindus mukorossi*, *Populus deltoides*, *Foeniculum vulgare*, *Syzygium aromaticum*, *Allium sativum*, *Psidium guajava*, *Mentha piperita* and *Eucalyptus globulus* were capable of inhibiting methanogenesis and ciliate protozoa in an *in vitro* gas production test. Although these plant extracts exhibited more than 50% inhibition in *in vitro* experiments, the same plants/plant extracts either showed no effect or a very poor effect in *in vivo* experiments. Probable reasons include the different concentrations of plant secondary metabolites used in *in vivo* and *in vitro* experiments and large variations in the chemical composition of different accessions of the plant products. Therefore, detailed experiments are needed to optimise the doses of plant secondary metabolites required to produce significant inhibition of CH<sub>4</sub> emissions without adversely affecting animal performance.

**Key words:** methane emissions, tropical plant extracts, secondary metabolites, gas production test, open circuit respiration calorimetry.

## INTRODUCTION

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 a small number of yaks, mithuns, mules etc. (FAO, 2006), which account for 11.95% of the global livestock population and produce 12.45% of the total enteric CH<sub>4</sub> emissions. If emissions from livestock excreta are included, this proportion is reduced to 10.76% of the global emis-

sions because waste management in western countries (especially USA and Europe) is by anaerobic processes which produce more CH<sub>4</sub> whereas solid waste management is practised in India which is an aerobic process producing lower CH<sub>4</sub> emissions. Although the situation does not appear to be so alarming, keeping in view the future commitments of the Kyoto Protocol, mitigation of CH<sub>4</sub> emissions is essential to protect the environment. By doing so, better feed conversion efficiency can be obtained as a bonus.

The CH<sub>4</sub> emission factors for different categories of animals under Indian conditions are much lower than those calculated by the IPCC (2001 and 2006). Based on studies conducted at the Indian Veterinary Research Institute (IVRI), Izatnagar, India, the CH<sub>4</sub> emission factors (CH<sub>4</sub> emission, kg/h/y) for cattle, buffalo, sheep and goat vary between 25.6–47.6, 28.9–52.7, 2.6–4.1 and 3.3–4.3, respectively (Table 1). The variations might be due to different body weights of the animals, types of feed and individual behaviour (high or low CH<sub>4</sub> producer) of the animals. These CH<sub>4</sub> emission factors have been calculated on the basis of experiments conducted under conditions where animals are fed according to prescribed feeding standards, but the majority of animals at the farmers' door are fed much lower quality nutrients. Therefore, the estimates of CH<sub>4</sub> emissions calculated above are an over estimates to the tune of 20–25%.

Ruminants in India are fed primarily on a diet rich in cellulose, hemicellulose and lignin. Unfortunately, ruminants do not produce any enzyme which can hydrolyse these polymers into monomers for further utilisation as a source of energy. Therefore, these animals depend upon microbes which live in the gastrointestinal tract in an ecto-symbiotic relationship with the animal and help to bioconvert these polysaccharides into a usable of energy i.e. volatile fatty acids. This bioconversion process is very complex, being accomplished by the synergistic activities of different groups of microbes present in the reticulo-rumen e.g. bacteria, protozoa, fungi, archaea and bacteriophages.

The major substrates to be fermented in the reticulo-rumen consist of structural (cellulose, hemicellulose) and non-structural (soluble sugars, starch and pectin) carbohydrates, which are hydrolysed to mono- and disaccharides by microbial activity as an initial step in fermentation of feed. These monomers and dimers of sugars are absorbed by the microbes and partially oxidised to volatile fatty acids (VFAs [acetate, propionate, butyrate, lactate, valerate and other iso-acids]) and yield energy for their survival and growth. In the process of microbial fermentation under anaerobic conditions, reduced cofactors like NADH and NADPH are produced. For re-use in the anaerobic system, these reduced cofactors are oxidised to NAD<sup>+</sup> and NADP<sup>+</sup> by electron transfer to terminal acceptors other than oxygen. As CO<sub>2</sub> is already available in the rumen, CH<sub>4</sub> generation is preferred over the other hydrogen sinks (e.g. SO<sub>4</sub>, NO<sub>3</sub>, fumarate etc.) and therefore a significant portion of energy is wasted in

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**Table 1. Methane emissions from Indian livestock (data from IVRI, Izatnagar).**

Animal	BWt (kg)	Methane (L/h/d)	Methane (%GE)	Methane (kg/h/y)
Sheep	13–44	10–15.7	3.0–12.7	2.6–4.1
Goat	20–32	12.6–16.5	5.2–7.5	3.3–4.3
Cattle	220–360	98–221	3.6–8.4	25.6–47.6
Buffalo	200–501	111–202	3.9–9.0	28.9–52.7

the form of CH<sub>4</sub> (Johnson and Johnson, 1995) as it cannot be oxidised further in the rumen under anaerobic conditions. As the global warming potential of CH<sub>4</sub> is 23 times greater than that of carbon dioxide (IPCC, 2001), it is essential that its level in the atmosphere is kept within safe limits.

This paper describes studies to evaluate different strategies to control CH<sub>4</sub> emissions from the animals while promoting economic and eco-friendly livestock production.

## STRATEGIES TO MITIGATE METHANOGENESIS

### Replacement of Non-productive Animals

Non-productive or low productive animals should be replaced with either high producing indigenous cattle/buffalo or high producing crossbred cattle. This is not an easy task to accomplish in Indian conditions as non-productive and low producing animals cannot be removed immediately due to the ban on cow slaughter. However, this might be achieved slowly by replacing existing poor performers with high producing cattle. If a target is set to increase the number of high producing cattle from the present figure of 23 million to 46 million within the next five years, excretion of wastes and CH<sub>4</sub> would increase by only 3.2% and 1.7% respectively, but excreta and CH<sub>4</sub> produced/unit of livestock productivity would be considerably reduced.

### Dietary Manipulations

Energy level in the diet and the type of roughage used (wheat straw, paddy straw or sugarcane bagasse) have significant effects on the extent of methanogenesis, while the level of protein does not appear to be important if it meets the minimum requirement of the animals (Chatterjee et al., 2006). Sugarcane bagasse caused the highest production of CH<sub>4</sub>/unit digested dry matter (DM) and wheat straw the minimum, while CH<sub>4</sub> generation was intermediate with paddy straw as the roughage source. Sugarcane bagasse and paddy straw as substrate produced 11% and 4% more CH<sub>4</sub> than wheat straw. The *in vitro* evaluation of oilseed cakes revealed minimum CH<sub>4</sub> production with castor bean cake and karanj seed cake (20–21 ml/g DM), maximum with soybean cake (31 ml/g DM) and intermediate production with mustard, cotton and groundnut cakes. However, reductions in CH<sub>4</sub> production were accompanied by lower *in vitro* degradability which might be due to the presence of antinutritional factors in these two cakes (Kumar et al., 2007).

Improvement in the digestibility of lignocellulosic feeds with different treatments also results in lower methanogenesis by livestock. Wheat straw treated with urea (4 kg urea/100 kg wheat straw) or urea plus calcium hydroxide (3 kg urea + 3 kg calcium hydroxide/100 kg wheat straw) and stored for 21 d before feeding, reduced significantly the CH<sub>4</sub> emissions by sheep (Sahoo et al., 2000).

Beauchemin and McGinn (2006) reported that adding canola oil at the rate of 4.6% DM intake (DMI) inhibited CH<sub>4</sub> emissions by 32%

and as a percent gross energy intake by 21%, but the decreases were attributed primarily to reduced feed intake and lower total digestibility of feed, especially the fibre component. On the other hand, in a study conducted by Cosgrove et al. (2008), infusion of a blend of linseed and sunflower oils (3:1) in the rumen of eight-month old wether sheep at the rates varying from 1.2% up to 6.2% of DMI did not affect CH<sub>4</sub> emissions, DMI and the concentrations and proportions of VFAs. There was, however, a loss of 7.5% gross energy in the form of CH<sub>4</sub> which was attributed to the presence of long chain fatty acids in these oil plants.

Fumaric acid is a precursor of propionic acid during feed fermentation in the rumen and may act as an alternative hydrogen sink. Its inclusion in the diet increased total VFA concentration, increased propionate proportion and decreased the acetate:propionate ratio (Beauchemin and McGinn, 2006), but the levels required to inhibit methanogenesis to a significant extent may cause a drop in pH which might adversely affect feed fermentation. Wallace et al. (2006) reported that encapsulating fumaric acid in a shell of hydrogenated vegetable oil prevented a fall in pH, but retained its ability to inhibit methanogenesis. Free fumaric acid (10% in the ration) and an equivalent amount of encapsulated fumaric acid decreased CH<sub>4</sub> emission by 49% and 75% respectively compared with control sheep. In wether lambs there was a CH<sub>4</sub> emission (g/d) which decreased linearly with increasing dose of fumaric acid (zero to 10% of the diet), but when expressed in terms of g/kg DMI, emissions were similar in all groups. Interestingly there was also a linear increase in rumen pH with increasing dose of fumaric acid, a finding which contrasts with other studies (Molano et al., 2008).

### Plant Secondary Metabolites

Plant secondary metabolites (saponins, tannins, lignins, essential oils etc.) have anti-microbial activities to protect plants against invasion by microbes. This property has been exploited for controlling undesirable microbes in the rumen. Initial screening experiments have indicated that extracts of saponin-rich plants like *Sapindus mukorossi*, *S. saponaria* and *Acacia concinna* (Hess et al., 2004; Agarwal et al., 2006; Patra et al., 2006b), essential oil-rich plants like *Allium sativum*, *Coriandrum sativum*, *Eucalyptus globulus*, *Foeniculum vulgare*, *Mentha piperita*, *Ocimum sanctum*, *Populus deltoids* and *Syzygium aromaticum* (Busquet et al., 2006; Patra et al., 2006c; Agarwal et al., 2009) and tannin-rich plants like *Bergenia crassifolia*, *Emblia officinalis*, *Peltiphyllum peltatum*, *Populus deltoides*, *Quercus incana*, *Rheum undulatum*, *Terminalia bellerica*, *Terminalia chebula* and *Vaccinium vitis-idaea* (Patra et al., 2006a and b; Kamra et al., 2006; Jayanegara et al., 2009) are examples of products which exhibited antimethanogenic and antiprotozoal activities. However, some of them also had adverse effects on feed degradability and nutrient utilisation by the animals.

Based on the results of *in vitro* screening experiments, a few plants and some mixtures of plants were selected for inclusion in



**Table 2. Effect of plant secondary metabolites on *in vivo* CH<sub>4</sub> emissions and feed DM digestibility.**

Plant	Methane inhibition (%)	DM Digestibility (%)	Animal	Reference
Mixture of <i>Allium sativum</i> , <i>Syzygium aromaticum</i> , <i>Foeniculum vulgare</i> and <i>Mentha piperita</i> (3 g/100 kg BWt)	No effect	No effect	Buffalo	Agarwal et al., 2007
<i>Terminalia chebula</i> (1% DMI)	24.0	11.3 (+)		
<i>Allium sativum</i> (1% DMI)	11.9	11.1 (+)	Sheep	Patra et al., 2008
<i>Terminalia chebula</i> and <i>Allium sativum</i> (0.5% each of DMI)	23.5	10.6 (+)		
<i>Allium sativum</i> and <i>Mentha piperita</i> (1% and 0.1% of DMI on alternate d) (Mix 1)	7.0	No effect	Buffalo	Verma et al., 2009
Mixture of three plants (2% of DMI on alternate days) (Mix 2)	12.0	No effect	Cattle calves	Unpublished
Mixture of three plants (1% of DMI/d) (Mix 3)	11.0	No effect		
Mixture of three plants (2% of DMI/d) (Mix 3)	15.5	No effect	Buffalo calves	Chaudhary et al., 2009
Mixture of three plants (3% of DMI/d) (Mix 3)	27.8	No effect		
Canola oil	32.0	Reduction	Growing beef cattle	Beauchemin and McGinn (2006)
A blend of linseed and sunflower oils (3:1) (Infused at 1.2% - 6.2% DMI)	No effect	No effect	sheep	Cosgrove et al., 2008

the diets of ruminants to study their effect on *in vivo* CH<sub>4</sub> emissions using open circuit respiration calorimetry. 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 3 g/100 kg BWt (Table 2). Each plant individually inhibited *in vitro* methanogenesis, but in *in vivo* studies there was no effect on CH<sub>4</sub> emissions, VFAs, microbial profiles and nutrient digestibilities (Agarwal et al., 2007). This might be due to insufficient levels of plant secondary metabolites being fed to the animals, since the level of secondary metabolites present in the additive and the nature of diet are some of the important factors influencing animal responses (Calsamiglia et al., 2007).

*Terminalia chebula*, *Allium sativum* and a mixture of the two, when fed to sheep at the rate of one percent DMI, decreased CH<sub>4</sub> production (L/kg digestible DMI) by 24%, 11% and 23.5%, respectively, but CH<sub>4</sub> energy losses as a percentage of digestible energy intake decreased ( $P = 0.08$ ) in the groups fed *T. chebula* and the mixture compared with the control and *A. sativum* fed groups (Patra et al., 2008). Both these plants separately and as a mixture caused an improvement of between 10.6% and 11.3% in feed DM digestibility (Table 2). *T. chebula* is a rich source of tannins (4.89% DM), whereas *A. sativum* is rich in essential oils. The data indicated that *T. chebula* was more effective than garlic. The low *A. sativum* activity might be explained by the instability of allicin, the main secondary metabolite responsible for the antimicrobial activity of *A. sativum*.

Murrah buffaloes fed a wheat straw and concentrate mixture (50:50) and supplemented with a feed additive (a mixture of *Allium sativum* [1%] and *Mentha piperita* oil [0.1%] of DMI, [Mix 1]) on alternate d reduced CH<sub>4</sub> emissions by seven percent (L/kg DMI), but these reductions were attributed to lower DMI (Verma et al., 2009). There was no adverse effect on rumen fermentation pattern, enzyme and microbial profiles. In another experiment, a mixture of three plants (Mix 2) fed to calves on alternate d at the rate of 2% DMI caused a 9.4% fall in CH<sub>4</sub> emissions and BWt gain was 8.7% (448 g/day vs 412 g/d) higher than in controls, with no adverse effect on digestibility of nutrients (unpublished data).

In the authors' laboratory, a mixture of three plants (Mix 3) fed to buffalo calves at the rate of one, two and three percent of

DMI, resulted in a dose-dependent inhibition of CH<sub>4</sub> emissions (L/kg digestible DM) without affecting DM digestibility at any feed additive levels (Chaudhary et al., 2009). Volatile fatty acid and fibre degrading enzyme activities were not affected, although there were a few changes in rumen microbial profiles as estimated by real time-polymerase chain reaction (RT-PCR), but these were not responsible for any significant change in rumen fermentation.

## CONCLUSIONS

Experiments conducted so far indicate that the type of diet, feed additive, roughage level and type, the nature of the oil cake including their residual fatty acids are some of the important factors which affect rumen fermentation and CH<sub>4</sub> emissions by livestock. Therefore, it is very important to formulate diets by selecting ingredients which are poor CH<sub>4</sub> producers but are easily available to the farmer.

The results of *in vivo* experiments indicate that plants containing secondary metabolites which show promising results in *in vitro* experiments, do have a potential as rumen modifiers for controlling CH<sub>4</sub> emissions by ruminants. The levels used in *in vitro* experiments are usually very high and may not be usable in *in vivo* experiments. There is an interaction between feed additive and the diet fed to the animal. Therefore, levels of plant additives have to be standardised for different diets for practical application, in order to obtain significant inhibition of methanogenesis without adversely affecting nutrient utilisation.

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# Correlation Between Milk and Blood Urea Nitrogen in High and Low Yielding Dairy Cows

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## ABSTRACT

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, 30–90 d in milk) and low yielding cows (LY, >180 d in milk). The objective of the study was to evaluate the effect of milk yield and time after feeding on milk and blood urea-nitrogen (BUN) concentration, and to establish a correlation between these two parameters. On Olocuilta, HY cows had the highest BUN and milk urea nitrogen (MUN) concentrations. Blood urea nitrogen least squares concentration was 12.77 mg/dL and 13.98 mg/dL for the LY and HY cows, respectively; while the MUN average concentration was 12.30 mg/dL and 14.82 mg/dL for the LY and HY cows, respectively. BUN and MUN concentrations were similar at 30 min, one and two h post-feeding but by four h post-feeding BUN concentrations had decreased and were significantly lower than those 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 the MUN average concentration was 10.18 mg/dL and 8.83 mg/dL for the LY and HY cows, respectively. The reason for these differences seems to be related to protein balance. For instance on the Los Conacastes farm, protein balance was negative in the HY group (-88 g/d) while on Olocuilta farm the balance was positive. The correlation between BUN and MUN for the Olocuilta farm had a regression coefficient of 0.84, and a correlation ( $r^2$ ) of 0.7543. For Los Conacastes these values were 1.04 and 0.9017, respectively. It should be noted that BUN and MUN concentrations were better correlated at 30 min, one h and two h after feeding and that the correlation decreased at four h post-feeding due to a drop in BUN concentration. It is concluded that BUN and MUN concentrations are not related directly to milk yield but with the protein balance. There was a high correlation between BUN and MUN concentrations; hence, either of these parameters can be used to monitor protein nutrition in dairy farms.

**Key words:** *Holstein cows, milk yield, time of feeding, blood urea nitrogen, milk urea nitrogen, protein nutrition.*

## INTRODUCTION

Dairy cows need appropriate quantities of protein for milk production and for this reason diets are supplemented with additional protein.

However, excess protein can negatively affect production and reproduction, and pollute the environment (Sonderegger and Schurch, 1977; Peabody, 2004). When an excess of degradable protein relative to energy is present in the rumen, the concentration of rumen ammonia increases and elevates rumen pH (Gómez and Fernández, 2002). Some of the ammonia liberated in the rumen cannot be fixed by the microorganisms; this excess is absorbed and taken into the blood. The liver converts ammonia to urea which is excreted by the animal in the urine (Garriz and López, 2002). High concentrations of urea reflect an excess of protein in the diet, which can adversely affect fertility (Ropstad and Refsdal, 1987; Melendez et al., 2000; Nousiainen et al., 2004). An urea-nitrogen concentration higher than 20 mg/dL of milk suggests an excess protein supply in the diet which can decrease production and cause fertility problems (Ferguson and Chalupa 1989; Hojman et al., 2004). This also makes the diet more expensive and increases nitrogen excretion to the environment (Jonker et al., 1998). Measurement of urea nitrogen in blood and milk has been proposed as a tool to monitor protein nutrition (Ferguson et al., 1993; Hof et al., 1997).

Blood urea gets transported into milk and therefore urea is a normal constituent of milk (Ferguson, 2002; Acosta and Delucchi, 2002). The relationship between the levels of (BUN) and MUN in dairy cattle depends on the degradability of the different protein sources and nitrogen compounds (Acosta and Delucchi, 2002). Since it is both simpler and less stressful to take milk than blood samples (Acosta and Delucchi, 2002; Acosta et al., 2006), measurement of MUN is an easy method for determining BUN levels and for assessing the protein and energy supply in the diet. By determining MUN levels, milk producers could be advised on the appropriateness of different diets for providing a proper protein to energy ratio.

The main objective of this investigation was to establish correlations between BUN and MUN concentrations in dairy cows at two levels of milk production i.e. high and low, and at different times after feeding.

## MATERIALS AND METHODS

### Animals and Feeding

The study was carried out from December 2006 to July 2007 on two dairy farms: Ranch Olocuilta and Ranch Los Conacastes located in the central region of El Salvador. The herds had more than 80% of Holstein genetic make up and were producing more than 15 kg milk/cow/d. The cows were kept and managed in free stall barns. The feed offered consisted of forage (silage or green grass) and concentrate. Thirty cows were selected on each farm. Fifty percent of the cows on each farm were between 30–90 d in milk (high production) and the other 50% were > 150 d in milk (low production).

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## Collection and Analysis of Milk

Milk and blood samples were taken at 30 min, one, two, and four h after feeding. Blood samples were taken from the jugular vein into vacutainer tubes without an anticoagulant. Milk samples (50 mL) were obtained directly from the udder.

After clotting, blood samples were centrifuged at 7 000 rpm for 15 min., and sera collected and stored in cryovials at -20°C for later analysis. BUN concentration was determined by means of the Liq-uicolor Enzymatic Colorimetric Test (Human®, Damstad, Germany). A total of 240 samples were analysed.

MUN concentrations were determined in the clear medium obtained after precipitation of proteins using trichloroacetic acid for 15 min and then centrifuged at 4 000 rpm for 10 min and after filtering the supernatant and diluting it to 1:100 with distilled water. Urea nitrogen was analysed using the technique described by Merck® (Darmstadt, Germany). Urea standards were prepared at concentrations of 1–5 ppm. A graph relating urea concentration (ppm) and absorbance was drawn to obtain the equation  $y = ax + b$ ; in which  $x$  represents the urea concentration and  $y$  represents the absorbance. Urea (mg/dL) =  $(Absorbance - b) / a$ . The urea level in mg/dL was multiplied by 4.16 to obtain MUN.

## Feed Analyses

Feeds were analysed for crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Information on the characteristics of the cows and the proportions and composition of ingredients was entered into the NRC Dairy Cattle Program (NRC, 2001) to obtain reports on the protein and energy balances of the high and low producing cows.

## Statistical Analyses

The effect of milk yield (high and low) and time after feeding (0.5, one, two and four h) on BUN and MUN concentrations were analysed using repeated measures analysis and the MIXED procedure of SAS (SAS Institute, Version 9.1.3, 2006). Variables for which variance analysis were significant at  $P < 0.001$ , were subjected to a test of comparison using the Student's *t*-test. Data were also subjected to linear regression to find the correlation and regression coefficients between BUN and MUN. In this case the GLM procedure of SAS was used.

## RESULTS AND DISCUSSION

### Protein Balance

On Olocuilta, the protein content in the diet was higher for the high yielding cows than for the low yielding and the same pattern was found for the protein balance (Table 1). On Conacastes, the protein

percentage was similar for the two groups, while protein balance was positive in the low yielding cows but negative in the high yielding cows. These results showed that high producing cows are not necessarily those that receive more protein or have a more positive protein balance.

### Blood Urea Nitrogen (BUN)

High yielding cows on Olocuilta had higher average values of BUN than low yielding cows (13.94 vs 12.75 mg/dL,  $P < 0.05$ ). These levels are within the acceptable range of 10–20 mg/dL (Ferguson et al., 1993), and reflect the fact that the protein levels in the diet were adequate. On Conacastes on the other hand, it was found that high producing cows had lower values of BUN than low producing cows (9.09 vs 11.20 mg/dL,  $P < 0.05$ ); this could be explained by the negative protein balance in high yielding cows.

### Milk Urea Nitrogen (MUN)

The pattern of MUN was similar to that of BUN. On Olocuilta, high yielding cows had higher ( $P < 0.05$ ) values of BUN (average 14.99 mg/dL) than those of low yielding ones (average 12.26 mg/dL) throughout the sampling period (Figure 1). However, for both productive states the values were within the normal range (10–20 mg/dL).

On Conacastes, higher mean values for MUN were obtained in the low yielding group (10.15 mg/dL). The MUN for the high yielding group was 8.91 mg/dL. It has been reported that MUN concentra-

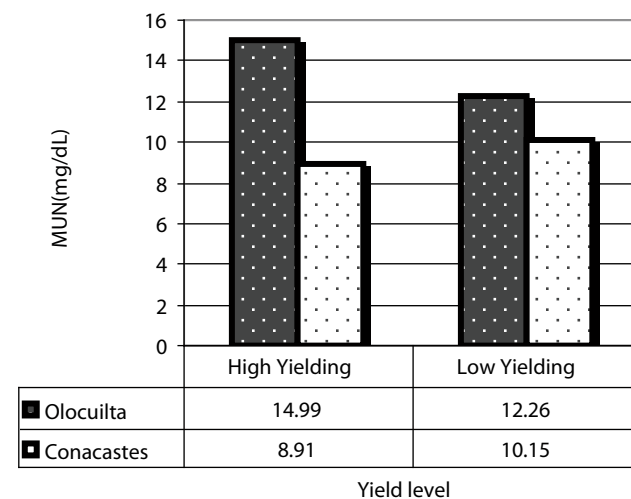


Figure 1. Milk urea nitrogen (mg/dL) for high and low yielding dairy cows on two farms.

Table 1. Milk production, nutritional composition of the diet and protein balance on two 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 <sub>i</sub> (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

tions usually fall at the beginning and the end of lactation rather than in mid lactation. Probably these observed variations respond more to changes in the nutrient demand during the postpartum period than to changes in the diet (Acosta and Delucchi, 2002).

### Time after-Feeding

Urea concentrations were compared in blood and milk at different times after feeding. For the Olocuilta farm these values ranged from 12.8 to 13.8 mg/dL (Figure 2). BUN and MUN values were similar for high and low producing cows at 30 min, one and two h after feeding. However, by four h post-feeding, BUN concentrations had decreased and were lower than those in milk ( $P < 0.05$ ). This differ-

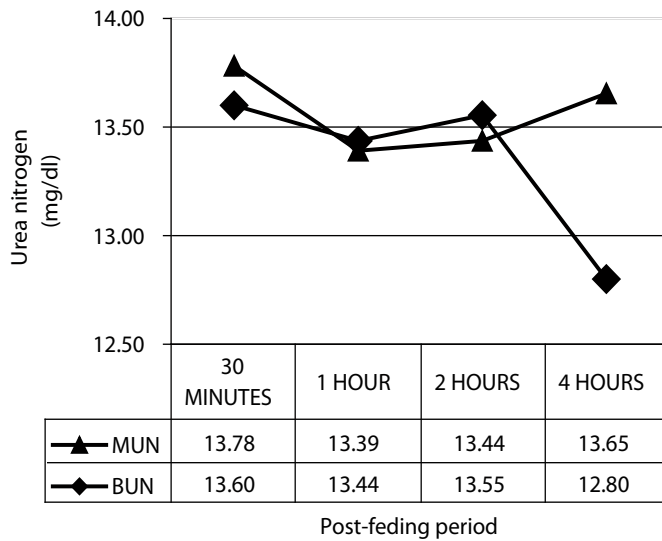


Figure 2. BUN and MUN concentrations at different times after feeding dairy cows on Olocuilta farm.

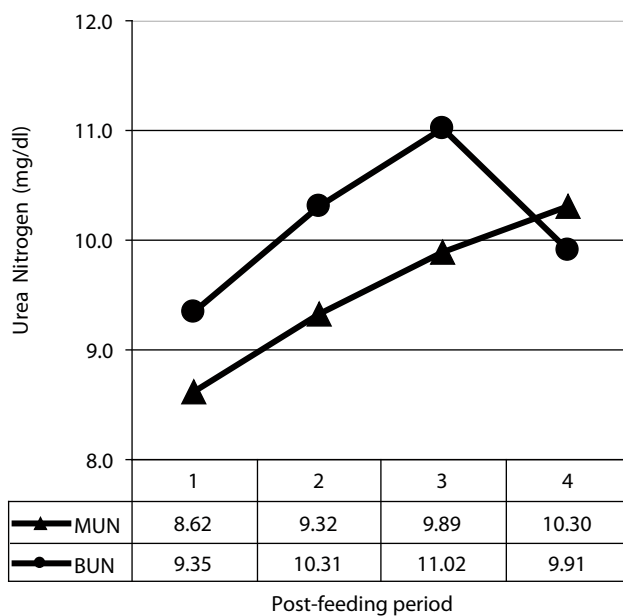


Figure 3. BUN and MUN concentrations at different times after feeding dairy cows on Conacastes farm.

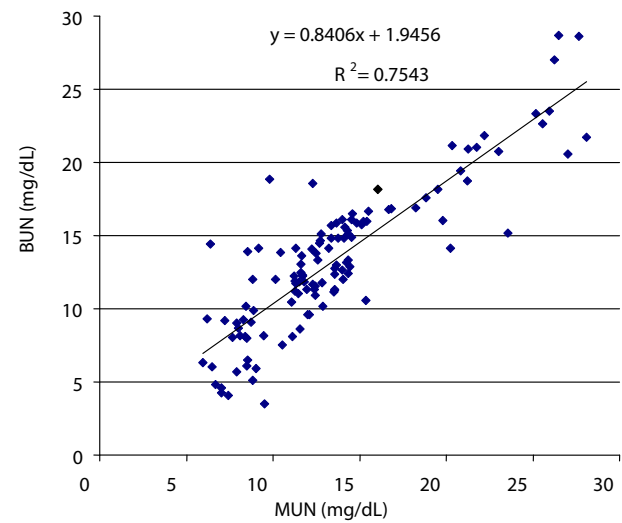


Figure 4. Correlation between BUN and MUN concentrations for dairy cows in Olocuilta farm.

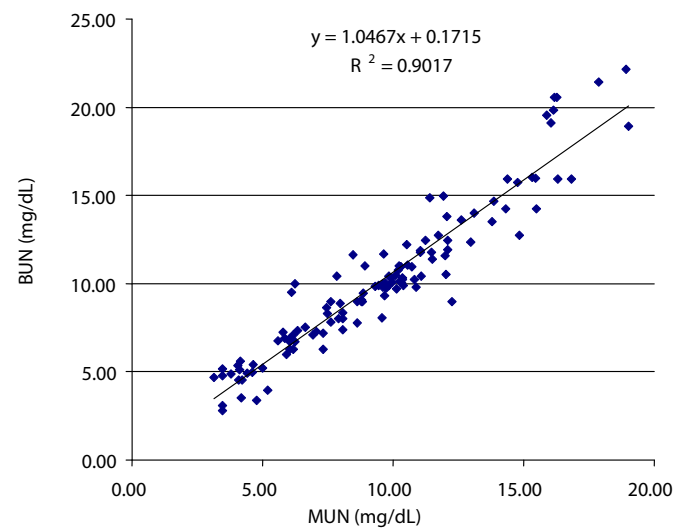


Figure 5. Correlation between BUN and MUN concentrations for dairy cows on Los Conacastes farm.

ence could be due to changes in absorption or production of urea that are quickly reflected in blood levels but not in previously synthesised and stored milk.

On Los Conacastes farm, BUN concentrations tended to be higher than those of MUN (Figure 3) and as observed on Olocuilta farm, a decrease was observed in concentration at four h post-feeding. However, BUN and MUN concentrations were statistically similar at all times after feeding. Butler (1998) reported elevations of BUN at four and eight h after feeding a 19% CP total mixed ration. However, the CP content in the present study was lower (Table 1).

Noticeable was how BUN values increased up to three h post-feeding and then started to decrease while MUN values increased steadily. These results suggest that MUN measurements have an advantage over BUN since milk samples can be taken at any time

after feeding. In other words, MUN values are better for predicting the nutritional status of the animals.

### Correlation between BUN and MUN

**Figure 4** shows the correlation between BUN and MUN concentration considering the values at all time periods after feeding. For Olocuilta farm, the regression coefficient between BUN and MUN was 0.84 (slope of the curve), while the correlation was 0.7543 ( $r^2$ ) (**Figure 4**). It should be noted that BUN and MUN concentrations were better correlated at 30 min, one, and two h after feeding.

The relationship between BUN and MUN for animals on Conacastes farm is shown in **Figure 5**. In this case the correlation was 0.90 ( $r^2$ ) and the regression coefficient was 1.04. The values for MUN and BUN were almost identical (9.53 mg/dL and 10.15 mg/dL, respectively).

When all blood and milk determinations were compared using the Student's test the association between both methods was high ( $P < 0.05$ ), and when averages were compared they are almost the same for MUN (13.53 mg/dL) and BUN (13.35 mg/dL). Therefore, either measurement can be used to determine protein status using urea nitrogen although milk sampling is both simpler and causes no stress on the animals. Nonetheless, BUN values tend to decrease when sampling four h post-feeding, while that variation was not seen when determining MUN concentrations.

It has been established that urea balances quickly with other body fluids including milk, and that a relationship between BUN and MUN can be calculated (Broderick and Clayton, 1997). MUN values represent 83–98% of BUN values and hence by dividing MUN by 0.85 a good estimate of BUN can be obtained (Arias and Nesti de Alonso, 1999). The results obtained in this study are in accordance with this statement.

When comparing BUN concentration for the two farms, the values differed significantly ( $P < 0.05$ ) with means being higher in farm Olocuilta (13.34 mg/dL vs. 10.14 mg/dL).

### CONCLUSIONS

The determination of urea in milk is a useful tool for monitoring protein nutrition in dairy cows since is a reliable technique and highly correlated with BUN. In the nutritional management of dairy cows diets should be balanced based on the analysis of the feedstuff to have a good approach of the balance of the nutrients in the animal. For determinations of BUN, it is better to carry out the samplings before two h after feeding; for MUN, sampling can be carried out until four h after feeding without significantly altering the values.

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# Use of Sodium or Calcium Salts of Fatty Acids as Sources of Energy in Buffalo Rations during Late Pregnancy

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## ABSTRACT

Thirty pregnant buffaloes expected to calve within 60-75 days were divided into three groups balanced by bodyweight and expected date of parturition. The first group received a control ration consisting of concentrate diet (75% concentrate feed mixture with 25% yellow corn) plus berseem (*Trifolium alexandrinum*) hay and rice straw. In the second and third groups, yellow corn was replaced with either sodium salts of fatty acids (Na-SFA) or calcium salts of fatty acids (Ca-SFA). The content of acid ether extract (AEE) in Ca-SFA was lower than that of Na-SFA, while TFAs in Ca-SFA were higher. Degradability rates of dry matter (DM), organic matter (OM), crude protein (CP), effective degradability (ED) and potential degradability (PD) decreased with the ration containing Na-SFA. Undegradable values of DM, OM and CP increased with adding Na-SFA compared with adding Ca-SFA or the control diet. Digestion coefficients of DM, OM, CP and cell wall constituents (CWC) were lower with feeding the ration containing Na-SFA compared with that containing Ca-SFA, while no significant differences were found between the control and Ca-SFA-containing rations. Values for total digestible nutrients (TDN) and digestible crude protein (DCP) were reduced ( $P < 0.05$ ) with the ration contained Na-SFA compared with Ca-SFA. Feed intake was not affected by feeding rations containing Na-SFA or Ca-SFA, but bodyweight (BWt) was higher after feeding rations containing Ca-SFA or Na-SFA compared with the control. pH values, propionic acid and free fatty acids (FFAs) in the rumen were higher ( $P < 0.05$ ) when feeding the ration containing Na-SFA compared with that containing Ca-SFA or the control, while total volatile fatty acids (TVFAs), acetic, Ac:Pr ratio and NH<sub>3</sub>-N were significantly decreased. Adding Na-SFA in the ration decreased glucose and total protein concentrations in blood compared with Ca-SFA or in the control. Concentrations of albumin, globulin and their ratio were not affected with feeding rations containing either Na-SFA or Ca-SFA while levels of total lipid (TL), triglyceride and FFAs were higher ( $P < 0.05$ ) with feeding rations containing fat than with the control ration.

**Key words:** *Buffaloes, soapstock, fatty acids, feed intake, bodyweight, pregnancy.*

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## INTRODUCTION

Diets based on bulky feed resources are unsuitable for pregnant animals if they are not supplemented with a high proportion of concentrates (Mahouachi et al., 2004). It is expected that added fat is generally favourable to foetal development, mammary adipose tissue and subsequent milk production, especially for late pregnant buffalo. Soapstock (sodium salts of fatty acids, Na-SFA) is the waste generated in the mill during refining of crude oil when sodium hydroxide reacts with the free fatty acids in the oil (Khatab et al., 2001). Significant amounts of soapstock are produced from the processes of refining seeds for oil, and while these by-products are potentially harmful to the environment, they are by-products which are potentially available as dietary fat sources (Shain et al., 1993). Soapstock as fatty acids has a higher inhibitory effect on rumen microbes than in the form of triglycerides (Wu et al., 1993). Adding either 2.5 or 5% dietary soapstock on a DM basis to the diets of beef cattle tends to decrease rumen digestibilities of CP and crude fibre (Perry and Weatherly, 1976).

The aim of this study was to examine the impacts on rumen functions and performance of adding Na-SFA or Ca-SFA as an energy source instead of corn grains to the rations of late pregnant buffaloes.

## MATERIALS AND METHODS

Palm oil and sunflower oil soapstocks were air dried and the resulting lumps were then broken in a hammer mill and mixed 1:1 on a DM basis with other concentrate ingredients in a granular form of 3 mm diameter or converted to calcium salts of fatty acids (Ca-SFA) according to El-Bedawy et al. (2005).

## Animals and Diets

Thirty pregnant buffaloes in their third lactation and that were expected to calve within 60–75 d were divided into three groups according to BWt and expected date of parturition and penned in ventilated sheds. Three concentrate portions of the diets were formulated and pelleted in a feed mill, Diet 1 consisted of a 75% concentrate feed mixture (CFM) containing 29% cottonseed meal, 26% yellow corn, 35% wheat bran, 6% molasses, 3% limestone and 1% common salt, and 25% yellow corn. In Diets 2 and 3, either Na-SFA or Ca-SFA replaced 100% of corn energy, respectively. The diets were formulated and adjusted biweekly according to changes in BWt by adding berseem hay and rice straw to cover animal requirements according to NRC standards (1988).

## Digestibility Trials

Digestibility trials were carried out at the end of the experimental period using three replicates and applying the acid insoluble ash (AIA) technique suggested by Van Keulen and Young (1977). Acidified ether extract (AEE) was determined by a modified method described by (Abo-Donia et al., 2003). Samples of feeds or residues, faeces and urine were subjected to proximate analysis (AOAC., 1990). Fibre fractions as neutral detergent-fibre (NDF), acid detergent fibre (ADF) and acid detergent-lignin (ADL) were determined according to Goring and Van Soest (1970). Hemicellulose and cellulose were calculated as the difference between NDF, ADF and ADL. Gross energy (GE) of feed and faeces were determined using a Gollen Kump ballistic bomb calorimeter, (catalogue No CCBB: 33-0101). Total fatty acids (TFAs) in Na-SFA and Ca-SFA were determined according to AOCS (2000), while in feed and faeces as described by Sukhija and Palmquist (1988).

## Blood Analyses

Blood plasma samples were withdrawn from three buffaloes at the end of the feeding period 4 h after feeding. Total protein, albumin,

glucose, total lipids (TLs) and triglycerides (TGs) were determined calorimetrically using commercial kits (Bio Merieux 69280 Marcy-1, Etoile/France). Globulin was obtained by subtracting the albumin value from the total protein concentration and the albumin:globulin ratio was calculated by dividing albumin by corresponding globulin values. Total fatty acids (long-chain) in blood serum were determined according to Itaya and Ui (1965).

## Rumen Degradability and Analysis of Rumen Fluids

Six male sheep equipped with a permanent rumen cannula (50 mm. inner diameter) were used for the *in-situ* trials and fed berseem hay (*Trifolium alexandrinum*) to cover their maintenance requirements. Nylon cloth (100% polyester) with a mean pore size of 120 µm was used for constructing the *in-situ* bags (8 × 10 cm) with nylon threads. Double bags containing approximately 4 g of dried experimental rations were incubated for 8, 16, 32, 48, 64 and 72 h to determine DM and OM degradability rates. A further 4 g were incubated for measuring protein degradability at the same times. Dry matter, OM and N were estimated according to the methods of AOAC (1990).

The data were fitted to the model of McDonald (1981)  $Y = a + b(1 - e^{-c(t-t_l)})$  where:

**Table 1. Chemical composition of Na-SFA and Ca-SFA (% DM basis).**

Composition	DM	OM	AEE	TFAs <sup>a</sup>	OL <sup>b</sup>	Ash	CE Mcal/kg
Na-SFA <sup>c</sup>	61.73	92.60	81.00	68.95	12.05	7.40	6.562
Ca-SFA <sup>d</sup>	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, and d = calcium salts of fatty acids.

**Table 2. Formulation and chemical composition of different rations and roughages (DM basis).**

Ingredients	Control	Na-SFA	Ca-SFA	BH <sup>d</sup>	RS <sup>e</sup>
Content of ingredients (%)					
Con1 <sup>a</sup>	42.86	—	—	—	—
Con2 <sup>b</sup>	—	42.86	—	—	—
Con3 <sup>c</sup>	—	—	42.86	—	—
BH	14.29	14.29	14.29	—	—
RS	42.86	42.86	42.86	—	—
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 = concentrate without fat, b = concentrate containing Na-SFA, c = concentrate containing Ca-SFA, d = berseem hay and e = rice straw.



$Y$  = degradability at time ( $t$ ),  $a$  = the zero time intercept,  $b$  = potentially degradable fraction,  $c$  = rate of degradation of  $b$  and  $t_l$  = lag time.

Samples of rumen fluids were withdrawn individually before feeding then after 4 h and 8 h after feeding from cannulated rams that were fed the experimental rations (3 for each group) for two weeks as an adaptation period at the end of the feeding trials. Rumen pH was immediately determined by the HANNA-Ph meter, model HI8424, total VFA concentration as described by Eadie et al. (1967) and VFA fractions (C2, C3 and C4) analysed according to Erwin et al. (1961). Free fatty acids (long-chain) in rumen liquor were determined using the method of Itaya and Ui (1965), and ammonia concentrations using the Conway method (1978).

### Statistical Analyses

Results obtained were subjected to analysis of variance according to SAS (2000), and treatment means were ranked using Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Composition of Soapstocks, Rations and Roughages

The data in **Tables 1** and **2** show that the AEE content in Ca-SFA was lower than that in Na-SFA, while the reverse was the case for TFA's. The lower content of AEE in Ca-SFA was due to its higher ash content, while the lower content of TFA's in Na-SFA was due to the higher content of other lipids such as pigments, wax, etc, which are concentrated in Na-SFA during refining oils. As a result of the loss of large amounts of other lipids with washing water while converting Na-SFA to Ca-SFA, the levels were 1.27% and 12.05% for Ca-SFA and Na-SFA, respectively. The higher ash content in the

ration containing Ca-SFA was related to higher ash content in Ca-SFA (18.61%). The content of NDF and ADF in experimental rations was lowered by adding either Na-SFA or Ca-SFA.

### Rumen Breakdown of Rations

Rates of DM, OM and CP disappearance (**Table 3**) at different times after incubation in the rumen for rations contained Na-SFA were lower ( $P < 0.05$ ) than the corresponding values for Ca-SFA or control. However, no differences ( $P > 0.05$ ) were found between Ca-SFA and the control. Reduced DM, OM and CP breakdown in the rumen with added Na-SFA may be due to fatty acids interfering with the digestibility of feed components by inhibiting the activity of rumen microorganisms concerned with cellulose digestion or by reducing the retention of calcium due to excessive excretion of soap in the faeces (Roberts and McKirdy, 1965). Foaming and physical coating of dietary fibre with added Na-SFA has been proposed as a possible mechanism for the sometimes depressed DM, OM and CP disappearances observed under these conditions (Devendra and Lewis, 1974).

### Degradation Kinetics

The rapidly degradable fraction ( $a$ ) and slowly degradable fraction ( $b$ ) of DM, OM and CP were similar with the Ca-SFA and control rations, and were reduced ( $P < 0.05$ ) with the ration containing Na-SFA (**Table 4**). These results might be due to Na-SFA having a higher inhibitory effect on rumen microbes than the triglyceride form, as suggested by Wu et al. (1993). Perry and Weatherly (1976) reported that either 2.5 or 5% dietary soapstock (DM basis) tended to decrease ruminal digestibilities of CP and CF when fed to beef cattle. Effective degradability (ED) and potential degradability (PD) of DM, OM and CP were significantly lowered ( $P < 0.05$ ) with adding Na-SFA compared with Ca-SFA rations. Undegradable DM, OM and CP were

**Table 3. Mean values of fitted DM, OM, CP, NDF and ADF % disappearance for experimental rations at different intervals.**

Disappearance	8h	16h	32h	48h	64h	72h
<b>Dry matter (%)</b>						
Control	23.97 <sup>a</sup>	37.73 <sup>a</sup>	56.93 <sup>a</sup>	68.73 <sup>a</sup>	75.93 <sup>a</sup>	78.43 <sup>a</sup>
Na-SFA	20.37 <sup>b</sup>	32.07 <sup>b</sup>	48.37 <sup>b</sup>	58.40 <sup>b</sup>	64.53 <sup>b</sup>	66.63 <sup>b</sup>
Ca-SFA	23.90 <sup>a</sup>	37.60 <sup>a</sup>	56.73 <sup>a</sup>	68.47 <sup>a</sup>	75.67 <sup>a</sup>	78.17 <sup>a</sup>
± SE	± 0.76	± 1.20	± 1.82	± 2.20	± 2.43	± 2.52
P<	*	*	*	*	*	*
<b>Organic matter (%)</b>						
Control	26.77 <sup>a</sup>	42.90 <sup>a</sup>	64.37 <sup>a</sup>	76.63 <sup>a</sup>	83.67 <sup>a</sup>	85.97 <sup>a</sup>
Na-SFA	21.47 <sup>b</sup>	34.40 <sup>b</sup>	51.60 <sup>b</sup>	61.43 <sup>b</sup>	67.07 <sup>b</sup>	68.93 <sup>b</sup>
Ca-SFA	26.00 <sup>a</sup>	41.70 <sup>a</sup>	62.60 <sup>a</sup>	74.50 <sup>a</sup>	81.37 <sup>a</sup>	83.57 <sup>a</sup>
± SE	± 0.44	0.71 ±	± 1.04	± 1.26	± 1.35	± 1.40
P<	*	*	*	*	*	*
<b>Crude protein (%)</b>						
Control	19.77 <sup>a</sup>	31.07 <sup>a</sup>	46.87 <sup>a</sup>	56.60 <sup>a</sup>	62.53 <sup>a</sup>	64.57 <sup>a</sup>
Na-SFA	17.40 <sup>b</sup>	27.40 <sup>b</sup>	41.37 <sup>b</sup>	49.87 <sup>b</sup>	55.13 <sup>b</sup>	56.97 <sup>b</sup>
Ca-SFA	19.73 <sup>a</sup>	31.00 <sup>a</sup>	46.83 <sup>a</sup>	56.53 <sup>a</sup>	62.50 <sup>a</sup>	64.50 <sup>a</sup>
± SE	± 0.31	± 0.49	± 0.74	± 0.90	± 0.99	± 1.04
P<	*	*	*	*	*	*

<sup>a</sup>, <sup>b</sup> means in the same column for each category with different superscript are significantly different ( $P < 0.05$ ).

**Table 4. Rumen degradation kinetics (%) of tested rations incubated *in sacco*.**

Degradation	WL (%)	a (%)	b (%)	c (%/h)	PD (%)	ED (3%h-1)	B (%)	UND (%)
<b>Dry matter (%)</b>								
Control	13.96 <sup>a</sup>	6.43 <sup>a</sup>	81.00 <sup>a</sup>	0.031	87.43 <sup>a</sup>	47.27 <sup>a</sup>	73.47 <sup>a</sup>	26.53 <sup>b</sup>
Na-SFA	11.90 <sup>b</sup>	5.47 <sup>b</sup>	68.83 <sup>b</sup>	0.031	74.30 <sup>b</sup>	40.17 <sup>b</sup>	62.40 <sup>b</sup>	37.60 <sup>a</sup>
Ca-SFA	13.97 <sup>a</sup>	6.40 <sup>a</sup>	80.70 <sup>a</sup>	0.031	87.17 <sup>a</sup>	47.13 <sup>a</sup>	73.20 <sup>a</sup>	26.80 <sup>b</sup>
± SE	± 0.45	± 0.22	± 2.58	± 0.00	± 2.80	± 1.51	± 2.36	± 2.36
P<	*	*	*	*	*	*	*	*
<b>Organic matter (%)</b>								
Control	15.74 <sup>a</sup>	5.43 <sup>a</sup>	87.67 <sup>a</sup>	0.035	93.07 <sup>a</sup>	52.53 <sup>a</sup>	77.33 <sup>a</sup>	22.67 <sup>b</sup>
Na-SFA	12.63 <sup>b</sup>	4.37 <sup>b</sup>	70.27 <sup>b</sup>	0.035	74.63 <sup>b</sup>	42.13 <sup>b</sup>	62.00 <sup>b</sup>	38.00 <sup>a</sup>
Ca-SFA	15.30 <sup>a</sup>	5.30 <sup>a</sup>	85.20 <sup>a</sup>	0.035	90.50 <sup>a</sup>	51.07 <sup>a</sup>	75.20 <sup>a</sup>	24.80 <sup>b</sup>
± SE	± 0.25	± 0.10	± 1.42	± 0.00	± 1.53	± 0.87	± 1.26	± 1.26
P<	*	*	*	*	*	*	*	*
<b>Crude protein (%)</b>								
Control	11.50 <sup>a</sup>	5.30 <sup>a</sup>	66.70 <sup>a</sup>	0.031	72.00 <sup>a</sup>	38.90 <sup>a</sup>	60.50 <sup>a</sup>	39.50 <sup>b</sup>
Na-SFA	10.17 <sup>b</sup>	4.67 <sup>b</sup>	58.80 <sup>b</sup>	0.031	63.50 <sup>b</sup>	34.33 <sup>b</sup>	53.33 <sup>b</sup>	46.67 <sup>a</sup>
Ca-SFA	11.50 <sup>a</sup>	5.27 <sup>a</sup>	66.60 <sup>a</sup>	0.031	71.90 <sup>a</sup>	38.90 <sup>a</sup>	60.40 <sup>a</sup>	39.60 <sup>b</sup>
± SE	± 0.18	± 0.09	± 1.06	± 0.00	± 1.15	± 0.62	± 0.96	± 0.96
P<	*	*	*	*	*	*	*	*

<sup>a</sup>, <sup>b</sup> means in the same column for each category with different superscript are significantly different (P<0.05).

a = the zero time intercept, b = potentially degradable fraction, c = rate of degradation of b, B = degradation of water insoluble fraction, UND = undegradable components, ED = effective degradability and PD = potential degradability.

**Table 5. Nutrient digestibility, cell wall constituent and nutritive value for tested rations.**

Item	Control	Na-SFA	Ca-SFA	± SE	P<
<b>Nutrient digestibility, (%)</b>					
DM	69.64 <sup>a</sup>	64.89 <sup>b</sup>	68.92 <sup>a</sup>	± 0.73	*
OM	72.11 <sup>a</sup>	67.67 <sup>b</sup>	70.92 <sup>a</sup>	± 0.86	*
CP	61.25 <sup>a</sup>	56.83 <sup>b</sup>	60.90 <sup>a</sup>	± 0.71	*
AEE	69.26 <sup>b</sup>	75.21 <sup>a</sup>	77.39 <sup>a</sup>	± 1.92	*
Energy	68.66 <sup>a</sup>	64.67 <sup>b</sup>	67.33 <sup>a</sup>	± 0.74	*
<b>Cell wall constituent (%)</b>					
NDF	68.62 <sup>a</sup>	62.26 <sup>b</sup>	67.96 <sup>a</sup>	± 1.35	*
ADF	65.45 <sup>a</sup>	60.46 <sup>b</sup>	64.92 <sup>a</sup>	± 1.10	*
Cellulose	71.71 <sup>a</sup>	66.30 <sup>b</sup>	71.20 <sup>a</sup>	± 1.21	*
Hemicellulose	79.76 <sup>a</sup>	68.56 <sup>b</sup>	78.61 <sup>ab</sup>	± 3.03	*
<b>Nutritive value, (%)</b>					
TDN	67.97 <sup>a</sup>	64.03 <sup>b</sup>	66.66 <sup>a</sup>	± 0.74	*
DCP	7.27 <sup>a</sup>	6.54 <sup>b</sup>	7.09 <sup>a</sup>	± 0.08	*

<sup>a</sup>, <sup>b</sup> means in the same row with different superscript are significantly different (P<0.05).

higher (P<0.05) with the ration containing Na-SFA compared with both the ration containing Ca-SFA and the control ration, but similar (P > 0.05) between the control and Ca-SFA rations. These results indicate that rumen degradabilities of DM, OM and CP with the Na-SFA ration were less compared with the Ca-SFA ration.

The data in **Table 7** show increased FFAs in the rumen with feeding a diet containing Na-SFA which in turn led to a decreased concentration of NH<sub>3</sub>-N, a result reflecting the impact of added Na-SFA on protein degradability in the rumen. At the same time, the values for TVFAs show the impact of feeding Na-SFA on fibre degradability

**Table 6. Feed intake and change in BWt for pregnant females fed experimental rations.**

Item	Control	Na-SFA	Ca-SFA	± SE	P<
Feed intake on basis DM (kg)					
Concentrate, CFM	3.00	3.00	3.00	—	—
Berseem hay, BH	1.00	1.00	1.00	—	—
Rice straw, RS	3.00	3.00	3.00	—	—
TDMI	7.00	7.00	7.00	—	—
Concentrate: roughages	0.75	0.75	0.75	—	—
Changes in BWt (kg)					
BwtBP	565.40	567.70	566.10	± 7.79	ns
BwtPP	522.80	523.30	518.70	± 7.75	ns
Changing	42.60 <sup>c</sup>	44.40 <sup>b</sup>	47.40 <sup>a</sup>	± 0.41	*
Av. BWt	544.10	545.50	542.40	± 7.77	ns
Av. BWt <sup>0.75</sup>	122.70	112.90	112.40	± 1.24	ns
Birth weight (kg)	34.30 <sup>c</sup>	36.00 <sup>b</sup>	38.80 <sup>a</sup>	± 0.38	*
Duration (day)	67.20 <sup>a</sup>	63.40 <sup>ab</sup>	61.80 <sup>b</sup>	± 1.61	*

<sup>a</sup>, <sup>b</sup>, <sup>c</sup> means in the same row with different superscript are significantly different ( $P < 0.05$ ).

BwtBP = BWt before parturition at beginning of experiment; BwtPP = BWt postpartum.

resulting from the breakdown of fibre in the rumen. Eastridge (2002) reported that both chemical and physical forms of fat sources can affect digestion and reduce fibre digestibility in the rumen by inhibiting cellulolytic microorganisms.

**Table 5** shows that the digestion coefficients of DM, OM, CP and energy were lower ( $P < 0.05$ ) with feeding the ration containing Na-SFA compared with that containing Ca-SFA, while no significant differences were found between the ration containing Ca-SFA and the control. These results are in good agreement with the data obtained from the *in sacco* study, where DM, OM and CP disappearances were significantly decreased with added Na-SFA compared with Ca-SFA. By contrast, undegradable DM, OM and CP were significantly higher as shown in **Table 4**. Palmquist (1994) and Kattab et al. (2001) found that the effect of added fat on digestibility depended on the type and level of fat supplementation. Jenkins (1994) suggested that when fat supplementation decreased protein digestibility in the whole tract, less nitrogen is absorbed across the rumen as ammonia and therefore available for use by body tissues for production. The reduced digestion coefficient of protein is attributed to depression of degradation in the rumen (Boggs et al., 1987).

Feeding rations containing Na-SFA or Ca-SFA significantly increased the digestion coefficient of AEE. The higher ( $P < 0.05$ ) digestibility of lipids associated with fat supplementation in Na-SFA and Ca-SFA rations might be related to the higher digestibility of the supplemented fat (Devendra and Lewis, 1974; El-Bedawy et al., 2005).

Digestibilities of NDF, ADF and cellulose were significantly lower with the ration containing Na-SFA compared with the Ca-SFA and control rations; however, no significant difference was found between the Ca-SFA-containing and control rations. These results might be due to the effect of long chain fatty acids (LCFA) on microbial growth in the Na-SFA ration, and in turn on rumen fermentation which affects fibre digestibility (El-Hag and Miller, 1972). Growth of the cellulolytic species *Butyrivibrio fibrisolvens*, *Ruminococcus albus* and *Ruminococcus flavefaciens* was inhibited by oleic acid in the presence of the soluble substrate cellobiose (Palmquist, 1988).

Nutritive values expressed as TDN and DCP were significantly lower with the ration containing Na-SFA compared with that containing Ca-SFA, while no significant difference was found between the control group and that containing Ca-SFA. Jenkins (1993) found that if the ability of microorganisms to ferment fibre is inhibited by fat, the fibre energy is lost in faeces.

#### Feed Intake and Bodyweight Changes

The concentrate: roughage ratio was fixed in the experimental rations to avoid changes in feed intake and to study the effect of rations containing Na-SFA or Ca-SFA on intake. No significant differences were found for bodyweight among tested groups at the beginning or the end of the experimental period (**Table 6**). Bodyweight increased to a significantly greater extent with feeding a ration containing fat than with the control diet, while animals fed the ration containing Ca-SFA had higher rates of gain ( $P < 0.05$ ) than those fed a ration containing Na-SFA. These results suggest that fat is generally favourable for foetal development in late pregnant buffaloes, especially Ca-SFA which contains calcium for developing the foetal skeleton. The data on birth weights supports this suggestion.

Changes in the live weight of dams during gestation are often assumed to be indicative of pre-natal foetal development (Amoah et al., 1996). Akingbade et al. (2001) reported that during late pregnancy there is preferential nutrient utilisation for foetal growth at the cost of mobilising maternal body tissue, which results in weight loss of does if the dietary supply of nutrients is inadequate (Al-Totajji and Lubbadah, 2000). The pattern of foetal growth rate, calculated as the weight difference of dams before and after parturition during the experiment, corroborates these observations and also indicates that the last month of gestation is the period of most rapid foetal growth.

#### Rumen Parameters

The data in **Table 7** show increased FFAs in the rumen with feeding a diet containing Na-SFA which in turn led to a decreased concentration of  $\text{NH}_3\text{-N}$ , a result reflecting the impact of added Na-SFA on protein degradability in the rumen. At the same time, the values for

**Table 7.** Rumen parameters of sheep fed the experimental rations.

Item	Time (h)	Control	Na-SFA	Ca-SFA	± SE	P<
pH	0	6.50 <sup>a</sup>	6.97	6.57	± 0.168	ns
	4	5.84 <sup>b</sup>	6.79	6.03	± 0.207	ns
	8	6.13 <sup>Bab</sup>	6.82 <sup>A</sup>	6.60 <sup>A</sup>	± 0.072	*
P<		*	ns	ns	—	—
TVFAs (meq/dL)	0	12.22 <sup>A</sup>	8.99 <sup>Bb</sup>	10.72 <sup>Ab</sup>	± 0.302	*
	4	14.43 <sup>A</sup>	11.06 <sup>Ba</sup>	12.51 <sup>ABa</sup>	± 0.505	*
	8	11.89	9.88 <sup>ab</sup>	11.81 <sup>ab</sup>	± 0.384	ns
P<		ns	*	*	—	—
Acetic (%)	0	59.32 <sup>A</sup>	52.51 <sup>B</sup>	57.24 <sup>ABb</sup>	± 1.180	*
	4	62.05	53.84	62.11 <sup>a</sup>	± 2.320	ns
	8	60.22 <sup>A</sup>	51.40 <sup>B</sup>	60.81 <sup>Aa</sup>	± 0.442	*
P<		ns	ns	*	—	—
Propionic (%)	0	25.05 <sup>b</sup>	25.41	25.40 <sup>b</sup>	± 0.342	ns
	4	25.95 <sup>a</sup>	29.03	25.99 <sup>a</sup>	± 0.833	ns
	8	25.95 <sup>a</sup>	28.72	25.83 <sup>ab</sup>	± 0.587	ns
P<		*	ns	*	—	—
Ac:Pr	0	2.37	2.07	2.25 <sup>b</sup>	± 0.006	ns
	4	2.39	1.87	2.39 <sup>a</sup>	± 0.117	ns
	8	2.32 <sup>A</sup>	1.79 <sup>B</sup>	2.35 <sup>Aa</sup>	± 0.038	*
P<		ns	ns	*	—	—
Butyric (%)	0	10.63 <sup>b</sup>	10.58 <sup>b</sup>	10.94	± 0.367	ns
	4	12.17 <sup>a</sup>	13.79 <sup>a</sup>	12.32	± 0.538	ns
	8	11.16 <sup>Bab</sup>	12.48 <sup>Aab</sup>	12.28 <sup>A</sup>	± 0.180	*
P<		*	*	ns	—	—
NH <sub>3</sub> -N (mg/dL)	0	10.36 <sup>b</sup>	9.26 <sup>Bb</sup>	11.08 <sup>Ab</sup>	± 0.267	*
	4	13.38 <sup>a</sup>	10.67 <sup>Ba</sup>	12.59 <sup>Aa</sup>	± 0.204	*
	8	12.33 <sup>a</sup>	9.55 <sup>Bb</sup>	12.40 <sup>Aa</sup>	± 0.108	*
P<		*	*	*	—	—
FFAs µmol/L	0	2.41 <sup>B</sup>	7.14 <sup>A</sup>	3.13 <sup>B</sup>	± 0.313	*
	4	2.86 <sup>B</sup>	7.34 <sup>A</sup>	4.45 <sup>B</sup>	± 0.333	*
	8	2.54 <sup>B</sup>	6.86 <sup>A</sup>	3.51 <sup>B</sup>	± 0.247	*
P<		ns	ns	ns	—	—

A, B, C means in the same row with different superscript are significantly different (P<0.05); a, b means in the same column within each category with different superscript are significantly different (P<0.05).

TVFAs show the impact of feeding Na-SFA on fibre degradability resulting from the breakdown of fibre in the rumen. Eastridge (2002) reported that both chemical and physical forms of fat sources can affect digestion and reduce fibre digestibility in the rumen by inhibiting cellulolytic microorganisms.

Mean pH values, propionic acid and FFAs in the rumen increased significantly while TVFAs, acetic, Ac:Pr ratio and NH<sub>3</sub>-N significantly decreased when feeding a ration containing Na-SFA compared with that containing Ca-SFA or the control (Table 7). Butyric acid was not affected by added fat compared with the control diet. Increased release of FFAs in the rumen when feeding Na-SFA decreased both NH<sub>3</sub>-N and TVFAs. Fatty acids, especially unsaturated fatty acids

are antimicrobial and interfere with the normal function of rumen microbes (Palmquist, 1988). Devendra and Lewis (1974) reported that rumen fermentation is negatively affected as fatty acids become more unsaturated and/or are released faster from feedstuffs. The effects of adding fat on rumen fermentation depend on the source and content of fibre in the ration (Jenkins, 1994), and the type and level of fat (Abo-Donia et al., 2003).

#### Changes in Blood Parameters

Including Na-SFA in the ration decreased total protein concentrations in the blood of late pregnant buffaloes compared with Ca-SFA or the control rations (Table 8). This result is consistent with the data

**Table 8. Blood parameters of buffaloes fed rations containing Na-SFA or Ca-SFA during the late pregnant period.**

Parameter	Control	Na-SFA	Ca-SFA	± SE	P<
Total protein (g/dL)	6.51 <sup>a</sup>	6.21 <sup>b</sup>	6.49 <sup>a</sup>	± 0.06	*
Albumin (g/dL)	2.61	2.59	2.67	± 0.05	ns
Globulin (g/dL)	3.88	3.80	3.83	± 0.04	ns
Albumin / Globulin	0.69	0.69	0.71	± 0.02	ns
Total lipids (g/dL)	5.00 <sup>b</sup>	6.14 <sup>a</sup>	6.31 <sup>a</sup>	± 0.15	*
Triglyceride (mg/dL)	66.60 <sup>b</sup>	72.97 <sup>a</sup>	75.43 <sup>a</sup>	± 0.89	*
FFA's (µmol/L)	19.35 <sup>c</sup>	26.14 <sup>b</sup>	30.47 <sup>a</sup>	± 0.44	*
Glucose (mg/dL)	58.74 <sup>a</sup>	52.74 <sup>b</sup>	52.55 <sup>b</sup>	± 0.72	*

<sup>a</sup>, <sup>b</sup>, <sup>c</sup> and means in the same row with different superscript are significantly different (P<0.05).

in Tables 3, 5 and 6 where protein degradability and digestibility decreased with the ration containing Na-SFA compared with that containing Ca-SFA or the control ration. Concentrations of albumin, globulin and their ratio were not affected by feeding rations containing either Na-SFA or Ca-SFA. The effect of Na-SFA on blood protein concentration might be dependent on the kind of fatty acids in Na-SFA (Aiad et al., 2005). Concentrations of total lipids, triglycerides and free fatty acids were significantly increased with feeding a ration containing fat compared with the control. No significant difference was found between Na-SFA and Ca-SFA except with respect to FFAs which were significantly higher with the Ca-SFA compared with the Na-SFA ration. The higher blood lipids might be due to inhibited lipogenic enzyme activities in the liver and adipose tissues of animals fed fat-containing rations (Storry, 1981). These results are related to the high content of fatty acids in Na-SFA and Ca-SFA. The present results are supported by those reported by Aiad et al. (2005). Palmquist and Conrad (1978) attributed the high blood plasma lipids of fat-supplemented cows to the greater quantity of fatty acids absorbed. All serum parameters were within the normal range as reported by William (1997). Glucose concentrations were significantly decreased with feeding a ration containing Na-SFA compared with feeding Ca-SFA or control rations.

## CONCLUSIONS

Feeding diets supplemented with fat is suitable for foetal development, mammary adipose tissue and subsequent milk yield in late pregnant buffaloes. Soapstock as Na-SFA is a potential dietary fat source. Converting it to Ca-SFA reduces its negative effects and could be used as a source of energy in the rations of buffaloes especially during the late period of pregnancy.

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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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## ABSTRACT

The objective of this study was to determine the effects of nutrition on milk production and fertility in lactating multiparous Friesian and Sahiwal cows. Forty in-calf cows comprising 20 Friesians and Sahiwals were selected and upon calving were randomly assigned to five dietary groups consisting of concentrate supplementation at the rates of zero to four kg fed twice daily after grazing on pasture for 24 weeks postpartum. Each group consisted of four Friesians and four Sahiwals. Pastures and concentrates were analysed using proximate analysis, milk samples were collected weekly to determine composition using infrared spectroscopy while blood was collected bi-weekly to determine progesterone levels using radioimmunoassay. Parameters recorded included milk yield and composition (percentages of fat, protein, solids not fat (SNF) and density. Reproductive data included d to beginning of luteal activity (progesterone >3nm/L), d to first insemination and conception to first service. Data were analysed by GLM of SAS. Level of supplementation, breed, parity and BWt of cow significantly affected milk yield. Heavier cows produced more milk with a mean increase of 0.2 kg for each kg increase in weight. Animals receiving four kg supplements twice daily had the highest mean milk yield per week ( $P < 0.05$ ) in both breeds averaging  $72.2 \pm 4.4$  and  $43.1 \pm 1.7$  L for Friesians and Sahiwals respectively. Breeds differed also in terms of d to reach peak milk production and peak milk yield with Friesians and Sahiwal cows averaging  $31.6 \pm 6.0$  and  $42.2 \pm 3.8$  d to reach peak milk yield, while peak milk yields were  $79.5 \pm 5.9$  and  $58.4 \pm 2.7$  L respectively. Significant breed differences were also observed for percent fat, protein, SNF and density of milk. Sahiwal cows exhibited better reproductive performance than Friesians. It was observed that 18% of in-calf cows lost their foetus before term and 25% of them never showed heat by 120 d postpartum. Of these, 15% never showed any luteal activity, while 10% had silent heat. Sahiwals came into heat and started cycling earlier ( $P < 0.05$ ) than Friesians but more Friesian cows ( $P < 0.05$ ) conceived at first insemination and showed luteal activity later than the Sahiwals. There were within-breed differences between supplementation regimes ( $P < 0.05$ ) for d to first heat and to start of luteal activity. However, the outcomes were quite variable and there were no clear patterns for effects of supplementation in both breeds. It is concluded that

breed effects were more important than nutritional effects in determining milk production, composition and reproductive performance.

**Key words:** *Friesian and Sahiwal cows, nutritional supplementation, milk yield and characteristics, reproductive performance, breed effects, nutritional effects.*

## INTRODUCTION

The lifetime productivity of a dairy cow depends on the number of calves born and the amount of milk produced during its active reproductive phase (De Vries, 2006). Efficient reproduction requires that calving intervals are optimised and there is adequate nutrition to support milk production and calf growth to reach puberty early in life (Gong et al., 2002).

Under tropical conditions, Friesian cattle have been reported to produce 72.5 L milk/week with a butterfat content of 3.7% and to have a lactation length averaging 290 d (Irungu and Mbugua, 1998) compared with Sahiwal cattle which produce 67.2 L/week and have a lactation length of 280 d (Muhuyi and Lokwaleput, 1998). Walshe et al. (1991) reported a range of 5–15 kg milk/cow/d in sub-Saharan Africa, and an average of 8.7 kg/cow/d from 69 herds in Kenya. The low milk production was attributed to various factors including the genetic potential of the animal, the nutritional inadequacy of the diet, parasitism and disease and late lactation.

Nutrition and other environmental factors have a profound influence on the production and reproductive performance of ruminants, but little is known about the complex relationship between nutrition and reproduction which is variable (Gong, 2002). Energy, protein, minerals and vitamins can all affect reproduction and insufficient intake of these nutrients is associated with suboptimal reproductive performance (Beam and Butler, 1998). Inadequate energy intake results in delayed puberty, prolonged postpartum intervals to first ovulation, increased incidences of silent heat, reduced conception rates and birth weights (Lopez et al., 2004).

Lactating cows are in a state of negative energy balance postpartum, because the energy required for milk production and to maintain body functions exceeds energy ingested and metabolic and endocrine changes lead to enhanced mobilisation of depot fat and breakdown of skeletal muscle to provide substrates for milk synthesis (Bauman, et al., 1988; Santos, et al., 2009). The concentrations of fat and protein increase during the advanced stages of lactation in pasture-fed dairy cows (Auld et al., 1998). In exotic cattle peak milk yield occurs 50–70 d postpartum (Roche et al., 2006).

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The composition of milk varies considerably with breed, stage of lactation, feed, season of the year, and many other factors (Charles, 1998). Since the cow's diet is the ultimate source of most of the material used in milk synthesis, the conditions of feeding and the rations fed to the cow influence milk yield and composition (Robinson, 1997). To optimise milk composition, the nutritional status of cows can be adjusted through proper grazing and feeding management (Robinson 1997).

Low milk yields and poor fertility in tropical cattle are a result of nutritional factors due to seasonal variation of forage quantity and quality (Mukasa-Mugerwa et al., 1997). Topps (1994) showed a deficit of up to 15 MJ/d of metabolisable energy and effective rumen degradable protein of 235 g/day to support desired levels of milk output. The shortage of protein was considered to be more critical than energy. However, it has been shown that under good nutrition, average milk yields of 12–15 kg/d can be achieved, representing increases of 140–300% over the median daily milk yield (van der Valk, 1992).

This study was designed to determine the influence of nutrition on lactation and reproductive performance of lactating Friesian and Sahiwal cows in Kenya.

## MATERIALS AND METHODS

### Animals and Diets

Forty multiparous cows from two research station herds comprising 20 Friesians and 20 Sahiwal cows were selected based on relative weight within breed, parity (between three and five) and pregnancy status. The cows were two months to parturition at the beginning of the experiment and were randomly assigned to five dietary groups upon calving comprising eight cows per group i.e. four Friesians and four Sahiwals. They were grazed together on pasture leys of predominantly Rhodes grass and upon calving they were individually fed a concentrate supplement, the amounts ranging from zero to four kg twice daily. The cows were adapted to their diets for 14 d and the study conducted from the time of calving up to 24 weeks (6 months) postpartum.

### Analysis of Diets

The pastures were sampled monthly using a quadrant and analysed using the Van Soest method to determine their dry matter (DM), fibre and protein content (AOAC, 1995). The concentrate component of the diet was also analysed to determine DM, neutral detergent fibre (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and crude protein (CP).

### Observations, Sampling and Measurements

After parturition, body condition was determined, the cow and calf were weighed using a weighing scale and randomly assigned to a dietary group. The calf was allowed to suckle for one week after which it was withdrawn; hand milking was done twice daily. Cow parameters recorded included date of calving, age at first calving, age, parity, breed, and daily milk yield (summed for the week).

The cows were then observed for behavioural signs of oestrus and served using artificial insemination (AI) by two AI practitioners using locally processed Friesian or Sahiwal semen. Cows observed on heat in the morning were served in the afternoon while those exhibiting heat in the later part of the afternoon were served the following morning. The cows were inseminated on up to four occasions, after which they were considered open.

Reproductive data included: d from calving to beginning of luteal activity as determined by progesterone profiles ( $\geq 3$  ng/mL) and d

to first service and conception to first service. After insemination, the cows were monitored for return to heat. Pregnancy diagnosis was done by rectal palpation 90 d after insemination and confirmed by progesterone profiles. Conception rates were determined as the proportion of cows bred that became pregnant after the first AI. Other reproductive parameters recorded included embryonic or foetal loss as determined by progesterone profiles and rectal palpation. Blood samples were collected bi-weekly (20 mL) from each cow for 6 months after calving via jugular venipuncture using tubes containing ethylenediaminetetra-acetate (EDTA) at the rate of 1.8 mg/mL as an anticoagulant for plasma collection. Samples were centrifuged for 15 min at 1 600 rev/min to separate the plasma from the solid blood components. The plasma was then pipetted into 2 mL plastic vials and stored at  $-20^{\circ}\text{C}$  after which progesterone was determined using radioimmunoassay with  $^{125}\text{I}$ iodine as the tracer (FAO/IAEA, 1999).

Homogenised milk samples (20 ml) were collected weekly (morning and afternoon milk) from each cow into plastic tubes for a period of 6 months after parturition. The milk samples were analysed immediately to determine milk components and characteristics which included percent butter fat (BF), protein (P), SNF, density and freezing point using infrared spectroscopy (Tietz, 1986). The percentage milk fat:protein and protein:SNF ratios were calculated to determine their association with reproductive and hormone parameters.

Daily milk yields were recorded for the morning and evening and eventually summed for the week for each cow postpartum. Lactation curves were then derived over the lactation period using polynomial regression equations.

### Statistical Analyses

The data were analysed using the SAS program package release 8.2 (SAS, 2001). Analysis of variance was performed using the GLM procedure of SAS. Differences were considered to be significant if  $P \leq 0.05$ . Data are presented as means  $\pm$  SEM. The model used was as follows:

$$Y_{ijkl} = \mu + b_i + t_j + (bt)_{ij} + p_k + d_m + \varepsilon_{ijklm}$$

where:  $Y_{ijkl}$  are the dependent observations of percentages of fat, protein and SNF, density (g/L), freezing point  $^{\circ}\text{C}$ , milk yield (L/week),  $d$  to peak milk, peak milk and fat:protein and SNF ratios;  $\mu$ ,  $b$ ,  $t$ ,  $p$  and  $d$  are the overall mean and the fixed effects of breed, treatment, parity and body condition scores of each experimental animal while  $\varepsilon$  is the residual error.

Weight at calving, current age, age at first calving and d in milk were included in the model as covariates. Correlations between the various variables of the model were also calculated to determine degree of association.

## RESULTS AND DISCUSSION

### Feed Analyses

Results from the forage and concentrate analyses are shown in **Table 1**. These values are similar to those obtained in the East African region as reported by Smith et al. (2006) in Ethiopia and by Irungu and Mbugua (1998) in Kenya.

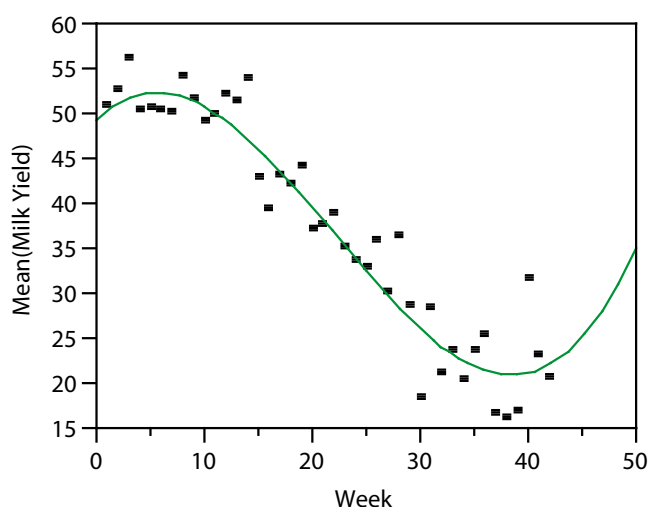
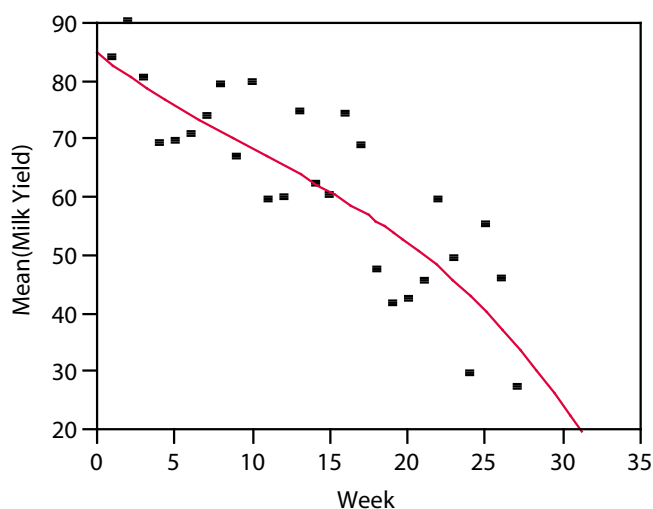
### Milk Yields and Lactation Curves

Lactation curves of third degree polynomials were fitted and are represented in **Figures 1** and **2** for the two breeds. Most of the lactation curves are characterised by an increase in milk production up to peak milk output followed by a steady decline. These polynomials show fits for the Sahiwals whose  $r^2$  is 0.91 while those of the Friesians had



**Table 1. Mean  $\pm$  SD of nutritional values (%) of feed components.**

Nutritional Component	Forage	Concentrate
Dry Matter	86.8 $\pm$ 2.5	89.4 $\pm$ 3.7
Crude Protein	4.7 $\pm$ 2.2	16.8 $\pm$ 1.1
Neutral Detergent Fibre	41.7 $\pm$ 6.7	45.7 $\pm$ 9.5
Acid Detergent Fibre	42.6 $\pm$ 2.5	22.4 $\pm$ 4.9
Acid Detergent Lignin	4.7 $\pm$ 1.6	3.1 $\pm$ 0.6
Ash	8.9 $\pm$ 1.3	9.42 $\pm$ 2.1

**Figure 1. Sahiwal lactation curves.****Figure 2. Friesian lactation curves.**

a  $r^2$  of 0.71; this could be an indication of inherent breed differences. These fits are similar to those found by Garcia and Holmes (2001). Friesians showed a shorter persistence with milk yield reaching 27% of peak milk yield at week 30 while that of the Sahiwals reached this level at week 40 postpartum; this is similar to the findings of Ilatsia et al. (2007) who found an average lactation length of 40.6 weeks for Sahiwals in Kenya, and to the 30% that was reported by Mech et al. (2008) as an indicator of the drying-off phase.

As per the model, breed, diet, breed by diet, parity, body condition and weight of cow had significant effects on milk yield. When milk yield is regressed on BWt of cows, it was found that heavier cows produced significantly more milk with a mean increase of 0.2 kg of milk for each increase in kg live weight. This was expected as older cows were heavier and had more developed udders. It has also been suggested that large cows have a larger rumen volume relative to their metabolic needs and are likely to channel more nutrients towards production rather than for maintenance (Preston, 1989).

There were breed and treatment differences in the lactation curves as depicted by milk yields and d to reach the peak (**Table 2**). The weekly mean milk yields irrespective of treatment were not significantly different ( $P > 0.05$ ), being 51.9  $\pm$  5.3 and 49.8  $\pm$  2.5 L/week for Friesian and Sahiwal cows respectively (**Table 2**).

Cows in Group 4 had the highest mean milk yield/week ( $P < 0.05$ ) in both breeds which averaged 72.2  $\pm$  4.4 and 43.1  $\pm$  1.7 L for Friesians and Sahiwals respectively and could be assumed to be the optimal supplementation for dairy cows not selected for high milk production. These findings contrast with those of Irungu and Mbugua (1998) who reported yields of 92.4 kg/week for Friesians with similar supplementation while Ilatsia et al. (2007) found lower values averaging 33.9 L/week for Sahiwals. Supplementation with three kg concentrate was able to provide adequate nutrients to tap the individual cow's potential which eventually fell to 58.4 L/week and 42.8 L/week for Friesians and Sahiwals respectively upon provision of four kg of concentrate to cows in Group 5 (**Table 2**). This reduction could be due to the cows becoming overweight thus impacting negatively on milk production. On average, both breeds produced similar amounts of milk/week (mean 50.4 L., **Table 2**), but breed peaks differed significantly ( $P < 0.05$ ), with Friesians attaining 79.5 L/week after 32 d postpartum while Sahiwals attained a peak of 58.4 L/week at 42 d postpartum (**Table 2**). These peaks are earlier than the 50–70 d postpartum reported by Roche et al. (2006). This could be due to the differences in energy balance for the two breeds after parturition (Buttler et al., 2002).

Diet, breed by diet, parity, body condition and BWt of cow had significant effects on milk yield. There were significant ( $P < 0.05$ ) breed differences for peak milk production and d to peak milk production in both breeds as indicated in **Table 2**. Friesian and Sahiwal cows averaged 31.6  $\pm$  6.0 and 42.2  $\pm$  3.8 d to reach peak milk yield respectively when diet was not considered, while the mean peak milk yields were 79.5  $\pm$  5.9 and 58.4  $\pm$  2.7 L (**Table 2**). These findings agree with Nebel and McGilliard (1993) who found that most cows reach peak yields between four and eight weeks postpartum and cows with high milk yield peaks also took longer to reach peak milk yield and vice versa.

There were significant interactions between breed and diet for d to reach peak yields and peak milk production but no consistent pattern emerged between these parameters as levels of supplementation increased.

### Milk Composition and Characteristics

There were significant differences for the percentage of fat, protein, SNF, density and freezing point of milk between the two breeds, with Sahiwals having higher ( $P < 0.05$ ) values than those of the Friesians (**Table 3**). These findings agree with those of Mwenya (1993), who indicated that local breeds of cattle produce relatively less milk but have higher values for milk components than exotics. Chenoset and Sansoucy (1998) found that these differences in composition could be due to differences in feed conversion and rumen function, being dependent on the quantities of volatile fatty acids and microbial proteins produced.

**Table 2. Milk production by diet and breed.**

Dietary Group	Breed	Milk Yield (L/week)	Peak Milk Yield (L/week)	Days to Peak Yield
1	Friesian	70.7 ± 4.7 <sup>a</sup>	103.6 ± 3.4 <sup>a</sup>	31.5 ± 3.6 <sup>a</sup>
	Sahiwal	33.9 ± 1.3 <sup>b</sup>	47.8 ± 0.8 <sup>b</sup>	44.7 ± 2.5 <sup>b</sup>
2	Friesian	57.9 ± 5.2 <sup>a</sup>	59.2 ± 5.8 <sup>a</sup>	25.6 ± 1.7 <sup>a</sup>
	Sahiwal	47.1 ± 2.3 <sup>b</sup>	65.7 ± 1.9 <sup>a</sup>	31.1 ± 0.5 <sup>b</sup>
3	Friesian	62.6 ± 4.3 <sup>a</sup>	82.7 ± 2.3 <sup>a</sup>	66.4 ± 3.8 <sup>a</sup>
	Sahiwal	44.6 ± 1.9 <sup>b</sup>	61.3 ± 0.1 <sup>b</sup>	25.9 ± 0.5 <sup>b</sup>
4	Friesian	72.2 ± 4.4 <sup>a</sup>	92.6 ± 3.1 <sup>a</sup>	62.9 ± 7.3 <sup>a</sup>
	Sahiwal	43.1 ± 1.7 <sup>b</sup>	61.1 ± 1.2 <sup>b</sup>	51.5 ± 1.1 <sup>b</sup>
5	Friesian	58.9 ± 4.3 <sup>a</sup>	88.1 ± 0.9 <sup>a</sup>	20.7 ± 0.2 <sup>a</sup>
	Sahiwal	44.0 ± 2.1 <sup>b</sup>	59.2 ± 1.4 <sup>b</sup>	46.3 ± 0.9 <sup>b</sup>
Overall	Friesian	51.9 ± 5.3 <sup>a</sup>	79.5 ± 5.9 <sup>a</sup>	31.6 ± 6.0 <sup>a</sup>
	Sahiwal	49.8 ± 2.5 <sup>a</sup>	58.4 ± 2.7 <sup>b</sup>	42.2 ± 3.8 <sup>b</sup>

Group 1 — pasture only; Group 2 — pasture +1 kg supplement twice daily; Group 3 — pasture +2 kg supplement twice daily; Group 4: pasture +3 kg supplement twice daily; Group 5 — pasture +4 kg supplement twice daily.

<sup>a,b</sup> Values with different superscripts within rows differ significantly ( $P < 0.05$ ).

**Table 3. Least square means ± SEM of milk components for the two breeds.**

Breed	% Fat	% Protein	% SNF	Density (kg/L)	Freezing Point (°C)	Fat:Protein
Friesian	3.55 ± 0.22 <sup>a</sup>	3.07 ± 0.03 <sup>a</sup>	8.12 ± 0.08 <sup>a</sup>	1.026 ± 0.04 <sup>a</sup>	-0.53 ± 0.05 <sup>a</sup>	1.17 ± 0.33 <sup>a</sup>
Sahiwal	4.52 ± 0.14 <sup>b</sup>	3.22 ± 0.02 <sup>b</sup>	8.50 ± 0.05 <sup>b</sup>	1.027 ± 0.26 <sup>b</sup>	-0.55 ± 0.03 <sup>b</sup>	1.40 ± 0.21 <sup>b</sup>

<sup>a,b</sup> Values with different superscripts within columns differ significantly ( $P < 0.05$ ).

It is apparent that the metabolism of these two breeds allows for different nutrient partitioning and that their nutrient requirements are also different which could be an inherent attribute of the Sahiwal considering its Zebu type (Charles, 1998). Fat, protein and fat:protein are important parameters of nutrient balance (Heuer et al., 1999). Milk fat percentage tends to increase and milk protein percentage tends to decrease in association with a negative energy balance post-partum due to mobilisation of adipose tissues. The fat:protein ratio has been suggested as a potential indicator of lack of dietary energy supply, and critical ratios between 1.35 and 1.5 have been suggested (Grieve et al., 1986). The ratios in **Table 3** show that Sahiwals were in greater energy deficit than Friesians which was surprising since Friesians might have been expected to be in a greater negative energy balance due to their higher maintenance requirements. However, their relatively low milk yield might suggest that the nutrients saved for milk production by this lower yield produced the favourable energy balance recorded while the Sahiwals on average exceeded their milk yield potential (Ilatsia et al., 2007).

The freezing point of Friesian milk was similar to the -0.53 °C reported by Henno et al. (2008) in Estonian Friesians, but lower for the Sahiwal cows (**Table 3**), and could be due to the inherent breed differences which showed Sahiwals with consistently higher solid components in their milk than the Friesians. A decrease of 0.1% in milk protein results in an increase of 0.0024 °C in freezing point. The freezing point depression and density of milk depends upon its concentration of water-soluble components (Sherbon, 1988). The density of Sahiwal milk was higher ( $P < 0.05$ ) than that of Frie-

sians and so was the depression of freezing point (**Table 3**). This could be explained by the significantly higher total solids (% BF plus SNF) in Sahiwal milk causing the bigger depression of freezing point and a larger specific gravity as indicated by the measurements of density (**Table 3**). For all treatments it can be argued that breed effects far outweighed the dietary effects for milk components because within-breed variations did not show clear patterns as concentrate intakes increased; this agrees with the findings of Gonthier et al. (2005).

Freezing point depression and density are useful indicators of the solids in milk and animals in Group 3 had significantly higher values, particularly the Friesians as indicated by **Figures 3** and **4**. This is when the milk solids are at their highest and indicates that the optimal milk quality for the two breeds would be at this treatment. However, there were no clear patterns with respect to diet influencing freezing point and density in either breed.

There was a strong negative correlation between percent fat and density for both the breeds (**Table 4**). This can be problematic for marketing because as fat levels increase, the milk density tends to decrease and *vice versa*, although regulatory standards require that both fat and density should be high for good quality milk.

However, the high association between protein and both density and freezing point suggests that the level of protein is the main factor influencing density and freezing point depression due to its contribution to SNF because of its higher molecular weight (**Table 4**). Solids not fat exhibited strong positive associations with density, freezing point depression and protein in both breeds (**Table 4**). This is expected because the higher the solid component of milk the greater

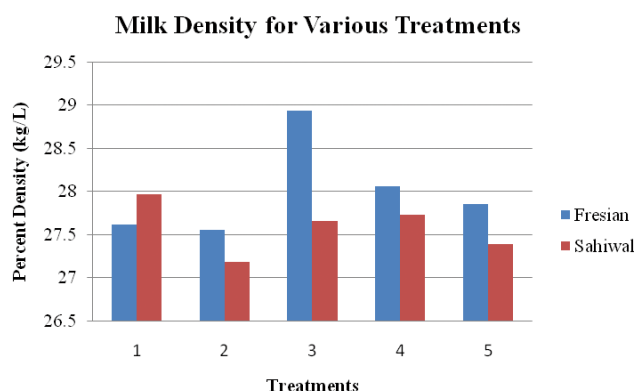


Figure 3. Milk density.

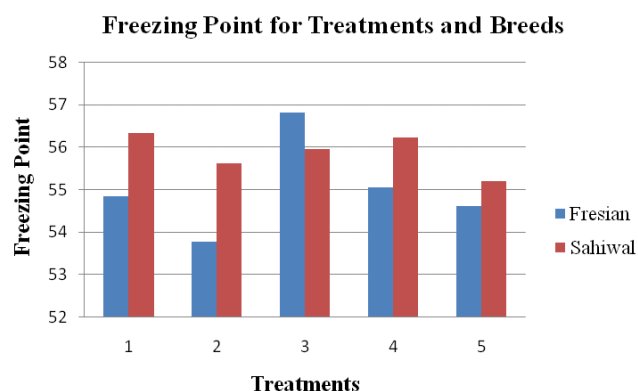
Figure 4. Milk freezing point (°C × 10<sup>-2</sup>).

Table 4. Correlations between milk constituents for cattle.

	Friesian	Fat	SNF	Density	Freezing point	Protein
Friesian						
Fat		1	0.07	-0.45	0.03	0.15
SNF			1	0.86	0.99	0.99
Density				1	0.88	0.82
Freezing Point					1	0.99
Protein						1
Sahiwal						
Fat		1	0.15	-0.39	-0.01	0.21
SNF			1	0.85	0.97	0.99
Density				1	0.91	0.81
Freezing Point					1	0.97
Protein						1

the freezing point depression and the higher the density. Fat and protein were moderately positively associated in both breeds.

Parity affected mean milk yield ( $P < 0.05$ ) which increased to  $68.8 \pm 1.9$  L/week for parity five and declined in subsequent parities for both breeds; similar trends were observed for peak milk yield where parity six peaked at  $92.1 \pm 1.1$  L/week. This was expected because the alveolar tissues in the mammary glands develop with increasing parity resulting in increased milk yield which takes longer to reach a peak. Similar findings were reported by Chase (1993). Parity also affected milk composition and its physical characteristics. Parity four milk had a significantly high mean protein content of  $3.3 \pm 0.02\%$ , density of 1.028, SNF of  $8.59 \pm 0.05\%$  and the lowest freezing point of  $-0.056$  °C. This could be explained by the udder being adequately developed to take full advantage of the nutrients supplied for improved milk quality and production.

Body condition scores had significant effects on lactation with body condition five being associated with the highest mean milk yield of  $75.5 \pm 2.01$  L which peaked at a mean of  $103.2 \pm 1.27$  L and took  $48.8 \pm 1.9$  d to reach peak milk yield. Milk composition and physical characteristics varied ( $P < 0.05$ ) with body condition scores. However, animals with body condition four showed higher mean levels for fat, protein and SNF, an average density of 1.027 and a freezing point depression of  $-0.056$  °C; this was the body condition under which both milk production and quality were optimal.

## Reproductive Performance

Sahiwal had a better reproductive performance than Friesians. It was observed that 18% of in-calf cows lost their foetus before term and 25% of them never showed heat by 120 d postpartum (Table 5). Of the cows that calved, 15% did not show any luteal activity, while 10% had silent heat postpartum. Sahiwals came into heat and started cycling earlier ( $P < 0.05$ ) than Friesians (Table 5) although, as indicated earlier, they were deemed to be in greater negative energy balance than Friesians. This finding could be due to adaptation i.e. being able to reproduce although the situation was less favourable given the fat:protein ratios. Alternatively, the Sahiwals could have a higher threshold for energy balance relative to the Friesians under tropical environments. Friesians had more ( $P < 0.05$ ) cows conceiving at first insemination and showed luteal activity later than the Sahiwals (Table 5); this may have arisen because of the demand for nutrients for milk production which peaked earlier in Friesians than in the Sahiwals. The relatively less favourable reproductive attributes of the Sahiwals could also be a reflection of the influence of metabolic size on reproductive performance.

The beginning of luteal activity and expression of heat are important factors that influence when insemination should be done for conception to occur. The earlier it occurs the better for reproductive efficiency. There were breed differences ( $P < 0.05$ ) for d to first heat and start of luteal activity for most of the dietary treatments (Table 6). However, these outcomes were quite varied and did not show spe-

**Table 5. Reproductive parameters of the two breeds.**

Reproductive Characteristics	Breed		
	Sahiwal	Friesian	Overall
Cows Calving (%)	85	80	82.5
Foetal Loss (%)	15	20	17.5
Non-return to heat six months postpartum (%)	18 <sup>a</sup>	31 <sup>b</sup>	24.5
Mean $\pm$ SEM d to first heat	72.6 $\pm$ 1.7 <sup>a</sup>	96.9 $\pm$ 5.4 <sup>b</sup>	84.8
Mean $\pm$ SEM d to start of luteal activity	55.5 $\pm$ 1.2 <sup>a</sup>	74.6 $\pm$ 1.9 <sup>b</sup>	65.1
Cows showing heat after parturition (%)	82 <sup>a</sup>	69 <sup>b</sup>	75.5
Cows conceiving at first insemination (%)	21 <sup>a</sup>	54 <sup>b</sup>	37.5
Cows showing luteal activity after 120 d (%)	88	81	84.5

<sup>a,b</sup> Values with different superscripts within rows differ significantly ( $P < 0.05$ ).

**Table 6. Mean  $\pm$  SEM d to first heat and start of luteal activity for the dietary groups.**

Dietary Group	Mean days to first heat		Days to start of luteal activity (> 3ng/L)	
	Sahiwals	Friesians	Sahiwal	Friesians
1	61.5 $\pm$ 0.1	53.3 $\pm$ 3.9	53.0 $\pm$ 1.6	49.3 $\pm$ 3.1
2	83.9 $\pm$ 0.8 <sup>a</sup>	51.5 $\pm$ 2.5 <sup>b</sup>	29.3 $\pm$ 2.0 <sup>a</sup>	63.4 $\pm$ 3.4 <sup>b</sup>
3	35.7 $\pm$ 0.3 <sup>a</sup>	116.1 $\pm$ 9.1 <sup>b</sup>	60.7 $\pm$ 3.2 <sup>a</sup>	72.2 $\pm$ 2.9 <sup>b</sup>
4	109.1 $\pm$ 3.9 <sup>a</sup>	42.4 $\pm$ 2.8 <sup>b</sup>	66.8 $\pm$ 2.3 <sup>a</sup>	58.7 $\pm$ 0.8 <sup>b</sup>
5	60.4 $\pm$ 2.4 <sup>a</sup>	243.3 $\pm$ 4.4 <sup>b</sup>	58.5 $\pm$ 2.1 <sup>a</sup>	101.6 $\pm$ 3.3 <sup>b</sup>

<sup>a,b</sup> Values with different superscripts within row differ significantly ( $P < 0.05$ ).

cific patterns in either breed, although on average the Friesians had more d to luteal activity and heat than the Sahiwals.

Overall, breed effects influenced milk production, composition and reproductive performance more than the nutritional effects. This can be attributed to the fact that the cows used in this study were not highly selected for increased milk production which could have skewed nutrient partitioning for milk production and reproduction. This reflects the adaptation of the two breeds to this production system.

## CONCLUSIONS

There are interactions between nutrition and breed which affect milk production, composition and reproductive performance but the effects of breed far outweighed the nutritional effects due to the lack of clear response of these parameters when concentrate intake levels were gradually increased from zero to twice daily supplementation with four kg concentrates. These findings may be attributed to the fact that the cows used in this study were not highly selected for milk production which would result in higher responses to supplementation as more nutrients are supplied to enhance milk production and reproduction.

## ACKNOWLEDGEMENTS

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# Milk Production and Reproductive Performances of Murrah Buffaloes in Tamil Nadu, India

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## ABSTRACT

Data on the production and reproductive traits of Murrah buffaloes (1980 lactation records of 698 animals) were collected from the Central Cattle Breeding Farm, Alamadhi, Tamil Nadu, India. The overall least-squares means ( $\pm$ SE) for peak milk yield, days to attain peak yield, 305-d yield, lactation length and milk yield, service period, calving interval and dry period were  $8.87 \pm 0.05$  kg,  $53.4 \pm 0.8$  d.,  $1804.9 \pm 14.7$  kg.,  $297.8 \pm 1.9$  d.,  $1855.6 \pm 16.1$  kg.,  $225.0 \pm 5.5$ ,  $532.8 \pm 5.5$  and  $230.2 \pm 4.9$  d respectively. Period of calving had a highly significant ( $P < 0.01$ ) effect on all the traits studied except days to attain peak yield, where it had only significant ( $P < 0.05$ ) effect. Season of calving had a significant ( $P < 0.05$ ) effect on peak yield and lactation milk yield and a highly significant ( $P < 0.01$ ) effect on days to attain peak yield, 305 d milk yield, milk yield/day of lactation and all the reproductive traits studied. The lowest calving interval was observed in southwest monsoon calvers and they differed significantly ( $P < 0.05$ ) with winter and summer calvers. Parity had a highly significant effect ( $P < 0.01$ ) on all the traits studied. Pair-wise comparison revealed that the lactation milk yield was lowest in first parity and differed significantly ( $P < 0.05$ ) from other parities. In general, reproductive traits such as service period, calving interval and dry period were slightly higher than those observed elsewhere and hence better breeding management and introduction of genetic evaluation programmes are needed for genetic improvement of these traits.

**Key words:** *Murrah, buffaloes, production, reproduction, performance, non-genetic factors.*

## INTRODUCTION

According to the 2003 livestock census, India possesses 185.2 million cattle and 97.9 million buffaloes, which is about 13.7% of the total cattle and 57.5% of the total buffalo population of the world. The dairy industry in India has made significant progress in the last few decades. Today, India is the largest producer of milk in the world. Milk production rose to about 88.1 million tonnes in 2003–2004 from 17.0 million tonnes in 1950–1951. At present, India's contribution to total world milk production is about 14.3% and the national per capita milk availability is 231 g/d. In India, although the proportion of buffaloes to cattle is 1:2, buffaloes contribute around 57% of the total milk obtained from cattle and buffaloes. Tamil Nadu with

9.14 million cattle and 1.66 million buffaloes produces an estimated 4.75 million tonnes of milk (Report, 2006). The Murrah breed is the finest genetic material of milk-producing buffalo not only in India but also probably in the world (Taneja, 1998). This breed has been used extensively throughout the country to upgrade the nondescript buffalo stock to improve the milk production. The breeding policy of Tamil Nadu State is to use Murrah or Surti as the breeds of choice to improve the nondescript buffaloes. As a result of these measures, the Murrah and graded Murrah populations have increased over the years. Although the water buffalo in the tropics out-produces other domestic animals, commercial milk production is adversely affected by a large number of factors such as late age at first calving, seasonality of oestrus, long calving interval and dry period. Therefore, it is necessary to evaluate the relative importance of various fixed environmental and physiological effects in influencing the milk production and reproductive traits for devising appropriate feeding and other managemental practices.

From the literature reviewed, it was found that the bulk of scientific information on buffaloes has come from the analysis of records made available from institutional and government farms in north India (Sethi and Khatkar, 1997; Dass and Sadana, 2000; Gogoi et al., 2002; Kundu et al., 2003a and b; Yadav et al., 2007). Information from the southern peninsular region, especially under the hot and humid coastal regions of Tamil Nadu is scanty. The home tract of Murrah buffaloes is a hot and dry climatic region in the north-western part of India. Breeding these buffaloes in the southern peninsular region of India, which is hot and humid, may affect their performance and adaptability. Hence the present study to both understand the performance and the influence of various non-genetic factors affecting economic traits of Murrah buffaloes in the coastal regions of Tamil Nadu and to suggest suitable managemental practices, selection and breeding strategies for genetic improvement of Murrah buffaloes under hot and humid climatic conditions of India.

## MATERIALS AND METHODS

The study was based on data pertaining to Murrah buffaloes born and bred at the Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu, India from 1979 to 2006 (28 years). This farm is located approximately at 13°N latitude and 80°E longitude at an altitude of about 20 m above mean sea level. The climate is generally hot, humid and tropical in nature. The mean annual maximum and minimum temperatures were 33°C and 24.7°C respectively and the mean relative humidity ranged between 69.2% and 76.2%. The buffaloes were housed in permanent sheds with open-type ventilation and maintained under stall-fed conditions. Roughage was provided in the form of green fodder and paddy straw. In addition, concentrate mixture was provided to all age groups as per the standard require-

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ments. Cows were hand-milked twice daily in the morning and evening.

Data on production and reproductive performance of Murrah buffaloes (1980 lactation records from 698 Murrah cows) were extracted from history and pedigree sheets. The traits studied were peak yield, days to attain peak yield, 305 d milk yield, lactation length and milk yield, milk yield/d of lactation, service period, calving interval, dry period and number of services/conception. Period and season were the fixed environmental effects considered for all the traits studied. As the calvings were less in a year, year-season analysis was not done. To utilise all available data the entire duration was divided into seven periods each with an interval of five years assuming that there would not be any major management changes within a period. Further, each calendar year was sub-divided into four seasons, viz. winter (January and February), summer (March to May), southwest monsoon (June to September) and northeast monsoon (October to December). The LSMLMW and MIXMDL PC-2 version computer program of Harvey (1990) was used to study the effect of various non-genetic factors and the means were compared using Duncan's multiple range test.

The model used for analysis was

$$Y_{ijkl} = m + P_i + S_j + O_k + e_{ijkl}$$

where

- $Y_{ijkl}$  is the  $j^{\text{th}}$  observation in  $i^{\text{th}}$  period,  $j^{\text{th}}$  season and  $k^{\text{th}}$  parity,  
 $m$  is overall mean when equal sub-class frequencies exist,  
 $P_i$  is effect of  $i^{\text{th}}$  period ( $i = 1$  to 7),  
 $S_j$  is effect of  $j^{\text{th}}$  season ( $j = 1$  to 4),  
 $O_k$  is effect of  $k^{\text{th}}$  parity ( $k = 1$  to 6) and  
 $e_{ijkl}$  is random errors NID (0,  $s_e^2$ ).

## RESULTS AND DISCUSSION

### Production Traits

Least-squares means ( $\pm$ SE) of different production traits are presented in **Table 1**. Period of calving played a highly significant ( $P < 0.01$ ) effect on the variation of peak yield, 305 d milk yield, lactation length, lactation milk yield and milk yield/d of lactation and it had only significant ( $P < 0.05$ ) effect on days to attain peak yield. The highest peak yield, 305 d milk yield and lactation milk yield were observed in period six (1999–2002) and they differed significantly with the rest of the periods, except with periods five and seven, where differences were not significant. The lowest lactation length was observed in period three (1987–1990) and differed significantly ( $P < 0.05$ ) from other periods except period seven (2003–2006), where it was not significant. There was a steep increase in peak yield, 305 d milk yield and lactation milk yield from the second-sixth periods and declined slightly in period 7 (2003–2006) and the values observed in period two differed significantly ( $P < 0.05$ ) with the rest of the periods.

Season of calving had no effect on lactation length. However, it had significant ( $P < 0.05$ ) effect on peak yield and lactation milk yield and a highly significant ( $P < 0.01$ ) effect on days to attain peak yield, 305 d milk yield and milk yield/d of lactation. The peak yield of Murrah buffaloes that calved in the southwest monsoon season was the highest and it differed significantly ( $P < 0.05$ ) with northeast monsoon calvers. However, there was no significant difference among other seasons. On the other hand, the lowest 305 d milk yield, lactation milk yield and milk yield/d of lactation were observed in cows calving in the northeast monsoon season. Winter and summer calvers had higher 305 d milk yields and lactation milk yield than the monsoon calvers. The yields observed among winter, summer

and southwest monsoon calvers were not significantly different but they differed significantly ( $P < 0.05$ ) with northeast monsoon calvers.

Parity had a highly significant ( $P < 0.01$ ) effect on all the traits studied. First lactation peak yield was significantly ( $P < 0.05$ ) lower than the rest. The yield increased from first to fourth parity and thereafter it started declining. On the other hand, the d to attain peak yield decreased from the first to fifth parity followed by a moderate increase in the sixth parity. Days to attain peak yield in first lactation were significantly ( $P < 0.05$ ) higher (60.9 d) than the rest. The 305-d milk yield and lactation milk yield increased up to the third parity and they were maintained at the fourth parity and declined thereafter. Pair-wise comparison revealed that the 305-d and lactation milk yields observed in the first parity differed significantly ( $P < 0.05$ ) from other parities. Similar to days to attain peak yield, the lactation length decreased with the advancement of parity. 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 milk yield/d of lactation increased linearly with the advancement of parity up to the fourth parity and then declined, and the values observed in the first parity differed significantly ( $P < 0.05$ ) with the rest of the parities.

### Reproductive Traits

Least-squares means ( $\pm$ SE) of different reproductive traits are set out in **Table 2**. Analyses of variance revealed that the period of calving influenced service period, calving interval, dry period and number of services per conception in a highly significant ( $P < 0.01$ ) manner although there was no consistent trend over the periods. Lowest service periods, calving intervals and number of services per conception were found in period three (1987–90) and they differed significantly ( $P < 0.05$ ) with other periods except the first period (1979–1982). On the other hand, the lowest and the highest dry periods were observed in periods one and five respectively, and the average dry period observed in period five was significantly ( $P < 0.05$ ) different from the first, third and fourth periods.

Season of calving also influenced all the reproductive traits in a highly significant manner ( $P < 0.01$ ). Murrah buffalo heifers freshening in the southwest monsoon had significantly ( $P < 0.05$ ) shorter service periods, calving intervals, dry periods and number of services/conception than those calving in other seasons. Highest service periods, calving intervals and number of services per conception were observed in winter season calvers. The service periods and calving intervals observed in southwest monsoon calvers differed significantly ( $P < 0.05$ ) with other seasons except with northeast monsoon calvers. On the other hand, the dry periods and number of services per conception recorded for southwest monsoon season calvers differed significantly ( $P < 0.05$ ) with winter, summer and northeast monsoon season calvers.

The differences between service periods among parities were highly significant ( $P < 0.01$ ). Service periods decreased with the order of lactation up to the fifth parity and the lowest values of  $187.4 \pm 13.7$  d were observed at this parity. The reductions in service periods in the second and third parities were rather sharp and further decline was gradual. In general, pair-wise comparison revealed that the mean service periods of first and second parities differed significantly ( $P < 0.05$ ) from other parities as well as between them. Similarly, Murrah buffaloes calving for the first time had the longest calving intervals and dry periods, which declined thereafter. The calving intervals and dry periods recorded in the first parity were significantly ( $P < 0.05$ ) different from second and later parities. In addition, the mean calving intervals and dry periods observed between the sec-



Table 1. Least-squares means ( $\pm$  SE) for different milk production traits of Murrah buffaloes.

Effect	n	Peak yield (kg)	Days to attain peak yield	305-day milk yield (kg)	Lactation length (d)	Lactation milk yield (kg)	Milk yield/d of lactation (kg)
Overall mean ( $\mu$ )	1980	8.87 $\pm$ 0.05	53.4 $\pm$ 0.8	1 804.9 $\pm$ 14.7	297.8 $\pm$ 1.9	1 855.6 $\pm$ 16.1	6.16 $\pm$ 0.04
Period of calving		**	*	**	**	**	**
P <sub>1</sub> (1979–1982)	268	8.47 $\pm$ 0.12 <sup>b</sup>	50.7 $\pm$ 1.7 <sup>ad</sup>	1 670.2 $\pm$ 32.6 <sup>b</sup>	295.6 $\pm$ 4.2 <sup>b</sup>	1 706.2 $\pm$ 35.8 <sup>a</sup>	5.74 $\pm$ 0.08 <sup>b</sup>
P <sub>2</sub> (1983–1986)	491	7.96 $\pm$ 0.09 <sup>a</sup>	54.9 $\pm$ 1.3 <sup>bc</sup>	1 584.2 $\pm$ 23.6 <sup>a</sup>	300.2 $\pm$ 3.1 <sup>b</sup>	1 629.9 $\pm$ 25.8 <sup>a</sup>	5.38 $\pm$ 0.06 <sup>a</sup>
P <sub>3</sub> (1987–1990)	430	8.30 $\pm$ 0.08 <sup>b</sup>	52.0 $\pm$ 1.3 <sup>ab</sup>	1 632.0 $\pm$ 23.4 <sup>ab</sup>	285.1 $\pm$ 3.0 <sup>a</sup>	1 658.5 $\pm$ 25.6 <sup>a</sup>	5.72 $\pm$ 0.06 <sup>b</sup>
P <sub>4</sub> (1991–1994)	265	9.16 $\pm$ 0.11 <sup>c</sup>	52.2 $\pm$ 1.6 <sup>abc</sup>	1 872.9 $\pm$ 29.8 <sup>c</sup>	298.3 $\pm$ 3.9 <sup>b</sup>	1 925.2 $\pm$ 32.7 <sup>b</sup>	6.43 $\pm$ 0.07 <sup>c</sup>
P <sub>5</sub> (1995–1998)	171	9.47 $\pm$ 0.13 <sup>ce</sup>	57.2 $\pm$ 1.9 <sup>c</sup>	1 947.2 $\pm$ 36.1 <sup>cd</sup>	307.2 $\pm$ 4.7 <sup>b</sup>	2 030.3 $\pm$ 39.6 <sup>c</sup>	6.55 $\pm$ 0.09 <sup>c</sup>
P <sub>6</sub> (1999–2002)	236	9.52 $\pm$ 0.11 <sup>e</sup>	51.4 $\pm$ 1.7 <sup>ab</sup>	1 974.1 $\pm$ 31.1 <sup>d</sup>	305.8 $\pm$ 4.1 <sup>b</sup>	2 055.6 $\pm$ 34.1 <sup>c</sup>	6.66 $\pm$ 0.08 <sup>c</sup>
P <sub>7</sub> (2003–2006)	119	9.18 $\pm$ 0.16 <sup>ce</sup>	55.0 $\pm$ 2.3 <sup>bcd</sup>	1 953.7 $\pm$ 43.2 <sup>cd</sup>	292.5 $\pm$ 5.6 <sup>ab</sup>	1 983.2 $\pm$ 47.4 <sup>bc</sup>	6.66 $\pm$ 0.11 <sup>c</sup>
Season of calving		*	**	**	*	*	**
Winter (Jan–Feb)	276	8.81 $\pm$ 0.10 <sup>ab</sup>	53.0 $\pm$ 1.5 <sup>b</sup>	1 839.0 $\pm$ 28.6 <sup>b</sup>	301.8 $\pm$ 3.7	1 888.6 $\pm$ 31.4 <sup>b</sup>	6.19 $\pm$ 0.07 <sup>b</sup>
Summer (Mar–May)	150	8.84 $\pm$ 0.14 <sup>ab</sup>	57.9 $\pm$ 2.0 <sup>b</sup>	1 853.8 $\pm$ 38.1 <sup>b</sup>	293.3 $\pm$ 5.0	1 882.4 $\pm$ 41.8 <sup>b</sup>	6.34 $\pm$ 0.09 <sup>b</sup>
South-west monsoon (Jun–Sep)	724	9.03 $\pm$ 0.07 <sup>b</sup>	54.1 $\pm$ 1.1 <sup>b</sup>	1 793.1 $\pm$ 19.8 <sup>b</sup>	298.8 $\pm$ 2.6	1 849.6 $\pm$ 21.8 <sup>b</sup>	6.14 $\pm$ 0.05 <sup>b</sup>
North-east monsoon (Oct–Dec)	830	8.79 $\pm$ 0.06 <sup>a</sup>	48.3 $\pm$ 0.9 <sup>a</sup>	1 733.8 $\pm$ 17.7 <sup>a</sup>	297.4 $\pm$ 2.3	1 801.6 $\pm$ 19.4 <sup>a</sup>	5.99 $\pm$ 0.04 <sup>a</sup>
Parity		**	**	**	**	**	**
First	645	7.73 $\pm$ 0.08 <sup>a</sup>	60.9 $\pm$ 1.2 <sup>b</sup>	1 619.7 $\pm$ 22.6 <sup>a</sup>	310.4 $\pm$ 2.9 <sup>c</sup>	1 687.6 $\pm$ 24.8 <sup>a</sup>	5.38 $\pm$ 0.05 <sup>a</sup>
Second	457	8.85 $\pm$ 0.09 <sup>b</sup>	54.0 $\pm$ 1.3 <sup>a</sup>	1 832.8 $\pm$ 23.5 <sup>c</sup>	301.1 $\pm$ 3.1 <sup>b</sup>	1 894.5 $\pm$ 25.7 <sup>cd</sup>	6.22 $\pm$ 0.06 <sup>b</sup>
Third	311	9.37 $\pm$ 0.10 <sup>cd</sup>	51.1 $\pm$ 1.5 <sup>a</sup>	1 913.9 $\pm$ 27.7 <sup>d</sup>	302.3 $\pm$ 3.6 <sup>bc</sup>	1 967.3 $\pm$ 30.4 <sup>e</sup>	6.45 $\pm$ 0.07 <sup>c</sup>
Fourth	224	9.54 $\pm$ 0.12 <sup>d</sup>	50.6 $\pm$ 1.7 <sup>a</sup>	1 910.4 $\pm$ 32.3 <sup>d</sup>	299.0 $\pm$ 4.2 <sup>bd</sup>	1 966.5 $\pm$ 35.5 <sup>de</sup>	6.50 $\pm$ 0.08 <sup>c</sup>
Fifth	150	8.98 $\pm$ 0.14 <sup>b</sup>	50.0 $\pm$ 2.1 <sup>a</sup>	1 823.4 $\pm$ 38.7 <sup>c</sup>	289.8 $\pm$ 5.0 <sup>abd</sup>	1 853.7 $\pm$ 42.4 <sup>bc</sup>	6.33 $\pm$ 0.09 <sup>bc</sup>
Sixth and above	193	8.74 $\pm$ 0.13 <sup>b</sup>	53.5 $\pm$ 1.9 <sup>a</sup>	1 729.1 $\pm$ 35.1 <sup>b</sup>	284.3 $\pm$ 4.6 <sup>a</sup>	1 763.8 $\pm$ 38.5 <sup>ab</sup>	6.09 $\pm$ 0.09 <sup>b</sup>

n — number of observations.

\* P &lt; 0.05; \*\* P &lt; 0.01.

Means bearing same superscript within classes do not differ significantly (P &gt; 0.05).

Table 2. Least-squares means ( $\pm$ SE) for different reproductive traits of Murrah buffaloes.

Effect	N	Service period (d)	Calving interval (d)	Dry period (d)	Number of services per conception
Overall mean ( $\mu$ )	1 550	225.0 $\pm$ 5.5	532.8 $\pm$ 5.5	230.2 $\pm$ 4.9	2.31 $\pm$ 0.05
Period of calving		**	**	**	**
P <sub>1</sub> (1979–1982)	220	182.8 $\pm$ 11.4 <sup>af</sup>	488.8 $\pm$ 11.4 <sup>a</sup>	181.6 $\pm$ 10.2 <sup>a</sup>	1.92 $\pm$ 0.11 <sup>ad</sup>
P <sub>2</sub> (1983–1986)	423	241.3 $\pm$ 8.1 <sup>be</sup>	548.2 $\pm$ 8.2 <sup>be</sup>	242.6 $\pm$ 7.3 <sup>bc</sup>	2.45 $\pm$ 0.08 <sup>b</sup>
P <sub>3</sub> (1987–1990)	316	176.1 $\pm$ 8.5 <sup>a</sup>	481.9 $\pm$ 8.5 <sup>a</sup>	191.5 $\pm$ 7.6 <sup>a</sup>	1.87 $\pm$ 0.08 <sup>ad</sup>
P <sub>4</sub> (1991–1994)	210	218.9 $\pm$ 10.4 <sup>b</sup>	527.7 $\pm$ 10.4 <sup>b</sup>	228.6 $\pm$ 9.3 <sup>b</sup>	2.29 $\pm$ 0.10 <sup>b</sup>
P <sub>5</sub> (1995–1998)	114	272.8 $\pm$ 14.0 <sup>cd</sup>	580.8 $\pm$ 14.0 <sup>cd</sup>	268.7 $\pm$ 12.5 <sup>c</sup>	2.82 $\pm$ 0.13 <sup>c</sup>
P <sub>6</sub> (1999–2002)	199	252.9 $\pm$ 10.7 <sup>cde</sup>	559.7 $\pm$ 10.7 <sup>cde</sup>	253.3 $\pm$ 9.6 <sup>bc</sup>	2.55 $\pm$ 0.10 <sup>bc</sup>
P <sub>7</sub> (2003–2006)	68	230.5 $\pm$ 17.6 <sup>bdef</sup>	542.4 $\pm$ 17.7 <sup>bd</sup>	245.1 $\pm$ 15.8 <sup>bc</sup>	2.30 $\pm$ 0.16 <sup>bcd</sup>
Season of calving		**	**	**	**
Winter (Jan–Feb)	211	246.8 $\pm$ 10.3 <sup>b</sup>	554.4 $\pm$ 10.3 <sup>b</sup>	248.0 $\pm$ 9.2 <sup>c</sup>	2.56 $\pm$ 0.10 <sup>c</sup>
Summer (Mar–May)	109	245.2 $\pm$ 14.0 <sup>b</sup>	553.4 $\pm$ 14.0 <sup>b</sup>	254.1 $\pm$ 12.5 <sup>c</sup>	2.44 $\pm$ 0.13 <sup>bc</sup>
South-west monsoon (Jun–Sep)	587	197.5 $\pm$ 7.2 <sup>a</sup>	505.5 $\pm$ 7.2 <sup>a</sup>	201.5 $\pm$ 6.4 <sup>a</sup>	2.02 $\pm$ 0.07 <sup>a</sup>
North-east monsoon (Oct–Dec)	643	210.6 $\pm$ 6.4 <sup>a</sup>	517.9 $\pm$ 6.5 <sup>a</sup>	217.1 $\pm$ 5.8 <sup>b</sup>	2.24 $\pm$ 0.06 <sup>b</sup>
Parity		**	**	**	**
First	513	280.1 $\pm$ 8.0 <sup>c</sup>	586.6 $\pm$ 8.1 <sup>c</sup>	274.1 $\pm$ 7.2 <sup>c</sup>	2.82 $\pm$ 0.08 <sup>c</sup>
Second	367	237.1 $\pm$ 8.3 <sup>b</sup>	544.9 $\pm$ 8.3 <sup>b</sup>	240.3 $\pm$ 7.4 <sup>b</sup>	2.40 $\pm$ 0.08 <sup>b</sup>
Third	259	218.4 $\pm$ 9.6 <sup>ab</sup>	526.4 $\pm$ 9.6 <sup>ab</sup>	220.9 $\pm$ 8.6 <sup>ab</sup>	2.24 $\pm$ 0.09 <sup>ab</sup>
Fourth	175	205.9 $\pm$ 11.6 <sup>a</sup>	512.7 $\pm$ 11.6 <sup>a</sup>	207.9 $\pm$ 10.4 <sup>a</sup>	2.19 $\pm$ 0.11 <sup>ab</sup>
Fifth	118	187.4 $\pm$ 13.7 <sup>a</sup>	495.5 $\pm$ 13.7 <sup>a</sup>	201.2 $\pm$ 12.2 <sup>a</sup>	2.00 $\pm$ 0.13 <sup>a</sup>
Sixth and above	118	221.2 $\pm$ 14.0 <sup>ab</sup>	530.8 $\pm$ 14.1 <sup>ab</sup>	236.8 $\pm$ 12.6 <sup>ab</sup>	2.24 $\pm$ 0.13 <sup>ab</sup>

n — number of observations.

\* P < 0.05; \*\* P < 0.01.

Means bearing same superscript within classes do not differ significantly (P > 0.05).

ond and the fourth and fifth parities differed significantly (P < 0.05). The decline between the third and fifth parities was gradual and the differences between means were not significant.

## DISCUSSION

The average peak yield obtained in the present investigation was higher than the value reported by some earlier workers (Rao and Rao, 1994; Kundu et al., 2003b), although Chhikara et al. (1998) and Suresh et al. (2004) reported higher values for Murrah buffaloes than those observed in the present study. The time to reach peak yield in the present study was substantially higher than that reported for Murrah buffaloes at different places in India (Kundu et al., 2003b; Suresh et al., 2004). Lactation milk yield of cattle and buffaloes up to 305-d of lactation is the criterion most commonly used for the selection of dairy animals and a study of the performance of this trait is of paramount importance for carrying out selection. The overall 305-d milk yield obtained for buffaloes in this investigation was comparable with the value reported by Ulaganathan et al. (1983) and higher than the values reported by other researchers (Kandasamy, 1987; Suresh et al., 2004). The overall least-squares means of lactation milk yield obtained were higher than those observed by Ulaganathan et al. (1984) and Patnaik (1988) in the same herd. Differences in the estimates might be due to sampling errors, genetic constitution

of the herds, agroclimatic variations and management conditions. In general, the performance in terms of the first lactation milk yield of Murrah buffaloes at the Central Cattle Breeding Farm, Alamadhi is quite comparable (Sethi and Khatkar, 1997; Kumar et al., 2002) with those herds in Haryana indicating that there might not be any appreciable genotype x environment interaction.

The mean service period, calving interval and dry period recorded here were in agreement with other research reports on Murrah buffaloes (Kandasamy, 1987; Patnaik, 1988; Kundu et al., 2003b). However, much lower than the present estimates for the above reproductive traits were also reported by several researchers (Chhikara et al., 1995a; Dass and Sadana, 2000; Banik and Tomer, 2003). The mean number of services/conception recorded (2.31 $\pm$ 0.05) is also much higher than those reported by Kumar et al. (2003), but Dutt and Yadav (1988) and Chhikara et al. (1995b) found comparable estimates for Murrah buffaloes maintained at the National Dairy Research Institute, Karnal and the Buffalo Research Centre, Hisar in India.

The main factor controlling variations in the calving interval is the service period, which in turn depends on postpartum oestrus and number of services/conception. In addition, many other factors have been implicated in lengthened calving intervals such as embryonic mortality, high milk production, seasonal and environmental fac-

tors, age of cow and sire used for service. The coefficient of variation obtained for the service period in the present study (67.3%) indicates that the herd was more heterogeneous for this trait. This strongly suggests better opportunities for improvement through good breeding practices. Hence, every effort should be made to reduce the service period sufficiently to reduce the calving interval.

The highly significant influence of period of calving observed in the present study on different production and reproductive traits was supported by similar findings on Murrah buffaloes maintained at different places in India (Kandasamy, 1987; Sethi and Khatkar, 1997; Suresh et al., 2004; Yadav et al., 2007). The difference in performance of the animals among different periods might be attributed to differences in management practices, sires used for breeding, environmental conditions such as ambient temperature, humidity, rainfall etc., and variations in feed and fodder availability.

The significant to highly significant effects of season of calving on different production traits corroborates the findings of earlier workers (Chhikara et al., 1998; Dass and Sadana, 2000) and indicates that there was a pronounced seasonal influence on the traits under study. Buffaloes calving in the winter season had longer lactation lengths and higher lactation milk yields than those calving in rainy seasons. This confirms the findings of earlier reports on Murrah buffaloes (Rao and Rao, 1994; Dass and Sadana, 2000; Gogoi et al., 2002). The higher lactation milk yields in winter and summer seasons might be due to the buffaloes calving in those periods having less gestational stress as a result of the longer service period and delayed conception; also, during their descending stage of lactation, there was an abundant availability of fodders coinciding with the monsoon seasons. The lowest milk yield in monsoon calvers might be because they suffered from heat and humidity stress and the non-availability of quality fodder during a major part of the lactation period.

The significant effect of season of calving on service period and calving interval is in agreement with earlier findings reported in literature for Murrah buffaloes (Chhikara et al., 1995b; Kumar et al., 2003; Suresh et al., 2004). It is generally observed that buffalo cows are seasonally polyoestrus between October and February and they breed regularly during this period. This could explain the shorter service period during monsoon seasons.

The higher number of services per conception in winter calvers recorded here might be due to the fact that animals calving in winter exhibit postpartum heat in the summer months; hence there would be reduction in conception rate. Conception rate is related to oestrous behaviour, time of oestrus detection and insemination and site of semen deposition. Among the different factors, accurate detection of oestrus is of paramount importance in any reproductive management programme, which is difficult in buffaloes during the summer season since most of them exhibit silent oestrus. Thus accurate detection of oestrus and management interventions to ameliorate the effects of heat load on conception rate should be implemented to reduce the number of services per conception. This in turn will have a positive effect on service period and calving interval.

The significant influence of parity on different production and reproductive traits is in accordance with the results obtained by other researchers (Dass and Sadana, 2000; Kundu et al., 2003b) on Murrah buffaloes. The highest 305-d and lactation milk yields obtained in the third parity indicate that lactational maturity is attained in the third lactation and is similar to the reports of Ulaganathan et al. (1983) and Kandasamy (1987). The significant influence of calving sequence on service period and calving interval and the longer first calving interval than the rest found in the present study concurs with other reports on Murrah buffaloes (Kandasamy, 1987; Dass and Sadana, 2000; Kundu et al., 2003a; Suresh et al., 2004). The reduction in service period and calving interval over parities might be due

to differences in age of the animals and periodic culling of buffalo cows with longer calving intervals. The other plausible reason is that following the first two calvings, the physiological rhythm may be maintained (i.e. reduced postpartum oestrus days and better conception) which results in shorter inter-calving periods in pluriparous buffalo cows. Similarly, the longest dry periods in the first parity and significant reductions in later parities might be due to the reduced calving intervals, while the slight increase in reproductive traits from the fifth to sixth and later parities might be due to the lumping of all later records with the sixth parity.

## CONCLUSIONS

This study revealed that non-genetic factors such as period and season of calving had highly significant effects on all the traits studied. In general, milk production of the farm-bred Murrah buffaloes at the Central Cattle Breeding Farm, Alamadhi was comparable with that of animals maintained under other government and institutional herds in India. However, the comparatively lower performance of Murrah buffaloes with respect to fitness traits indicates a lower adaptability of the breed to the hot and humid coastal region. Since temporary environmental factors play a major role on these fitness traits, better breeding management is needed for improvement. In addition, multi-trait evaluation using a combination of production and reproductive traits is needed for simultaneous improvement of production and reproductive performances of Murrah buffaloes. It is therefore imperative to emphasise improvements in husbandry practices and introduction of genetic evaluation programmes at the same time.

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# Effects of Evaporative Cooling System on Productive and Reproductive Performance and some Physiological Parameters of Crossbred Holstein Friesian Cattle in Tropical Conditions

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## ABSTRACT

The productive and reproductive performances and physiological changes occurring in crossbred primiparous cows raised in an evaporative cooling housing system (EVAP) and an open housing system (NEVAP) were compared. In the first experiment, 20 primiparous cows were randomly allocated to two groups of 10 animals. Each group was kept individually in a two-tie stall housing system with all animals being fed with the same ration after parturition through 10 weeks of lactation. Average ambient temperature, relative humidity and temperature-humidity index in EVAP and NEVAP systems were 25.4°C and 28.5°C; 86 and 70; and 77 and 81 respectively. Respiration rate and rectal temperature of cows in EVAP were significantly ( $P < 0.01$ ) lower than cows in NEVAP, while dry matter intake (DMI) and DMI (% body weight [BWt]) were significantly higher ( $P < 0.05$  and  $P < 0.01$  respectively). Cows in the EVAP system had significantly ( $P < 0.01$ ) higher milk yields but significantly lower water intakes than cows in the NEVAP system; however, differences in milk composition were not recorded. Cows with EVAP housing spent more time ( $P < 0.05$ ) chewing than cows in the NEVAP system. In Experiment 2, 34 milking crossbred primiparous cows were randomly allocated into two groups of 17 animals kept under the same housing and feeding conditions as in Experiment 1. There was no significant difference in the interval to first postpartum ovulation between cows in EVAP and NEVAP or in follicular development although there was a tendency towards increased synchronisation and conception rates in the EVAP system. The results suggest that EVAP could improve the productive performance and to some extent also the reproductive performance of crossbred Holstein Friesian cows under tropical conditions.

**Key words:** *evaporative cooling system, heat stress, productive performance, reproductive performance, crossbred Holstein Friesian cows.*

## INTRODUCTION

Heat stress has a significant impact on dairy production. Heat stress occurs when the sum of the cow's physical heat production increases and the environmental heat becomes greater than cow's ability to dissipate heat. The principal climatic factors causing heat stress are temperature, humidity, solar radiation and wind speed (Armstrong, 1994). The most noticeable responses to heat stress are reduced feed intake, milk yield and impaired reproductive performance. Dry matter intake starts to decline and maintenance expenditure increases when environmental temperatures exceed 25°C (NRC, 1981). However, the temperature-humidity index (THI) may describe more precisely the effects of the environment upon the cow's ability to dissipate heat. Milk yield and total digestible nutrient (TDN) intake decline slightly when the THI exceeds 72 and decline sharply when an index of 76 is exceeded (Johnson et al., 1963). During hot periods, dairy cows show signs of disrupted behaviour and impaired physiological function (Hahn and Mader, 1997). A coping strategy of cattle during heat stress is to decrease metabolic heat production by lowering feed intake, which adversely affects productivity. The major changes involved in this acclimatisation are in respiration rate (RR) and rectal temperature (RT), both as well as pulse rate being increased in cattle under heat stress (Marai et al., 1997; Bernabucci et al., 1999). For example, significant increases of RT from 38.7°C to 40.6°C and respiration from 42.5 to 85.3 breaths/min were found when ambient temperatures increased from 18°C to 28°C (Itoh et al., 1998).

It has been shown that a rise of 1°C or less in RT is enough to reduce feed intake and milk production in dairy cows (Johnson et al., 1963). High environmental temperatures also increase water intake, which consequently reduce DMI due to gut fill (Bernabucci et al., 1999; Mallonee et al., 1985). Also, Ominski et al. (2002) found significant differences in DMI and water intake between thermoneutral and heat stress phases, with heat exposure resulting in a 6.5% decrease in DMI and milk production decreasing by 4.8% when animals were exposed to heat stress compared with that produced during the thermoneutral phase. The amount of milk produced depended on the amount of feed ingested and the status of the hormonal system involved in milk production. Heat stress reduced daily milk yield by 21% as THI values increased from 68 in the spring to 78 in the summer (Bouraoui et al., 2002). Detrimental effects of heat stress on the reproductive performance of dairy cows have also been reported including suppressed intensity of oestrus, reduced

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preovulatory LH surge (Howell et al., 1994), altered ovarian follicle development (Wilson et al., 1998) and decreased embryo development (Hansen and Arechiga, 1999).

The objectives of this study were to characterise the effects of modifying the housing conditions by using an evaporative cooling system in which air is forced through a cooling pad on productive and reproductive performances of crossbred Holstein Friesian cows raised under tropical conditions.

## MATERIALS AND METHODS

### Experiment 1: Productive Performance

#### Animals and Management

Twenty primiparous 87.5% crossbred Friesian cattle were used; their average age at calving was 31.5 months. After calving they were divided into two groups of ten animals each. They were kept in a 20 x 10 m housing unit, divided into two parts. One group was kept in an evaporative cooling housing system (EVAP), 20 x 5 m and a height of 2.5 m, in which the temperature was reduced by forcing air through a cooling pad which was soaked by water for the whole day. Air movement was moved through the cooling pad by two fans each of 150 cm in diameter which automatically started to work when the ambient temperature in the barn increased to over 28°C. Another group was kept in the open conventional housing system (NEVAP), 20 x 5 m and a height of 4 m. Both types of housing were individual tie-stall barns with individual feed and water troughs.

Ambient temperature and relative humidity of both housing systems were recorded between 0700 to 1900 h by using a dry-wet bulb thermometer for calculating the temperature humidity index (THI). THI was calculated according to NOAA (1976) in which:

$$\text{THI} = [1.8(\text{temp}) + 32] - [0.55 - 0.0055(\text{rh})][1.8(\text{temp}) - 26]$$

and where *rh* is relative humidity (dry-wet bulb thermometer).

Maximum and minimum temperature in both housing systems were recorded daily using a digital thermometer.

#### Diets, Body Weight, Feed and Water Intake Measurements

Diets were formulated to meet NRC requirements (NRC, 1989). All animals received feed in the form of a total mixed ration (TMR). The roughage:concentrate ratio was 45:55 (DM basis). The roughage used in this experiment was silage composed of corn stalk and pineapple waste at a ratio of 1:1. From the beginning to the end of the experiment, which covered the period from parturition to 10 weeks postpartum, animals of both groups were fed the same ration. Food and water were available *ad libitum*.

Body weight (BWt) of individual animals was measured weekly throughout the experiment, while DMI was measured daily from parturition until week ten postpartum. The amount of feed offered andorts were weighed daily. Orts were removed in the morning before the next feeding. Samples of feed were collected and dried at 105°C immediately to determine DM. Water consumption of each cow was measured daily at 0600 h for three consecutive days using individual water meters and an average calculated.

#### Rectal Temperatures and Respiration Rates

From 8–10 weeks postpartum, rectal temperature (RT) and respiration rate (RR) were measured in all animals every 2 h between 0700 to 1900 h for three consecutive days. RT was recorded with a digital electronic thermometer, while RR was measured by observing movement of the flank for 1 min three times from which an average was calculated.

#### Ruminal Fluid Collection and Analysis

At the end of experimental period, oro-ruminal intubation was used to collect ruminal fluid 2.5 h after feeding in the morning (Whitelaw et al., 1970). Ruminal content was obtained by sucking with an air pump, strained immediately using two layers of cheesecloth and a 60 mL aliquot of the filtered fluid preserved by adding 3 mL 6 N hydrochloric acid and kept at -20°C. Ruminal fluid was analysed for volatile fatty acids (VFA) by the method modified from Erwin (1961).

#### Eating Behaviour

Between weeks 8–10 of the experimental period, the behaviour of all animals was observed continuously for two consecutive d using video recorders. Tape records were used for assessing times spent eating, ruminating and chewing.

#### Milk Production and Composition

Milk production was recorded daily from parturition until 10 weeks postpartum. Milk samples were collected weekly on two occasions (a.m. and p.m.) and kept at -20°C for analysis. They were analysed for lactose, fat and protein using MilkoScan 133B (Foss Electric, Hillerød, Denmark).

### Experiment 2: Reproductive Performance

#### Animals and Management

The effect of EVAP and NEVAP on follicular development, the time of ovulation and conception rate were investigated in 34 primiparous 87.5% crossbred Friesian cows. They were separated into two groups of 17 animals and kept individually in the same type of housing and on the same feeds as in Experiment 1.

Starting at between 3 and 7 weeks postpartum the reproductive tract of all animals was monitored weekly by rectal palpation and ultrasonography using B-mode real time ultrasound (Aloka SSD500) and a 5 MHz linear array rectal transducer to measure follicular size and determine the presence of a corpus luteum. From 1–12 weeks postpartum blood samples (20 mL) were collected twice weekly at intervals of 3–4 d from the jugular vein. Plasma was separated and kept at -20°C for progesterone analysis by RIA (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). Calving to first ovulation interval was defined by the first increase in plasma progesterone ( $\geq 1.0$  ng/mL), ovulation being assumed to have occurred 7 d before elevation of progesterone concentration. Between 85–90 d postpartum oestrus synchronisation was performed by giving all animals an initial intramuscular injection of 100 µg of gonadorelin (gonadotropin-releasing hormone [GnRH]; Fertagyl®) followed 7 d later by 500 µg of cloprostenol ([prostaglandin] PGF<sub>2</sub>α agonist Estrumate®) intramuscularly. Ultrasonography of the ovaries was performed once daily from the time of initial treatment with GnRH to the time of PGF<sub>2</sub>α treatment and then every 4 h until 120 h post-treatment. Follicular size was measured and ovulation confirmed by disappearance of the follicle. Blood samples used to determine plasma progesterone were collected daily for 3 d after the PGF<sub>2</sub>α injection. Corpus luteum regression was defined as a cow having plasma progesterone concentration > 1.0 ng/mL and then declining to a level < 1.0 ng/mL. A cow was considered synchronised only if she met the criteria stated above for both ovulation and CL regression. Synchronisation rate was defined as the percentage of cows which showed regression of the corpus luteum in addition to ovulation of a dominant follicle.

At approximately 110 d postpartum, cows received 100 µg of gonadorelin followed seven d later by an intramuscular injection of 500 µg of cloprostenol; a second injection of 100 µg gonadorelin

was performed on d 9. Sixteen to 24 h after the second injection of gonadorelin the cows were inseminated (Ovisynch). Ultrasonography was used to identify the embryonic vesicle and conceptus at 22, 28, 35 and 42 and 60 d after insemination. The presence of embryonic fluid and a foetus in the uterine horn, a palpable amniotic vesicle and foetal membrane slip were considered positive indicators of pregnancy. The conception rate (CR) was defined as the proportion of cows where early establishment of pregnancy occurred. Blood was collected at d 22 after insemination for progesterone analysis. Early embryonic loss at d 18 after insemination was declared if progesterone was below 1.0 ng/mL at d 22. To declare early embryonic loss at d 28, both plasma progesterone at d 22 and ultrasonography of the uterus at d 28 were used.

### Statistical Analyses

All data were reported as the mean value  $\pm$  SE. The unpaired t-test was used to estimate the statistical significance of differences in values between groups. The proportional data of cows were analysed using Chi-square. Significant differences were declared at  $P < 0.05$  and  $P < 0.01$ .

## RESULTS

### Temperature, RH, THI and Physiological Changes

Mean environmental RH, THI and physiological changes during the experimental periods are presented in **Table 1**. The temperature under EVAP housing was lower than the temperature in the NEVAP system, the average difference being 3.1 °C. During the day, ambient temperature was higher than 24 °C in both type of housing, the level which has been suggested to be critical for dairy cattle. There was a large difference in mean RH between EVAP (86%) and NEVAP (70%), but when average THI was calculated, THI in the EVAP system tended to be higher (77 vs 81). Respiration rate and rectal temperature of cows raised in the EVAP system were significantly lower ( $P < 0.01$ ) than those of cows kept in the EVAP system.

### Dry Matter Intakes, Weight Gains, Milk Yields and Composition

The dry matter intake of animals in the EVAP system (13.3 kg/d) was significantly higher ( $P < 0.05$ ) than in the NEVAP system, more so ( $P < 0.01$ ) when expressed as a percentage of BWt. (**Table 2**) There

**Table 1. Mean environmental temperatures, relative humidity, temperature-humidity index, respiration rate and rectal temperatures under EVAP and NEVAP cooling systems (mean  $\pm$  SE, n = 20).**

Parameter	EVAP	NEVAP
Minimum temperature (°C)	22.2 $\pm$ 0.7	23.6 $\pm$ 0.6
Maximum temperature (°C)	29.1 $\pm$ 0.3	35.8 $\pm$ 0.3
Average temperature (°C)	25.4 $\pm$ 0.4	28.5 $\pm$ 0.3
Mean RH (%)	86 $\pm$ 0.7	70 $\pm$ 3.2
Mean THI	77 $\pm$ 0.5	81 $\pm$ 1.4
RR (breaths/min)	53 $\pm$ 0.7 <sup>a</sup>	67 $\pm$ 2.4 <sup>b</sup>
Rectal temperature (°C)	38.7 $\pm$ 0.03 <sup>a</sup>	39.4 $\pm$ 0.09 <sup>b</sup>

Different superscripts in the same row are significantly different <sup>ab</sup> ( $P < 0.01$ ).

**Table 2. Body weights (BWt), dry matter intakes, milk yields and water intakes of crossbred Friesian heifers maintained in EVAP and NEVAP cooling systems (mean  $\pm$  SE, n = 20).**

Parameters	EVAP	NEVAP
Initial BWt (kg)	374.3 $\pm$ 15.3	358.4 $\pm$ 10.9
Final BWt (kg)	392.5 $\pm$ 10.8	378.7 $\pm$ 15.4
Average BWt (kg)	384.1 $\pm$ 12.6	364.6 $\pm$ 13.9
DMI (kg)	13.3 $\pm$ 0.4 <sup>a</sup>	11.1 $\pm$ 0.5 <sup>b</sup>
DMI	15.4 $\pm$ 0.3 <sup>a</sup>	12.9 $\pm$ 0.4 <sup>b</sup>
DMI/BWt	3.47 $\pm$ 0.22 <sup>c</sup>	3.03 $\pm$ 0.06 <sup>d</sup>
Milk yield (kg/d)	16.9 $\pm$ 1.9 <sup>a</sup>	12.6 $\pm$ 0.6 <sup>b</sup>
4%FCM (kg/d)	14.6 $\pm$ 2.5 <sup>c</sup>	11.1 $\pm$ 0.5 <sup>d</sup>
DMI/4%FCM	0.92 $\pm$ 0.13	1.01 $\pm$ 0.04
Water intake (L/d)	54.4 $\pm$ 3.5 <sup>c</sup>	93.6 $\pm$ 8.0 <sup>d</sup>
Water intake/DMI (L/kg)	4.5 $\pm$ 0.3 <sup>c</sup>	10.6 $\pm$ 0.8 <sup>d</sup>

Different superscripts in the same row are significantly different <sup>ab</sup> ( $P < 0.05$ ), <sup>cd</sup> ( $P < 0.01$ ).

was, however, no difference between the groups in terms of weight gain over the experimental period.

Cows in EVAP produced significantly more milk than cows in NEVAP ( $P < 0.05$ ), but differences in milk composition were not significant except that cows in the EVAP system produced a higher amount of 4% fat corrected milk (FCM) than cows in the NEVAP system ( $P < 0.01$ ).

It was found that animals in EVAP drank 54.4 L water/d compared with 93.6 L/d in the NEVAP system ( $P < 0.01$ ). Expressed as L/kg DMI, water intake under the NEVAP system was also significantly higher ( $P < 0.01$ ).

There were no differences in eating and ruminating times under the two systems of housing (**Table 3**) but cows in the EVAP system tended to spent more time eating and ruminating and significantly more time chewing ( $P < 0.05$ ) than cows in NEVAP, and as a result spent less time resting ( $P < 0.05$ ). Production of VFAs was essentially the same under both systems (**Table 3**).

### Reproductive Performance

One cow in the control group was excluded from the study due to a foot problem. The reproductive performance of the cows under the two systems of housing are shown in **Table 4**. The success of oestrus synchronisation in cows under EVAP housing was higher than cows raising in NEVAP, but not significantly so. Follicular size and d to first ovulation were similar in both groups, but conception rates

by 22 d and 60 d after insemination were higher in cows housed under EVAP conditions, but again the differences were not statistically significant. Embryonic loss was somewhat lower on d 18 in cows housed under EVAP conditions but by d 28 the rate of embryonic loss in both groups was similar.

### DISCUSSION

THI is widely used in hot areas all over the world to assess the impact of heat stress on dairy cows. If the heat stress level classified by Hahn and Mader (1997) is used, it can be concluded that cows in the EVAP group were under mild stress while those kept under NEVAP conditions were under medium stress. However, cows kept under the former conditions suffered mainly from high RH while those in the NEVAP group experienced heat stress through the high ambient temperature. All animals in this experiment were therefore heat stressed although at different levels of severity. Nevertheless, environmental temperatures and THI were higher for the NEVAP group, especially during the day although it is likely that the THI of the EVAP group fell below 74 during the night and as a result cows in this group might compensate for their lower day-time consumption by eating more during the night. On the other hand, the THI under NEVAP conditions may have exceeded 74 due to increased RH at night. Cows in NEVAP would therefore have less chance to compensate their consumption during the cooling period. This would explain why animals kept under EVAP housing consumed 22.7% more feed

**Table 3. Chewing behaviour and volatile fatty acids of cows maintained in EVAP and NEVAP cooling systems (mean  $\pm$  SE, n = 20).**

Parameter	EVAP	NEVAP
Total eating time (min/d)	227.1 $\pm$ 6.9	192.4 $\pm$ 6.2
Total ruminating time (min/d)	349.5 $\pm$ 5.2	255.9 $\pm$ 13.7
Total chewing time (min/d)	576.7 $\pm$ 4.3 <sup>a</sup>	448.3 $\pm$ 12.8 <sup>b</sup>
Resting time (min/d)	863.3 $\pm$ 4.7 <sup>a</sup>	981.7 $\pm$ 14.7 <sup>b</sup>
Volatile fatty acids (VFA, mmol/mL)		
Acetate	119.2 $\pm$ 6.4	107.7 $\pm$ 8.9
Propionate	42.7 $\pm$ 3.4	43.8 $\pm$ 3.1
Butyrate	19.0 $\pm$ 1.0	19.8 $\pm$ 1.4

Different superscripts in the same row are significantly different <sup>ab</sup> ( $P < 0.05$ ).

**Table 4. Reproductive performance of cows maintained under EVAP and NEVAP housing systems (mean  $\pm$  SE, n = 17).**

Parameters	EVAP	NEVAP
Synchronisation rate (%)	82.4 (14/17)	52.9 (9/17)
Size of the largest ovulatory follicle at PGF2 $\alpha$ injection (mm)	11.5 $\pm$ 0.6	10.2 $\pm$ 0.5
Maximal size of the largest ovulatory follicle (mm)	14.6 $\pm$ 0.5	14.2 $\pm$ 0.4
Days to postpartum ovulation <sup>a</sup>	31.4 $\pm$ 4.3	26.1 $\pm$ 3.6
Conception rate (%) within 22 d after insemination <sup>b</sup>	43.8 (7/16)	23.5 (4/17)
Conception rate (%) within 60 d after insemination <sup>b</sup>	25.0 (4/16)	7.6 (3/17)
Embryonic loss (%) within 18 d <sup>c</sup>	56.2 (9/16)	76.5 (13/17)
Embryonic loss (%) within 28 d <sup>c</sup>	75.0 (12/16)	82.4 (14/17)

<sup>a</sup> one cow from each treatment did not show ovulation; <sup>b</sup> number of pregnant cows/number of inseminated cows; <sup>c</sup> number of nonpregnant cows/number of inseminated cows.



than animals under NEVAP conditions. The higher feed intake of cows in the EVAP group had a direct effect on milk production. These animals produced 4.3 kg/d more milk and 3.5 kg/d more of 4% FCM than cows kept under the NEVAP system. Cows in the EVAP group were therefore more productive.

These findings are consistent with the results of Bouraoui et al. (2002) who found negative correlations between daily THI and both milk yield ( $r = -0.76$ ) and feed intake ( $r = -0.24$ ) and that milk yield decreased by 0.41 kg/cow/d for each point increase of THI above 69. Also, Johnson (1985) and Du Preez et al. (1990) showed that milk production was not affected by heat stress when THI values were between 35 and 72 and that both milk production and feed intake began to decline only when THI reached 72 and continued to decline sharply at THI values of 76 or greater. Shearer and Beede (1990) reported that heat stress influenced milk composition through its effect on feed intake, which was the main response of dairy cattle to high environmental temperatures (Collier et al., 1981). No significant differences were recorded in milk composition in this study, but cows in the EVAP group tended to produce milk of superior composition. A similar response was reported by Strickland et al. (1989) and Abelardo et al. (2002) who although not finding an effect of cooling on milk fat percentage, recorded an increase in protein levels in animals kept under a cooling regime.

Indicators of heat stress in cattle include increases in RT, RR and pulse rate (Lemerle and Goddard, 1986; Itoh et al., 1998 and Marai et al., 1997). RT is a sensitive indicator of thermal balance and may be used to assess the negative effects of hot environments on growth, lactation and reproduction in cows (West, 1999). It has been shown that a rise of 1 °C or less in RT is enough to reduce intake and production in dairy cows. A cow normally has 15–30 breaths/min and RRs of 80–90 breaths/min are considered a clear indication of heat stress (Stowell, 2000). Eigenberg et al. (1999) reported a positive correlation between RR and ambient temperature and at these temperatures thermoregulation by increasing evaporative heat loss from the upper respiratory passages would be apparent (Thatcher and Collier, 1986). In the present study, there were significant differences between RT and RR values for cows in the EVAP and NEVAP groups, the significantly greater increases recorded for the latter being characteristic of heat stressed animals. Several studies have shown that evaporative cooled cows had lower values for RT and RR than those that were not cooled (Abelardo et al., 2002; Chen et al., 1993; Huber et al., 1994).

Increased water intake is a further major physiological reaction to heat stress. Bernabucci et al. (1999) reported that exposure to high temperatures was responsible for both increased water intake and reduced DMI. In this study, dairy cows maintained under NEVAP housing consumed more water than those exposed to evaporative cooling. A finding explained by established physiological knowledge that water consumption increases with increasing environmental temperatures because of the greater water losses incurred from sweating and water vaporisation arising from more rapid respiratory rates (panting), both effects aimed at increasing evaporative cooling for the cow (NRC, 1981).

Climatic conditions also influence the behaviour of dairy cows. Cows try to avoid activity during the hotter day, concentrating their grazing/eating during the relatively cooler early morning and late afternoon periods extending into the cool of the evening. Under the conditions of this study, there were no statistical differences between the groups in times spent eating and ruminating, although due to their lower level of feed consumption cows in the NEVAP group tended to spend less time eating and ruminating. Also, when total chewing time (eating time + ruminating time) was considered, NEVAP animals spent significantly less time chewing. This finding is in agreement with the study of Prasanpanich et al. (2002) who reported

that rising temperature and humidity contributed to declining eating activity, and with the finding of Cowan et al. (1993) that increasing temperature during the day forced the early cessation of grazing in lactating cows.

Heat stress can depress the reproductive performance of dairy cows including by decreasing intensity of oestrus, reducing the pre-ovulatory LH surge and decreasing secretion of luteal progesterone (Howell et al., 1994). Also, estradiol is necessary during the pre-ovulatory period to produce an LH surge and ovulation. Cows in EVAP which were in a better status of heat stress may have produced enough estradiol to initiate oestrus and ovulation. The time from giving PGF2 $\alpha$  to oestrus or ovulation is influenced by the oestrus cycle and the follicular development stage in the follicular wave at the time of PGF2 $\alpha$  treatment (Stevenson et al., 1998). In this study, follicular development at the time of PGF2 $\alpha$  treatment was expected to be similar in both groups because the dominant follicle was synchronised by the GnRH injection. However, the response rate to synchronisation of ovulation in cows kept in EVAP housing tended to be greater than in cows kept under NEVAP conditions. One of the deleterious effects on lactating cows which experience heat stress is a reduction in follicular growth (Wilson et al., 1998), but in this study no relationship was found between follicular size and type of housing. However, the size of largest ovulatory follicle at the time of PGF2 $\alpha$  treatment in cows maintained under the EVAP system tended to be greater than cows kept in the NEVAP system. These findings suggest that EVAP housing may improve hormonal status by virtue of increased oestradiol secretion arising from the greater size of the ovulatory follicle and the higher ovulation rate.

High ambient temperatures significantly increase embryonic loss (Sugiyama et al., 2003) and Ryan et al. (1993) reported that a high percentage of embryonic loss occurred between d 7 and 14. In this study cows kept under EVAP housing suffered lower embryonic losses during the earliest stages of pregnancy, suggesting that the cooling system used could alleviate the effect of heat stress on embryonic loss at the early stage of pregnancy. However, the advantage noted at conception declined after 18 d. Indeed, the degree of embryonic loss after d 18 was higher in cows housed in the EVAP system indicating that the cooling system was not good enough to get rid of whole heat stress which can affect the loss of embryo at the later stage of pregnancy. However, the conception rate in cows housed in EVAP system was greater at 60 d after insemination suggesting that EVAP could nevertheless improve conception among crossbred cows at the earlier stage of pregnancy.

## CONCLUSIONS

The EVAP housing system led to lower environmental temperatures and values for THI during the day time compared with the conventional housing system, leading to reduced RR and RT. In addition, DMI, milk yields and 4% FCM were higher in cows maintained under the EVAP system, and some reproductive indices were superior compared with those of cows kept under NEVAP housing. The results suggest that EVAP has the potential to alleviate the stress occurring from heat exposure, reducing thereby the deleterious effects of heat stress on the productive and reproductive performance of crossbred lactating cows. However, further study is needed to determine the effects of this cooling system on animal health, and more broadly its economic benefit under the conditions found in Thailand.

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# Molecular Characterisation of Bulgarian Livestock Genetic Resources and their Optimal Utilisation for Animal Production

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## ABSTRACT

This study was undertaken to determine the genetic structure and diversity among two 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 from 0.6333 (TGLA 53) to 0.9333 (TGLA227) for Grey cattle, with similar average values for the two groups (0.7858 and 0.7757). These results clearly suggest that these breeds are suitable to preserve as genetic resources.

**Key words:** *microsatellites, genetic structure, genetic diversity, linkage disequilibrium, animal production.*

## INTRODUCTION

One of the factors for improvement of livestock breeds in future is to develop new breeds with desirable traits based on cross-breeding between traditional livestock breeds resistant to important diseases and commercial breeds. If done effectively, this would yield animals with productive characteristics of the commercial breed and the disease resistance of the traditional breed. However, when livestock breeds become extinct, their unique genes are lost forever. Loss of local breed populations contradicts the principles for sustainable development of animal breeding and the correct management of animal genetic resources (AnGR). The old Bulgarian breeds, traditionally bred, have been adapted to local conditions and they are resistant to diseases. Due to this they are usually preferred for organic raising of animals (which excludes the use of veterinary medicines and preparations). Utilising local breeds is therefore an effective strategy for contributing to the achievement of local food security objectives.

The necessity to extend, maintain and conserve genetic diversity has been outlined in the First Report on the State of the World's Animal Genetic Resources (FAO, 2007). In this context a number of molecular techniques have provided new DNA markers for the study of genetic variation (Nijman et al., 1999; Hansen et al., 2002). During

recent years, different studies of cattle breeds based on microsatellite markers have aimed at characterising the genetic variation, genetic relationships between cattle breeds from Italy (Ciampolini et al., 1995, Spain (Martin-Burriel et al., 1999; Canon et al., 2001), Belgium (Mommens et al., 1999), Poland (Radko and Duniec, 2002), and the Czech Republic (Czernekova et al., 2006).

The use of microsatellites with high polymorphism information content for correctly identifying cattle, assists in better operation of breeding programmes and breed improvement.

Understanding the diversity, distribution, basic characteristics, comparative performance and the current status of Bulgaria's animal genetic resources is essential for their efficient and sustainable use, development and conservation. For sustainable management, diversity needs to be considered and understood at the species level, between and within breeds.

The aims of this study were therefore to describe the cattle production systems and assess the genetic diversity by analysing genetic variability of eleven microsatellite markers in two populations of Bulgarian local breeds with a view towards promoting their conservation.

## Bulgarian Animal Genetic Resources and their Productivity

Bulgaria is rich in animal genetic resources, i.e. in cattle, horses, pigs, sheep, goats, dogs and poultry. Two cattle breeds (Grey and Rhodope Shorthorn); nineteen sheep breeds (Blackhead Plevan, Local Stara Zagora, Local Karnobat, Splotch-Faced Maritza, White Maritza, Karakachan, Cooper-Red Shoumen, Replyan, Duben, Middle Rhodopean, Kotel, Middle Stara and Planina, Sofia (Elin-Pelin), Strandza, Koprivshitzta, Sakar, Teteven, West Stara Planina and Breznik); one goat breed (Local Long-Haired [Kaloferska] goat); four horse breeds (East Bulgarian, Karakachan, Danubian, and Plevan); one dog breed (Karakachan); and one poultry breed (Black Shoumenska hen) have been reported so far (Gelev et al., 2008 and 2009).

The high value of Bulgarian local breeds reflects the genes they possess which provide their excellent adaptive capabilities, high resistance to diseases and ability to produce high quality meat, milk and eggs. However, intensified use of commercial breeds has had serious effects on local types of domestic animals, which as a rule are less productive. Undoubtedly, the increased import of highly-selected breeds and the complete ignorance and undeserved lack of interest in local genotypes have contributed to their decline. There is a real danger that more of them will disappear forever.

Within Bulgaria there are three recognised breeds of domestic cattle: the Local Grey and its offshoot the Iskar-Grey which

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are selected mainly for productivity, and the Rhodope Shorthorn. The Rhodope breed is a representative of the brachyceros type of cattle, and the Grey is a result of prolonged cross-breeding between the brachyceros type and a primigenius type. This cross-breeding has taken place with varying degrees of intensity and continuity in different places in Bulgaria, the result of which in the recent past has been a rich diversity of combinations. It has been established that in lower-lying regions where forage conditions are better, larger and more productive primigenius types are predominant. The opposite is also true: the influence of the smaller, less productive brachyceros type increases with altitude.

They are bred mainly in the region of the rivers Iskar, Vit, Osam, and Rositsa, from whence Iskar - Grey cattle breed received its name. The population of this breed has declined from 74 152 in 1957 to just 281 today. Put another way, Iskar cows in 1957 represented 7.1% of the cows in the country, and today they represent practically nil.

As suggested by its name, the Rhodope Shorthorn evolved in the Rhodope mountains. Together with the Albanian and southern Montenegrin cattle, the Rhodope cattle is the last remnant of the prehistoric brachyceric cattle in Europe. It was domesticated over 8 000 years ago and is also referred to as the Thraco-Illyrian Brachyceros. Small in size, they are capable of using any type of mountain pasture. In the winter when food is scarce, they usually lose about 20% (50 kg) of their body weight, but regain it in less than one month in the spring by grazing.

In 1957, there were 429 975 Local Grey cattle (40–42% of the cattle in the country) and there were 52 956 Rhodope Shorthorn cattle in 1961 (Danchev 1994; Dimitrov and Dimitrova 1994; Dimitrov et al., 1998) but nowadays they are on the brink of extinction. According to the information in the EAAP-Animal Genbank of the European Association for Animal Production (<http://www.tiho->

[hannover.de/einricht/zucht/eaap/](http://www.tiho-hannover.de/einricht/zucht/eaap/)), both these breeds are classified as “critically endangered”. The calculated number of females (NFN) is 280 for Grey cattle and for Rhodope Shorthorn it is less than 250. According to the most recent monitoring by the Executive Agency of Selection and Reproduction in Bulgaria (Gelev et al., 2008 and 2009), for the Grey cattle breed there are 1 506 cows with 729 females under selection while for the Rhodope Shorthorn breed, there are 604 cows with 97 females under selection control.

The average live weight of Grey Iskar cows is 390 kg. Average milk-yield is 2 500–2 600 L, with a maximum of 6 929 L. The fat content in the milk is on average 4.2%, with a maximum of 5.7%. The fat content of the milk is on average 4.5%, with a maximum of 5.9%. They are exceptionally hardy and strong. In addition to milk, these animals are used to provide tractive power (up to the age of 15 or 20 years).

The average live weight of Rhodope Shorthorn cattle is around 220 kg., their average milk yield is between 1 100–1 200 L but varies from 400 to 1 900 L. Maximum yields are obtained between the fifth and 11th lactations.

Comparisons among all the local and improved breeds of cows in Bulgaria show that the Rhodope Shorthorn breed is second only to the Bulgarian Red cows in production per 100 kg live weight. Moreover, they live 2–3 times longer than the highly-selected breeds and can produce offspring and milk into an advanced age.

In order to avoid extinction, the Bulgarian government has formed breeding populations of these autochthonous domestic animals. Also, in 2003 the Executive Agency for Selection and Reproduction in Animal Breeding in Bulgaria formulated a programme for conservation of AnGR and relevant guidelines for performing selection activities. In addition, a National Gene Bank was established recently for conservation of semen, as was patenting of the autochthonous

**Table 1. Chromosome location and primer sequences of eleven microsatellite loci analysed.**

Cattle chromosome	Locus	Size range (bp)	Primer sequence	Reference
18	TGLA 227	64–115	F:5'-cga att cca aat ctg tta att tgc t-3' R:5'-aca gac aga aac tca atg aaa gca-3'	Barendse et al., 1994
2	BM 2113	116–146	F:5'-cgt gcc ttc tac caa ata ccc-3' R:5'-ctt cct gac aga agc aac acc-3'	Bishop et al., 1994
7	TGLA 53	147–197	F:5'-gct ttc aga aat agt ttg cat tca-3' R:5'-atc ttc aca tga tat tac agc aga-3'	Barendse et al., 1994
5	ETH 10	198–234	F:5'-ggt cag gac tgg ccc tgc taa ca-3' R:5'-cct cca gcc cac ttt ctc ttc tc-3'	Toldo et al., 1993
20	TGLA 126	104–133	F:5'-cta att tag aat gag aga ggc ttc t-3' R:5'-ttg gtc tct att ctc tga ata ttc c-3'	Barendse et al., 1994
21	TGLA 122	130–193	F:5'-aat cac atg gca aat aag tac ata c-3' R:5'-aat cac atg gca aat aag tac ata c-3'	Barendse et al., 1992
3	INRA 023	193–235	F:5'-gag tag agc tac aag ata aac ttc-3' R:5'-taa cta cag ggt gtt aga tga act c-3'	Vaiman et al., 1994
19	ETH 3	90–135	F:5'-gaa cct gcc tct cct gca ttg g-3' R:5'-act ctg cct gtg gcc aag tag g-3'	Toldo et al., 1993
9	ETH 225	135–165	F:5'-gat cac ctg gcc act att tcc t-3' R:5'-aca tga cag cca gct gct act-3'	Steffen et al., 1993
1	BM1824	170–218	F:5'-gag caa ggt gtt ttt cca atc-3' R:5'-cat tct cca act gct tcc ttg-3'	Bishop et al., 1994
15	SPS 115	234–258	F:5'-aaa gtg aca caa cag ctt ctc cag-3' R:5'-aac gag tgt cct agt ttg gct gtg-3'	Mommens et al., 1998

breeds as well as monitoring their populations (Gelev et al., 2008 and 2009). One of the requirements for strengthening protection of these AnGR is to apply DNA analysis for the purposes of identification and for paternity testing. With the growing availability of new techniques in molecular biology, it is nowadays possible to characterise genetic resources at the DNA level.

## MATERIALS AND METHODS

Blood samples were collected from 89 animals belonging to the two breeds, namely 30 unrelated Grey cattle (G) and 59 Rhodope Shorthorn cattle (R) from the regions of Sredetz and Smoljan. Total genomic DNA was extracted using GFX Genomic Blood Purification Kit (Amersham Biosciences, UK).

As recommended by the International Society of Animal Genetics (ISAG), for diversity studies in cattle 11 microsatellites (**Table 1**) were selected, using the Stockmarks for Cattle Bovine Genotyping Kit (Applied Biosystems). Multiplex amplification was carried out in a final volume of 15 µL containing of 50 ng of template DNA, 0.5 units of AmpliTaqGold™ polymerase, 3.0 µL Stockmarks buffer, 400 µL of each dNTP and 5.5 of primer mix. The reactions were carried out using a thermocycler (GeneAmp 9700, Applied Biosystems) in an initial denaturation phase of 15 min at 95 °C, followed by 31 cycles of 45 sec at 94 °C, 45 sec at 61 °C, and 1 min at 72°C. A final extension was carried out at 72°C for 1 h and then at 25 °C for 2 h. The amplified products were mixed with fluorescent dye group and analysed in a 5% (w/v) denaturing gel using an ABI PRISM™ 377

DNA Sequencer. The fluorescence data were collected by GeneScan™ Analysis 2.0 and analysed using Genotyper™ 2.0 software.

The GENEPOP package (Raymond and Rousset, 1995) was used to perform an exact test for deviation from Hardy-Weinberg equilibrium (HWE), allele frequencies, the expected (He) and observed heterozygosity (Ho), and genotypic linkage disequilibrium. Fisher's method was used to combine the two populations and 11 microsatellite loci with 4n degrees of freedom, computed as in Weir and Cockerham (1984) and Robertson and Hill (1984).

## RESULTS AND DISCUSSION

One hundred and seventy eight alleles were detected from the 11 loci surveyed — 118 alleles in Rhodope (R) and 60 in Grey (G) cattle yielding a mean value of 9.0 alleles and 7.5 per locus respectively. Since the evaluation of polymorphism is strictly dependent on the allele number and the frequency distribution of the alleles, estimates of allele frequencies are essential. The allele frequencies of 11 microsatellites are listed in **Tables 2** and **3**. These frequencies revealed that not all markers were equally informative.

All loci were polymorphic in both breeds. The alleles detected at different loci varied with disparate frequency. At the most polymorphic locus TGLA 53–13 alleles were in the range of 154 to 186 bp. Alleles of 160 bp and 170 bp were the most frequent in both breeds at this locus. The allele 166 bp was present in Rhodope but absent in Grey cattle and allele 186 bp was present in Grey and absent in Rhodope Shorthorn cows. The data for another high polymorphic locus - TGLA 122 showed the absence of alleles 138bp, 140 bp and

**Table 2. Allele frequencies of the 11 microsatellites loci analysed in Bulgarian Grey cattle.**

TGL227		TGL53		TGL122		ETH3		BM2113	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
78	0.014	154	0.086	142	0.100	103	0.071	125	0.157
80	0.043	160	0.329	144	0.343	109	0.029	127	0.029
82	0.114	162	0.043	148	0.029	115	0.071	133	0.300
84	0.200	164	0.043	152	0.028	117	0.229	135	0.200
86	0.114	168	0.171	154	0.371	119	0.143	137	0.114
90	0.072	170	0.029	156	0.029	121	0.029	139	0.129
92	0.172	172	0.029	164	0.043	123	0.100	141	0.071
94	0.071	174	0.029	172	0.028	125	0.286		
96	0.057	176	0.057	174	0.029	127	0.042		
98	0.014	180	0.086						
100	0.014	182	0.057						
104	0.015	184	0.041						

SPS115		TGLA126		INRA023		ETH 225		ETH10		BM1824	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
248	0.571	109	0.014	199	0.186	140	0.371	210	0.071	180	0.157
250	0.014	117	0.529	207	0.115	144	0.143	216	0.057	182	0.200
252	0.029	119	0.300	209	0.129	146	0.129	218	0.286	184	0.386
254	0.157	121	0.014	211	0.057	148	0.157	220	0.329	190	0.2571
256	0.086	123	0.043	215	0.471	150	0.157	222	0.257		
258	0.029	125	0.100	217	0.042	158	0.043				
260	0.114										

**Table 3. Allele frequencies of the 11 microsatellites loci analysed in Rhodope Shorthorn cattle.**

TGL53		TGL122		TGL227		INRA023		ETH3	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
154	0.014	138	0.008	80	0.100	175	0.017	109	0.017
160	0.043	140	0.017	82	0.343	197	0.008	113	0.008
162	0.114	142	0.195	84	0.029	199	0.144	115	0.119
164	0.200	144	0.288	86	0.028	205	0.068	117	0.331
166	0.114	146	0.008	90	0.371	207	0.161	119	0.127
168	0.072	152	0.034	92	0.029	209	0.102	121	0.017
170	0.172	154	0.144	94	0.043	211	0.119	123	0.034
172	0.071	156	0.051	96	0.028	213	0.017	125	0.305
176	0.057	164	0.051	98	0.029	215	0.237	127	0.034
178	0.014	172	0.017	100		217	0.102	131	0.008
180	0.014	176	0.136	102		219	0.025		
182	0.015	178	0.051						
184	0.015								

ETH225		BM2113		ETH10		SPS115		TGL126		BM1824	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
138	0.008	125	0.008	212	0.017	248	0.686	117	0.051	180	0.051
140	0.288	127	0.144	214	0.017	250	0.034	119	0.525	182	0.331
144	0.229	129	0.008	218	0.517	252	0.068	121	0.153	184	0.432
146	0.025	131	0.186	220	0.195	254	0.076	123	0.008	190	0.178
148	0.186	133	0.153	222	0.220	256	0.042	125	0.042	192	0.008
150	0.085	135	0.288	224	0.017	258	0.025	127	0.220		
156	0.008	139	0.169	226	0.017	260	0.068				
158	0.153	143	0.042	226	0.017						
160	0.017			226	0.017						

178 bp in the Grey cattle population. The most frequent allele in the studied populations was the 144 bp allele, while the rarest alleles were 138bp and 152bp (0.008) for the Rhodope population and 156 bp (0.017) for the Greys. ETH 3 amplified ten and nine alleles respectively for Rhodope and Grey cattle with the most common alleles being 117 bp and 125 bp. In both breeds the 121bp allele had the lowest frequency. Four to five alleles were detected in locus BM1824. The most common allele was 184 bp. Allele 192 bp was present only in the Rhodope Shorthorn population.

The results obtained in the present study concerning allele frequencies in Bulgarian local cattle are in agreement with the data obtained by Radko and Duniec (2002). However, Czernekova et al. (2006) reported higher numbers of alleles in the same loci for Czech cattle — from seven at BM1824 to 14 at TGLA 227. According to Peelman et al. (1998) and Cervini et al. (2006) who have analysed European cattle and *Bos indicus* Nellore cattle, the number of TGLA53 locus alleles in Holstein Friesian (13 alleles), Belgian Red Pied (12 alleles), East Flemish (12 alleles) and Belgian Blue (10 alleles) cattle and Nellore cattle (13 alleles) were very similar to that found in Rhodope Shorthorn (13 alleles) and Grey cattle breeds (12 alleles). High polymorphism was noted also at loci TGLA 122, with 12 and 10 alleles respectively for R and G cattle, TGLA 227 (11 and 12 alleles) and ETH 3, with 10 and 9 alleles for R and G cattle. The lowest number of alleles in both breeds was observed at locus BM1824. The same variation of number of alleles were shown by Zhou et al.

(2005) for five native Chinese cattle breeds, while the number of alleles at locus TGLA 227 in the present study is higher than observed by Martin-Burriel et al. (1998) and Armstrong et al. (2006) for Spanish native and Creole cattle breeds (seven alleles). The Rhodope Shorthorn population had a greater mean number of alleles (9.0) than the Grey cattle (7.5), although this may have been due, in part, to the much larger sample size.

Both populations were described according to the expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity (Table 4). The data in Table 4 show a common trend in the two groups. Expected heterozygosity ranged from 0.5424 (SPS 115) to 0.8983 (TGLA 227) for the Rhodope population and from 0.6333 (TGLA 53) to 0.9333 (TGLA 227) for Grey cattle. All the microsatellite loci showed an expected heterozygosity greater than 0.500. The overall mean heterozygosity across all populations and all markers had similar average values for both groups i.e. 0.7858 and 0.7757 respectively. In an analysis of six Spanish native breeds, Martín-Burriel et al. (1998) reported an average expected heterozygosity between 0.56 and 0.68. Additionally, Rendo et al. (2004) found an expected heterozygosity between 0.69 and 0.76 in four Western Pyrenees cattle breeds using 11 microsatellite markers, while in a study of 15 Portuguese cattle breeds Mateus et al. (2004) found an average expected heterozygosity between 0.63 and 0.74. Similar results were reported by Zhou et al. (2005) for five native Chinese populations which displayed a high heterozygosity, namely 0.51 to 0.86. The values obtained here for expected heterozygosity

**Table 4. Observed and expected heterozygosity and number of alleles in Bulgarian cattle breeds studied.**

Locus	Expected heterozygosity $H_e$	Observed heterozygosity $H_o$	Number of alleles N
TGLA 227			
R	0.8983	0.8536	11
G	0.9333	0.8885	12
BM 2113			
R	0.8475	0.8141	8
G	0.8000	0.8172	7
TGLA 53			
R	0.7966	0.8383	13
G	0.6333	0.8517	12
ETH 10			
R	0.6610	0.6505	7
G	0.8333	0.7425	5
SPS 115			
R	0.5424	0.5143	7
G	0.7000	0.6488	6
TGLA 126			
R	0.6610	0.6531	6
G	0.6667	0.6149	6
TGLA 122			
R	0.8814	0.8369	12
G	0.6667	0.7523	10
INRA 23			
R	0.8814	0.8635	11
G	0.8333	0.7351	6
ETH 3			
R	0.8644	0.7702	10
G	0.8000	0.8351	9
ETH 225			
R	0.8814	0.8045	9
G	0.8333	0.7787	6
BM 1824			
R	0.7288	0.6749	5
G	0.8333	0.7402	4
Mean			
R	0.7858	0.7513	9.0
G	0.7757	0.7641	7.5

were higher than those reported by Citek and Rehout (2001) and Czernekova et al. (2006) for endangered populations of Czech Red, Czech Pied, Polish Red, and German Red cattle, which were in the range of 0.396–0.495 and 0.650 to 0.764 respectively. Our study showed the highest level of heterozygosity at locus TGLA 227 in both populations. This means that this marker could be included in subsequent genetic diversity studies of cattle populations. Also, the heterozygosity found in our samples of Bulgarian local cattle breeds was considerably higher than that found in studies on commercial breeds that used similar microsatellites. For example, Hansen et al. (2002), and Maudet et al. (2002) showed that highly selected commercial breeds were much less diverse and more inbred than local breeds, which reinforces the importance of local breeds as reserves of genetic diversity for sustainable agriculture.

According to classical genetics, a population is in Hardy–Weinberg equilibrium (HWE) if the gene ( $p$  and  $q$ ) and genotype frequencies,  $p^2$  (AA),  $2pq$  (Aa), and  $q^2$  (aa) are not changing from one generation to another. Evolutionary factors such as genetic drift,

selection, mutation, and migration are the forces which can modify HWE in the population. Genetic drift is due to a small population size, where mating of related individuals is conducive for enhanced inbreeding. The selection of non-neutral alleles and mutations contribute to genetic differentiation among populations. In this study, deviation from proportions of HWE were noted for all the loci except for TGLA 53 ( $P < 0.05$ ) in Rhodope Shorthorn and for BM 2113, ETH 3, TGLA 122 and TGLA 53 ( $P < 0.01$ ) in Grey cattle breeds. Ciampolini et al. (1995) reported that Hardy–Weinberg equilibrium was not maintained in a study of microsatellites for four Italian beef breeds. Migration (gene flow) from an external population is a possible factor contributing to the observed deviation from HWE in the present investigation.

Tests of genotypic disequilibrium across populations resulted in 110 comparisons. Significant linkage disequilibrium ( $P < 0.05$ ) was found between locus pairs BM2113 and TGLA53 on the one hand and ETH 3 on the other in the Rhodope Shorthorn breed. In the second local Bulgarian breed a genetic disequilibrium was observed between the BM2113 and ETH3 and ETH 225, BM1824 and ETH10 loci. ( $P < 0.05$ ). Gametic disequilibrium can arise from a variety of causes including epistatic selection, physical linkage, genetic hitchhiking. Because the markers studied were mapped to different chromosomes (respectively BM1824 on chromosome 1, BM2113 on chromosome 2, ETH 3 on chromosome 9, ETH 225 on chromosome 10 and TGLA 53 on chromosome 16), one can exclude physical linkage. The most probable reason may therefore be gametic selection.

## CONCLUSIONS

This study extends knowledge of the genetic diversity, genetic structure and molecular characterisation of small populations of local cattle breeds in Bulgaria that are on the brink of extinction. Grey and Rhodope Shorthorn cattle breeds were characterised genetically using DNA markers. All loci were polymorphic indicating that the microsatellite markers used are suitable for studying genetic diversity. The comparison between the two local breeds shows that they displayed a remarkably high variability. This clearly suggests that these breeds have however, potential value to be preserved as genetic resources.

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 the purposes of conservation and sustainable use.

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# Effect of Temperature and Humidity on Heat Stress Responses in Vietnamese Yellow Cattle

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## ABSTRACT

Four female cattle (local Yellow breed), eight months of age, were fed a diet of 50% urea-treated and 50% untreated rice straw *ad libitum*, with free access to water. The levels of ambient temperature/relative humidity (RH) were random combinations of 25, 29, 35 and 39 °C and 70, 80 and 90% RH, achieved in an experimental chamber fitted with air conditioners, heaters and ultrasonic humidifiers. The treatments were based on the natural conditions which frequently occur in an indoor animal house in Central Vietnam in summer. Feed intake decreased linearly while water intake increased with increasing ambient temperature. Heart rate increased in direct proportion to the air temperature but was not affected by RH levels. Body temperature only increased when the chamber temperature reached 39 °C and RH was 90%, while respiration rate increased when the ambient temperature exceeded 35 °C. Measurements of HSP70 (heat shock protein, a biochemical stress indicator) from leukocytes using PCR showed that HSP70 was evident when RH reached 90% with an ambient temperature of 25 °C, or with an RH of 70% and an ambient temperature of 39 °C.

**Key words:** Yellow cattle, heat stress, physiology indexes, HSP70.

## INTRODUCTION

In the life of animals the environment is important particularly ambient temperature and relative humidity (RH), which often vary. Hot weather and high humidity can reduce breeding efficiency, milk production, feed intake, weight gains, and sometimes cause death (Boyles, 2008). In addition to causing discomfort, high temperatures increase the maintenance energy required to keep the animal cool. An animal's ability to tolerate weather conditions depends on its type or breed and its body condition. The aim of this study was to obtain quantitative information on the changes in heat stress indicators in local Yellow cattle in response to changes in ambient temperature and relative humidity in Central Vietnam.

## MATERIALS AND METHODS

### Animals

Four female cattle (local Yellow breed) aged eight months were used; they had an average live weight of 80 kg at the beginning of the

study. The animals were housed individually in metabolism pens in one chamber and de-wormed before the experiment.

### Diets

The animals were given a diet consisting of 50% rice straw (90.3% DM and 6.6% CP (DM based) and 8 MJ/kgDM of ME) and 50% urea-treated rice straw (50.5% DM, 8.8% CP (DM based) and 10.3 MJ/KgDM of ME). All animals were fed *ad libitum* with free access to water.

### Environmental Treatments

The environmental conditions were achieved in an experimental chamber fitted with air conditioners, heaters and ultrasonic humidifiers with ventilators (Table 1). All animals were kept under the same conditions at the same time then changed to the next treatment using the following design:

The treatments were based on the natural conditions which frequently occur in an indoor animal house in Central Vietnam in summer. Temperature and humidity were recorded using thermo-hygrometers. The experiment was carried out from March to May.

During the first 15 d the cattle were kept outside the chamber while they adapted to the diet; they then spent five d adapting to the chamber. Following this, each treatment was imposed for a period of four d, i.e. three d of heat and humidity treatment in which the treatments were imposed from 7.00 to 17.00 h followed by an ambient temperature of 25 °C and RH of 70% from 17.00 to 7.00 h, during each d; these latter conditions were also imposed on the first d which was free from treatment in each 4-d period.

### Feeding

Fresh feed and water were supplied at 8.00 h and again at 18.00 h in the afternoon. Feed refusals and water intake were recorded at 17.00 and 06.00 h.

**Table 1. Relation between temperature, relative humidity and their index.**

Temp. °C/RH%	THI	Temp. °C/RH%	THI	Temp. °C/RH%	THI
(1)25/90	76	(5)29/70	80	(9)35/90	93
(2)39/80	97	(6)39/90	100	(10)25/80	75
(3)35/70	89	(7)35/80	91	(11)39/70	95
(4)29/90	83	(8)25/70	74	(12)29/80	81

Temp = temperature; RH = relative humidity; THI = temperature and humidity index.

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## Physiological Measurements

Feed and water intakes were measured. The respiration rate was counted by observing the rate of movement of the diaphragm, heart rate by listening to a stethoscope between the 3rd and 4th ribs, and body temperature using a 42 °C thermometer placed in the animal's rectum.

## Blood Sampling and RNA Isolation

Blood sampling was at 13.00 h on the last two d of heat treatment. Blood samples were collected from catheters fitted in the jugular vein into 10 mL heparinised tubes. Blood was kept on ice and immediately taken to the laboratory for isolation of total RNA from leukocytes. The procedure for isolating total RNA followed the Technical Manual provided by the manufacturer (Promega, 2007) using SV Total RNA Isolation Kits (50 prep). All RNA samples were frozen at -20 °C until the application of a real-time PCR (RT-PCR) technique to identify Hsp70 (heat shock protein, a biochemical stress indicator).

## Real-time PCR Protocol for Heat Shock Protein (Hsp70) Assay

Access Quick RT-PCR System Kits (100 reactions) from Promega (USA) were used. The RT-PCR component consisted of 25 µL 2x Access-Quick Master Mix, 1 µL of each primer (10 µM), 2 µL RNA template (50 ng), nuclease-free water to a final volume of 50 µL; 1 µL 5u AMV Reverse Transcriptase was added as the final component and mixed by gently pipetting. Reverse transcription was carried out at 42 °C for 60 min and the initial denaturation at 94 °C for five min; this was followed by 30 cycles of 30 s denaturation (94 °C), 30 s annealing (55 °C) and 1.30 min extension (72 °C), and a final cycle of seven min and final extension at 72 °C carried out in a thermocycler (iCycler, Bio-rad).

The Hsp primer pairs used for analysis by RT-PCR were designed using the bovine primers for Hsc70 described by Kennie (2000), from sequence data for Hsc70, Hsp70-1 and Hsp70-2 (DeLuca-Flaherty and McKay, 1990; Grosz and Skow, 1994) as follows:

The primers (Table 2) were purchased from Integrated DNA Technologies (USA).

## Data Processing

Excel software was used for data processing and a general linear model ANOVA followed by Minitab version 13.1 was used to assess significant differences ( $P < 0.05$ ) among treatments for the feed and water intake, the respiration rate, heart rate and body temperature.

## RESULTS AND DISCUSSION

The effects of changes in environmental temperature and relative humidity on the various parameters measured in the Yellow cattle are shown in Table 3.

### Heart Rate

The heart rate increased in direct proportion to temperature increases but not significantly with increases in humidity (Table 3). The pulse rate is an indicator of the number of times the heart beats in a minute (Wiggins, 2007). Pulse rates can increase as a result of stimulation of the sympathetic nervous system from a number of stresses, including thermal stress.

### Body Temperature

Body temperature did not vary with the levels of temperature in the chamber at humidity levels of 70% and 80%. However, at 90% humidity, the changes in body temperature following increases in chamber temperatures were significant ( $P < 0.001$ ).

If body temperature remains steady in hot conditions, the temperature regulatory mechanism is clearly able to cope with temperature and relative humidity environments to a point beyond which heat exchange to the environment is compromised in cattle. When environmental temperature increases, feed intake will decrease to reduce heat production from digestion in general and from rumen microbial fermentation in particular. Cattle can markedly increase subcutaneous evaporative water loss by sweating. In Vietnamese Yellow cattle, the sweat glands are well developed, and in hot environments the evaporation of water from sweat to the air can significantly cool and affect thermal balance (Withers, 1992) However, in this study, evaporative cooling through the sweat became limiting at 90% RH, resulting in an increase in body temperature ( $P < 0.001$ ).

### Respiration Rate

In this study the respiration rate became higher than normal (10–30 respirations/min) when the temperature in the chamber reached 35 °C (Tinh et al., 1996; Tho and Tien, 1990). This observation agrees with a recent study on Yellow cattle in Vietnam (Thanh and Wang, 2007).

An increase in lung ventilation not only increases gas transfer but also results in more loss of heat and water (Eckert and Randall, 1988) and Withers (1992) concluded that respiratory evaporative water loss is a major avenue for evaporative water loss, especially for non-sweating mammals. This is why respiration rate is sensitive to the thermal environment. Yellow cattle have an extensive sweat

Table 2. Heat shock protein primers.

Hsp Gene (species)	Primer Pair	PCR Product Length (base pairs)
Hsc70 S	5' AAGATGCTGGAACCTATTGCTGG 3'	1474 bp
Hsc70AS	5' ATCAACCTCTCAATGGTGG 3'	1474 bp
Hsp70-1 S	5' AGGACTTCGACAACAGGCTGGTGAA 3'	1098 bp
Hsp70-1 AS	5' CTCTTGTGCTCAAACCTGCCTTCT 3'	1098 bp
Hsp70-2 S	5' TCATCAACGACGGAGACAAGCCTA 3'	1165 bp
Hsp70-2 AS	5' ATCGATGTCGAAGGTACCTCGATCT 3'	1165 bp

S = sense DNA strand primer (forward); AS = antisense DNA strand primer (reverse).

**Table 3. Effects of ambient temperature and relative humidity on Yellow cattle.**

Heart Rate (Beats/min)					
RH%	Temp. °C				SE
	25	29	35	39	
70	65.7 <sup>ab</sup>	71.1 <sup>ab</sup>	88.0 <sup>cd</sup>	91.0 <sup>cd</sup>	1.7 <sup>***</sup>
80	73.2 <sup>ab</sup>	69.8 <sup>ab</sup>	83.5 <sup>cd</sup>	86.9 <sup>cd</sup>	1.7 <sup>***</sup>
90	66.8 <sup>ab</sup>	70.9 <sup>ab</sup>	86.8 <sup>c</sup>	93.7 <sup>d</sup>	1.7 <sup>***</sup>
Body Temperature (°C)					
70	38.7	38.7	38.8	39	0.1
80	38.6	38.7	38.8	38.9	0.1
90	38.6 <sup>abc</sup>	38.6 <sup>abc</sup>	38.7 <sup>abc</sup>	39.2 <sup>d</sup>	0.1 <sup>***</sup>
Respiration Rate (Respirations/min)					
70	25.2 <sup>ab</sup>	26.0 <sup>ab</sup>	42.9 <sup>cd</sup>	48.9 <sup>cd</sup>	2.4 <sup>***</sup>
80	24.2 <sup>ab</sup>	27.8 <sup>ab</sup>	42.1 <sup>c</sup>	51.4 <sup>d</sup>	2.1 <sup>***</sup>
90	24.2 <sup>ab</sup>	25.4 <sup>ab</sup>	42.5 <sup>c</sup>	60.1 <sup>d</sup>	2.9 <sup>***</sup>
Feed Intake (kg/d)					
70	4.02 <sup>d</sup>	3.54 <sup>c</sup>	2.87 <sup>ab</sup>	2.50 <sup>ab</sup>	0.1 <sup>***</sup>
80	4.21 <sup>d</sup>	2.94 <sup>abc</sup>	2.75 <sup>abc</sup>	2.73 <sup>abc</sup>	0.1 <sup>***</sup>
90	3.98 <sup>d</sup>	3.29 <sup>c</sup>	2.59 <sup>ab</sup>	2.47 <sup>ab</sup>	0.1 <sup>***</sup>
Water Intake (L/d)					
70	6.31 <sup>ab</sup>	6.93 <sup>ab</sup>	8.74 <sup>c</sup>	13.02 <sup>d</sup>	0.26 <sup>***</sup>
80	5.98 <sup>ab</sup>	6.35 <sup>ab</sup>	7.67 <sup>c</sup>	13.09 <sup>d</sup>	0.26 <sup>***</sup>
90	6.08 <sup>ac</sup>	7.13 <sup>bc</sup>	6.43 <sup>abc</sup>	13.78 <sup>d</sup>	0.24 <sup>***</sup>

<sup>abcd</sup> Means with different superscripts within rows are different at  $P < 0.05$ .

gland system, and are adapted to the hot and humid environment in Vietnam. However, cattle sweat at only 10% of the rate of humans and are therefore more susceptible to heat stress (Keown and Grant, 1993)

### Feed Intake

Feed intake fell by 12% after increasing the temperature from 25 °C to 29 °C ( $P < 0.001$ ) and fell by 38% at 39 °C and 80–90% RH (Table 3).

At ambient temperatures above the thermoneutral zone, feed intake is reduced in ruminants (McDonald et al., 1995), and cattle will automatically reduce their feed intake during hot weather (Keown and Grant, 1993; Linn, 1997). McDonald et al. (1995) found that feed intake fell by two per cent for every 1 °C rise in average daily temperature above 25 °C. The high reduction recorded here could be due to the high fibre content of the diet (only rice straw) since fibre digestion results in a higher heat increment of feeding (sum of heat produced from rumen fermentation and nutrient metabolism (Linn, 1997).

### Water Intake

Water intake increased with increases in chamber temperatures, being double at a chamber temperature of 39 °C compared with intakes at temperatures of 25 °C, even when RH levels reached 90%.

Because evaporation from the skin or respiratory epithelium is the most effective means of reducing heat stress, there is a close link between water balance and temperature control in hot environments (Eckert and Randall, 1988).

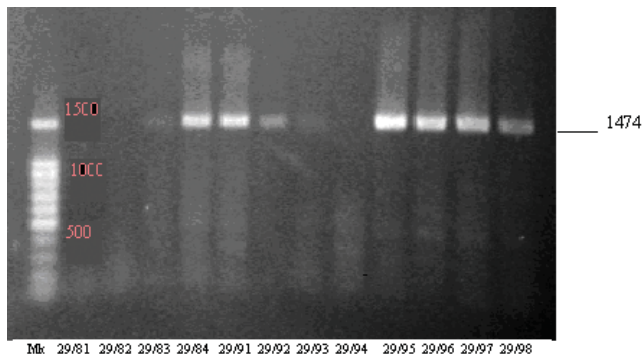
### Heat Shock Protein (Hsp70) in Leukocytes

Heat shock proteins (HSPs), also called stress proteins, are a group of proteins that are present in all cells in all life forms. They are induced when a cell is subjected to various types of environmental stresses like heat, cold and oxygen deprivation.

The Hsp70 family is one of the most studied due to the fact that one of its members, Hsp70, is highly heat-inducible and all organisms examined to date produce Hsp70 family in response to elevated temperatures. Two important members of the mammalian Hsp70 family are the cytosolic proteins, Hsc70 (heat-shock cognate protein) and Hsp70. They are very similar in biochemical properties and have a high sequence homology (about 95%).

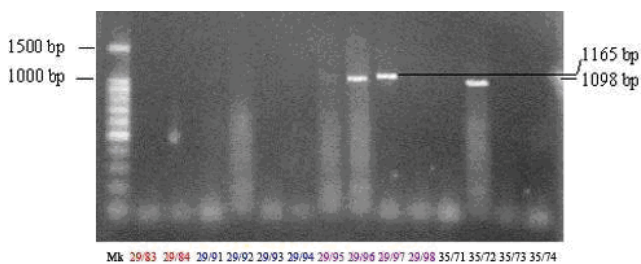
Reverse transcription-polymerase chain reaction (RT-PCR) has become the method of choice for quantifying mRNA expression in many laboratories for detecting gene(s), replacing Northern blots and *in situ* hybridisation. In RT-PCR, RNA is isolated from cells or tissues and then used as a template for reverse transcription of mRNA to complementary DNA (cDNA). The cDNA then acts as the template for the PCR using primers designed to amplify a selected cDNA region, known as the target sequence.

In this study, the RT-PCR and primers were used to identify the Hsc70, Hsp 70-1, and Hsp 70-2 RNA from cattle leukocytes while the cattle were undergoing the heat treatments. At an environment of 29 °C/80% RH (THI = 81), Hsc, an important member of Hsp70 was expressed (Figure 1). At the level of 29 °C/90% RH (at 29 °C/96% RH and 29 °C/97% RH samples) and 35 °C/70% RH (at 35 °C/72% RH sample) Hsp 70-1 and Hsp 70-2 were also present (Figure 2).



**Figure 1. Identification of the Hsp70 (PCR product length of Hsc70: 1474) in leukocytes.**

**29/81–29/84:** samples from four cattle kept at 29°C/80% RH d 1  
**29/91–29/94:** samples from four cattle kept at 29°C/90% RH d 1  
**29/95–29/98:** samples from four cattle kept at 29°C/90% RH d 2



**Figure 2. Identification of the HSP70–1 (1098 bp) and HSP70–2 (1165 bp) in leukocytes**

**29/83–29/84:** samples from two cattle kept at 29°C/80%RH d 1  
**29/91–29/94:** samples from four cattle kept at 29°C/90%RH d 1  
**29/95–29/98:** samples from four cattle kept at 29°C/90%RH d 2  
**35/71–35/74:** samples from four cattle kept at 35°C/70%RH d 1

## CONCLUSIONS

Yellow cattle are well adapted to hot and humid tropical conditions, but sudden changes in weather can cause heat stress in these cattle. In this study, when the ambient temperature reached 29°C and RH 80%, heart and respiration rates increased, feed intakes declined

and water intakes increased. Under these chamber conditions, heat shock protein, a chemical indicator of heat stress, were expressed in leukocytes of the cattle. This showed that Yellow cattle were stressed at a THI of 81 under the conditions in the chamber.

Farmers should be aware about this critical set point and pay attention to managing cattle to avoid heat stress and the resulting decrease in production. So what is needed is a house with shade, a well prepared diet, and access to water in the hot season. More studies are needed to identify diets that can be recommended to live-stock keepers during hot periods and especially when rapid climate changes occur that may reduce production and fertility.

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# Simple and Environmentally Friendly Options to Improve Livestock Performance under Smallholder Conditions

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## ABSTRACT

This paper describes a set of technical possibilities that are simple, inexpensive and efficient and that may improve livestock performance and contribute to environment protection. Sufficient evidence exists in the literature highlighting the beneficial effects of plant secondary metabolites, in particular of tannins and saponins, with respect to increasing animal productivity, health and product quality. Moreover, feeding of low levels of tannins to ruminants might also reduce methane (CH<sub>4</sub>) production, benefiting the environment. Many countries would benefit immensely from identifying plants and plant products having bioactive moieties that could be used as alternatives to antibiotics, growth promoters and antiparasitic drugs. The conservation of many agroindustrial by-products in the form of silage, feed blocks and pellets is another promising option to decrease feeding costs, increase animal productivity and mitigate environment pollution. Some shrub and tree species such as cactus (*Opuntia* spp.) and *Moringa oleifera*, and novel agroindustrial by-products such as *Jatropha* kernel meal from the non-toxic *Jatropha curcas*, the detoxified kernel meal from the toxic *J. curcas* and kernel meal from *M. oleifera* have considerable potential to increase livestock performance and improve farmers' incomes. Appropriate strategies for the transfer of various options and mechanisms to boost their adoption should be targeted. Participatory approaches based on the involvement of farmers, technicians, scientists, local institutions and policymakers are recommended to achieve this objective.

**Key words:** *Alternative feed resources, natural compounds, fodder shrubs and trees, feed blocks and pellets, smallholder farms, livestock performance.*

## INTRODUCTION

Ruminant production is one of the main sources of income for rural populations living in arid and semi-arid zones. The lack of adequate year-round feed resources is probably the most important factor contributing to the low productive and reproductive performances of

these animals (Ben Salem and Smith, 2008). In addition to rangeland degradation and global warming, the recent leap in the prices of concentrate feeds and the international economic crisis are seriously threatening the sustainability of livestock-based production systems. Until recently, antibiotics and other chemicals in feeds were used to improve livestock performance and health. However, the use of these additives has been banned in the EU since 2006 because of the risk to humans of chemical residues in food and of antibiotic resistance being passed on to human pathogens (Makkar et al., 2007).

A large number of recent research programmes focus on identifying alternative options that enhance animal performance and improve product quality without risk to human health (Makkar et al., 2007; Martin et al., 2010). Some promising cost-effective and environmentally friendly options which have recently proven to be efficient in improving ruminant performance and health include the use of plants (Wina et al., 2005; Makkar et al., 2007), plant extracts (Cheecke, 1999) or natural compounds (Makkar et al., 2007) as potential alternatives to growth promoters and antibiotics. Moreover, the development of simple and inexpensive techniques to improve the value of local feed resources (e.g. browses, cacti, agroindustrial by-products) could help smallholders to better manage livestock feeding throughout the year.

In this paper, we discuss some findings available in the literature on the benefits of alternative options based on locally available feed resources and how they can improve livestock performance and health while conserving the environment. Expected improvement of farmers' incomes and opportunities for their adoption by farmers are also highlighted.

## TECHNICAL OPTIONS TO IMPROVE LIVESTOCK PERFORMANCE AND PROTECT THE ENVIRONMENT

### Rumen Manipulation with Plant Secondary Compounds

Plant defensive compounds commonly but loosely known as plant secondary compounds include phenolics, saponins, alkaloids, non-protein amino acids, essential oils and glycosides. Tannins and saponins are the most widely occurring components from these groups. They have both beneficial and adverse effects depending upon their nature and the amount an animal consumes. In various studies, foliage as well as fruits and seeds of fodder shrubs and trees have been reported to suppress ruminal protozoal populations.

This natural defaunating (i.e. protozoa eliminating) activity of some multi purpose trees and shrub-derived feeds arises from their plant secondary metabolites (Leng et al., 1992). Digestion of feeds

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by ruminal microorganisms results in emission of CH<sub>4</sub> from livestock. The excretion of CH<sub>4</sub> from the rumen can represent a loss of up to 12% of the digestible energy, depending on the type of diet (Martin et al., 2010). Reduction of CH<sub>4</sub> emission (a greenhouse gas) from agricultural sources, representing 30–40% of total CH<sub>4</sub>, is a priority for both developed and developing countries. Reducing CH<sub>4</sub> production from ruminants can be of direct economic benefit because it is accompanied by greater energy use efficiency of the feed by the animal. Inclusion of feed additives of plant origin in particular plant secondary metabolites and oils in diets (Ben Salem et al., 2005; Jouany et al., 2008), feeding diets rich in unsaturated fatty acids (Martin et al., 2010), and modifying feeding practices and supplementing roughage-based diets with deficient nutrients have been investigated in some laboratories with the aim of reducing CH<sub>4</sub> emissions and increasing productivity (Martin et al., 2010). Dietary manipulations result in CH<sub>4</sub> reduction by decreasing fermentation of organic matter in the rumen (Martin et al., 2010) and shifting the site of digestion from the rumen to the intestines, diverting hydrogen away from CH<sub>4</sub> production during ruminal fermentation (Benchaar et al., 2001), inhibiting methanogenesis by ruminal bacteria (Moss et al., 2000) or by optimising rumen fermentation, thereby decreasing CH<sub>4</sub> emission/unit of organic matter digested.

### Tannins

The term 'tannin' refers to 'tanning' or preserving skins to create leather; tannins also contribute to the astringency of many popular drinks such as tea and wine. They are classified as:

- Hydrolysable tannins (HTs), which are potentially toxic and decrease the nutritive value of feedstuffs and thus have in general negative effects on livestock performance. In the Mediterranean area, these compounds can be found for example in *Quercus* spp. foliage. Although HTs have potential to bind feed proteins and increase the availability of proteins post-ruminally and thereby decrease CH<sub>4</sub> emission from ruminants, their *in vivo* effects have not been investigated;
- Condensed tannins (CTs), also known as proanthocyanidins, which are widespread in dicotyledonous species and occur infrequently in Graminaeae. They are present mainly in the foliage of a wide range of shrubs, trees and herbaceous roughages like Sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychis viciifolia*). CTs bind to proteins in the rumen, reduce protein degradation and when dietary crude protein (CP) concentrations exceed animal requirements for CP, these effects can improve performance (Min et al., 2003; Waghorn, 2008). However, when dietary CP levels are low and fibre concentrations are high, CTs are nearly always detrimental (Makkar, 2003).

In contrast to HTs, numerous studies have been carried out to identify and determine the bioactivity and study the effects of CTs (purified or CT-containing plants) on digestion (Min et al., 2003), productive and reproductive performances (Min et al., 2003; Waghorn, 2008) and product quality in ruminants (Vasta et al., 2008). It is now clear that depending mainly on their origin, level and structure, diet ingredients, animal species and physiological stage, CTs under specific conditions may have beneficial effects on ruminant performance (Ben Salem et al., 2005; Waghorn, 2008). Considering the objective of the current review, we report and discuss some examples illustrating the benefits from CTs in ruminant nutrition.

### CTs to Promote Protein Value of Feeds

Although generally regarded as antinutritional, certain tannins at low concentrations are known to alter rumen fermentation of carbohy-

drates and proteins and microbial protein synthesis to the benefit of ruminants (Min et al., 2003; Makkar et al., 2007; Waghorn, 2008). Since tannins are widely distributed in herbaceous and woody vegetation, identification of tanniferous feedstuffs having beneficial effects on ruminant digestion would provide useful means to exploit the use of such feedstuffs to improve efficiency of ruminant digestion.

Feeding a small amount of foliage from a the tanniferous legume shrub *Acacia cyanophylla* Lindl which is widespread in Tunisia with soyabean meal (SBM) resulted in a significant increase in the daily weight gain of Barbarine lambs (67 g/d vs 43 g/d) on oaten hay (Ben Salem et al., 2005). This positive effect was obtained at a total tannins:dietary protein ratio of 0.021 tannic acid equivalents (g/g protein) and when SBM (200 g/d) was given immediately after consumption of the entire amount of *Acacia* foliage (100 g/d) by animals. However, feeding *Acacia* and SBM at the same time or feeding SBM first and then *Acacia* foliage did not improve lamb growth when compared with animals on the control diet, i.e. hay + SBM. These findings were ascribed to decreased protein degradation and ammonia concentration in the rumen due to binding of tannins. Such a beneficial effect was also demonstrated on lambs and kids receiving oaten hay supplemented with fresh *Acacia* foliage and protein-rich concentrate (Ben Salem, unpublished). In a recent study (Ben Salem and Benyoussef, unpublished), lambs grazing on Sulla grew better than those on the same pasture but drenched with polyethylene glycol, MW 4 000 (PEG), a tannin deactivating reagent. Sulla contains moderate levels of CTs and at the same time is relatively high in CP. Therefore, the binding effect of Sulla-CT with proteins could explain the improved performance of lambs.

This strategy of feeding small amounts of CTs and dietary protein is simple and can promote the livestock sector under smallholder conditions by targeting the choice of plant species and management of grazing animals. For example, animals could be allowed to graze in an *Acacia* plantation (CT source) for a short time and then transferred to a grass dominated pasture (protein source, e.g. lucerne) for a longer period.

### CTs to Control Gastrointestinal Parasites (GIP) in Ruminants

The presence of GIP disturbs mainly protein metabolism (Min et al., 2003), and along with reducing food intake, this may explain the decreased growth of ruminants harbouring high parasitic loads (Hoste et al., 2006). Treatment against GIP is necessary to improve the performance of such animals. Commercial anthelmintics are used mainly in the commercial sector but seldom by smallholders, particularly in developing countries; since the drugs are expensive and their indiscriminate use may lead to anthelmintic resistance in worm populations (Hoste et al., 2006) particularly in small ruminants. Currently, alternative solutions are being sought which address public concern for more sustainable production systems by relying less on the use of chemicals to improve feeding efficiency and livestock health. Nutritional manipulation of the host animal in order to improve host resistance and/or resilience to parasitic infections is a promising option.

Recent studies showed that the incorporation of CT-containing feedstuffs in the diet reduced GIP (e.g. Akkari et al., 2008). Nevertheless, the extent of the GIP decrease varied among these studies probably because different CT sources and levels, diet composition and animals were used. According to Hoste (2005), tannins might interfere directly with the biology of various nematode stages and could also indirectly improve host nutrition by protecting dietary proteins from ruminal degradation which in turn could modulate worm biology. Recent studies in France and Tunisia showed that repeated feeding of sainfoin hay (Hoste et al., 2006) and *Acacia cyanophylla*

foliage (Akkari et al., 2008) reduced faecal egg counts in kids and lambs, respectively. It seems that some other natural secondary compounds such as flavonol glycosides and sesquiterpene lactones could also have anthelmintic activity (Hoste et al., 2006), although to our knowledge this hypothesis remains to be investigated.

The feeding of tannins also changes the partitioning of nitrogen excretion in that less is excreted in the urine and more in faeces. This decreases the loss of nitrogen to the environment from manure. In addition, the rate of release of nitrogen from the manure of animals on tannin-containing diets is lower, which is also advantageous for crop production (Makkar et al., 2007). Lower CH<sub>4</sub> emission from ruminants on tannin-containing feed has also been demonstrated (Waghorn and Clark, 2006).

### Saponins

Saponins are glycosides of aglycone linked to one or more sugar chains. These have a wide range of biological activities. For example they are antiprotozoal (see reviews by Makkar et al., 2007 and Jouany and Morgavi, 2007), and they interact with mucous membranes and influence nutrient transport (Cheeke, 1999) and absorption (Jouany and Morgavi, 2007). Also, the detergent action of saponins kills rumen protozoa which could lower ammonia levels in the rumen thereby improving the efficiency of microbial synthesis (Jouany and Morgavi, 2007; Wina et al., 2005). Defaunation has been shown to have several advantages for ruminants. Suppression or elimination of protozoa may enhance the flow of microbial protein from the rumen, increase the efficiency of feed utilisation and thereby improve animal nutrition. Saponins are also known to increase the permeability of the intestinal mucosal cells (Jouany and Morgavi, 2007). Reduced CH<sub>4</sub> production from rumen fermentation by saponins has also been demonstrated in various studies (Martin et al., 2010; Wina et al., 2005).

The possible use of natural plant products as growth promoters provides cheaper, safer and more consumer-acceptable alternatives to synthetic compounds. Lately inclusion of saponin-containing plants in the diets of ruminants has received wide interest due to the positive effects listed above (reviewed by Makkar et al., 2007; Wina et al., 2005). For example, preliminary results from a research programme on the effects of saponin-containing feedstuffs in ruminant feeding indicate that the administration of a small amount (30–40 g/d) of fenugreek (*Trigonella foenum-graecum* L.) seeds, which are relatively rich in saponins, increased lamb growth and milk production in dairy ewes (H. Ben Salem, unpublished). *Agavae americana* (Cactaceae family) is also high in saponins (ca. 80 g/kg DM), and in a recent study in Tunisia, an *Agavae* extract (120 ppm saponins) increased the growth rate of Barbarine lambs (Nasri and Ben Salem, unpublished).

The overall positive effects of tanniferous and/or saponin-containing forages on feed efficiency and for controlling GIP, and thereby improving the productive and reproductive performance of ruminants should encourage the uptake of practical options for using plants containing these natural plant secondary compounds in grazing systems. These options offer promising solutions to reduce the use of chemicals in livestock production systems, enhance livestock productivity and decrease both emission of CH<sub>4</sub> and discharge of nutrients to the environment.

### Essential oils

Many plant extracts contain essential oils (EOs), which are naturally occurring volatile components responsible for their characteristic essence and colour. These secondary metabolites have antimicrobial properties that make them potential alternatives to antibiotics for manipulating microbial activity in the rumen. Therefore, plant-derived

EOs could be used for improving the efficiency of nutrient utilisation in ruminants and reducing negative impacts on the environment. Compared with tannins and saponins, *in vivo* studies on the effect of EOs on rumen fermentation and performances of ruminants are scarce, although some Mediterranean institutes have recently initiated research on this topic. Available data suggest that EOs have potential to improve nitrogen and energy utilisation and inhibit ruminal methanogenesis (Benchaar et al., 2008).

## Promising Fodder Shrubs and Trees

### Cactus

The popularity of cactus (*Opuntia* spp.) as a feed in dry areas of some regions and countries (North Africa, Ethiopia and northern Brazil, among others) is increasing. Characterised by a remarkable tolerance to drought conditions, high water use efficiency, a rapid dissemination and growth, a high biomass yield and multipurpose uses, cactus is a promising range species that can promote the livestock sector in dry areas and improve farmers' incomes. Cactus cladodes are high in soluble carbohydrates, calcium and β-carotene (Ben Salem and Abidi, 2009), but they are low in fibre and CP (Stintzing and Carle, 2005). Therefore, provision of fibre and protein sources is recommended when feeding cactus to ruminants.

The following approaches have been tested for increasing the nitrogen content of cactus cladodes:

- Gonzalez (1989) noted that the CP content of fertilised cactus was almost double that of unfertilised cactus (99 g/kg vs 55 g/kg DM). However, the option of fertilising cactus fields has not been accepted by farmers in many countries especially when cactus is cultivated for forage rather than fruit production. Farmers would prefer to use manure or fertilisers for fruit trees and/or vegetable crops;
- Breeding is another way to select nitrogen rich clones of cactus. Some selected clones of cactus (e.g. clone TAMUK accession 1270) contained higher than normal CP contents of 110 g/kg DM (Felker and Inglese, 2003);
- Radiation induced mutation of cactus cladodes is being tested in Tunisia to increase their nitrogen content (INRAT-IAEA);
- Solid state fermentation seems a promising microbial process to produce protein from cactus. The microorganisms (algae, bacteria, fungi and yeasts) are considered a source of cell protein. They grow rapidly and could be cultivated on diverse substrates like cactus, rendering them rich in protein. The fermentation of cactus with *Aspergillus niger* resulted in a 12.8% increase in CP content (Oliveira, 2001). Also, Araújo et al. (2005) reported a remarkable increase (up to 400%) in the proportion of protein (260 g/kg DM) in cactus cladodes fermented with yeast (*Saccharomyces cerevisiae*). This procedure of protein enrichment of cactus is technically interesting, but its economic benefit should be evaluated before diffusion at the farm level.

Cactus cladodes are low in total extractable phenols and total tannins, condensed tannins and saponins (Ben Salem and Abidi, 2009). However, they are remarkably high in oxalates. Oxalates are present in a wide range of spiny and spineless cactus cultivars and clones at levels ranging from 70–150 g/kg DM (Ben Salem et al., 2002). It is well documented that oxalates form complexes with some minerals mainly calcium and magnesium and the ingestion of high amount of soluble oxalates is toxic to animals. However, since most cactus oxalates are present in insoluble form, cactus feeding has little adverse effects on the animal (Ben Salem and Abidi, 2009).

Replacing concentrate feeds (i.e. corn or barley) with cactus cladodes had no effect on digestion, lamb growth and cattle milk

production and quality, provided that energy from concentrate feeds was replaced by the equivalent energy from cactus cladodes (Ben Salem and Abidi, 2009). Total replacement of corn and barley with cactus could be achieved without any negative effects. However, with forages such as hay, straw and silage the replacement level should not exceed 50% otherwise digestion, daily gain and milk production is impaired (Ben Salem and Abidi, 2009).

Cactus could be used as fresh, dried or ensiled material:

- Cactus cladodes are fed mostly fresh to cows, sheep, goats and dromedaries. In order to avoid material loss, it is recommended to cut cladodes into small slices (using knives or electric choppers) before offering to animals. Tegegne et al. (2005) concluded that compared with a control diet (without cactus) sheep performed better when a proportion of grass hay offered was replaced by fresh cactus. Milk production of dairy cattle was not affected when fresh cactus replaced 12–36% of sorghum silage (Wanderley et al., 2002). Also, total replacement of barley (300 g) by fresh cactus (ca. 3.5 kg) had no effect on hay intake, *in vivo* organic matter digestibility and nitrogen balance in male lambs and kids (Abidi et al., 2009);
- Cactus cladodes could be dried and ground, and the meal obtained used as a supplement feed for animals. Veras et al. (2002) reported that lambs on elephant grass hay supplemented with corn or cactus meal exhibited similar organic matter (OM) intakes and OM and neutral detergent fibre (NDF) digestibilities. Although data on the replacement value of cactus meal for common feedstuffs (e.g. concentrate feeds) are limited, the benefit:cost ratio of this alternative strategy should be studied before diffusion to farmers;
- Cactus ensiling has been evaluated at the laboratory level and to our knowledge this technique is still not adopted at the farm level. Çürek and Özen (2004) evaluated the nutritive value of cactus cladodes which were chopped (1–2 cm), wilted (DM content 35%) and then ensiled. Based on pH and organic acids content, the quality of this silage was found acceptable. However, its nutritive value was low. It might be advantageous to ensile cactus mixed with other ingredients. Abidi et al. (unpublished data) ensiled fresh cactus cladodes with olive cake and wheat bran. Replacing oaten hay with this silage had no effect on digestible nutrient intakes but decreased the average daily gain of concentrate supplemented lambs from 50 g to 37 g. Cactus ensiling seems a simple technique, but its adoption would depend largely on the benefit:cost ratio which amongst others should include the costs of technology transfer efforts.

In addition to feed shortage, water scarcity compromises livestock performances in dry areas. Because of its succulence, cactus could overcome this constraint. Indeed, ruminants do not need to drink water when receiving cactus cladodes (ca. 35 g DM/kg metabolic weight) (Ben Salem and Abidi, 2009).

### *Moringa oleifera*

*Moringa oleifera* Lam. (syn. *Moringa pterygosperma* Gaert.) is native to the western and sub-Himalayan parts of northwest India, Pakistan and Afghanistan and is now widely cultivated across Africa (e.g. Nigeria, Senegal, Tanzania), South America and Southeast Asia (e.g. Malaysia, Indonesia). This plant is grown intensively in plantations and produces over 100 tons of dry green foliage/ha with a protein content of 18–25% which has a biological value comparable with soyabean (Foidl et al., 2001). In addition, this foliage has a number of antioxidants (Makkar et al., 2007). Almost every part of this plant has value as food or feed. For example *Moringa* leaves are exceptionally

rich in pro-vitamin A, vitamins B and C, Fe and several amino acids (Fuglie, 2001).

The beneficial effects of *Moringa* leaves on milk production and growth are well documented (Foidl et al., 2001). Also, proteins in the meal have an antibiotic effect (Makkar et al., 2007) and hence have the potential to modify rumen fermentation. These proteins have also been shown to decrease degradability of feed proteins in an *in vitro* rumen system (Hoffmann et al., 2003), and could enhance the post ruminal protein supply. In addition, defatted seed meal is free of most plant secondary metabolites such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin and cyanogenic glucosides, but contains glucosinolates (Makkar and Becker, 1997). Use of the defatted kernel meal in the diet as an additive at a level of 4 g/d increased body weight of lambs (Ben Salem and Makkar, 2009), and kernel meal proteins also reduced CH<sub>4</sub> production from ruminants (Makkar et al., 2009; patent). These beneficial effects are attributable to the presence of cationic proteins in the meal (Makkar et al., 2007).

### *Jatropha curcas*

*Jatropha curcas* L. belongs to the family Euphorbiaceae, and is native to tropical America and grown throughout the tropics. It is a drought-resistant shrub or small tree, commonly called Physic nut. The seeds contain 27–40% inedible oil which is easily converted into biodiesel and complies with USA and EU standards. The plant is easy to grow and widely used for many purposes, e.g. erosion control, fencing, firewood, green manure, various medicinal uses, feed, and soap production (Makkar and Becker, 2009). Because of the quality of its oil, *J. curcas* has created immense interest as a possible bio-fuel crop, and its plantations are expanding in many tropical countries including those in semi-arid areas. It even grows in upper Egypt in the hot desert sand when irrigated with sewage water from the city of Luxor (Becker and Makkar, 2008). There are two genotypes of *J. curcas*, a toxic and a non-toxic one. To the best of our knowledge, the non-toxic genotype is found only in Mexico. Today's global production of *J. curcas* from plantations is negligible. However, it is believed that approximately 25–30 million ha are currently being established, largely with the toxic genotype.

The kernel meal obtained from the non-toxic genotype has a CP content of approximately 60% with good amino acid composition (deficient only in lysine compared with soybean meal), but it contains heat labile antinutritional factors such as trypsin inhibitor and lectins (Makkar et al., 2007). The heat-treated kernel meal from the non-toxic genotype has been demonstrated to be an excellent fish feed, and it is also expected to be an excellent protein source for other high yielding farm animal species. Phorbol esters are absent in kernel meal from the non-toxic genotype but present in high concentrations in the kernel meal from the toxic genotype (Makkar et al., 2007). The toxicity of kernel meal from toxic *J. curcas* is therefore attributed to phorbol esters. Recently detoxification of kernel meal from the toxic genotype has been achieved at the University of Hohenheim, Germany (Makkar and Becker, 2010; patent). The detoxified kernel meal containing 62–64% CP was fed to common carp. The performance of the group in which 75% of fish meal protein was replaced by the detoxified *Jatropha* kernel meal was comparable with the group in which 75% of fish meal protein was replaced by soybean meal, but was lower than the group fed 100% fishmeal. On the other hand, the performance of the group in which 50% of fish meal protein was replaced by detoxified *Jatropha* kernel meal was better than that of groups in which 50% and 75% of fish meal protein was replaced by soybean meal and similar to that of the 100% fishmeal group. Histopathological studies showed no abnormalities



in liver, intestine and spleen and various haematological and biochemical parameters in blood were in the normal range, suggesting that detoxified *Jatropha* kernel meal could replace 50% of fish meal protein in carp diet (Kumar et al., 2008). Similar results have been observed for trout and tilapia (Makkar, unpublished).

### Better Use of Agro-industrial By-products (AGIBPs)

Huge amounts of AGIBPs are produced by the farming and wider food industries, but the use of these resources for livestock feeding is still limited because of their geographical separation from animals and associated high transport and storage costs, alternative uses and the relative opportunity costs, and the low managerial capabilities of the farmer. Some technologies have been developed to increase the use of these unconventional feed resources in ruminant feeding e.g. Ben Salem and Nefzaoui (2003) described a number of formulae for AGIBPs-based silages, feed blocks and pellets, and their positive effects on livestock performance and on decreasing feeding costs.

#### AGIBPs ensiling

For farmers with transportation facilities and flocks located in close proximity to food industries such as olive oil and fruit juice extraction, appropriate ensiling is a promising technique for efficient use of AGIBPs in livestock feeding. Micro silos (e.g. plastic bags) could be used to ensile these AGIBPs.

#### Feed blocks (FBs)

Feed blocks manufactured by the cold process are made from a mixture of one or more AGIBPs (e.g. olive cake, tomato pulp, etc.), binder (e.g. quicklime, cement or clay), water and common salt, and urea with or without molasses. This technique was used in the 1930s to overcome feed shortages and droughts in Tunisia. Nowadays, it is used in more than 60 countries. Makkar (2007) summarised the experiences of some countries in manufacturing and using FBs. Depending on their composition, FBs are used to partially or totally replace common concentrate feeds offered to ruminants receiving low quality diets or grazing in degraded natural rangelands. They have a role in stimulating the digestion of low quality feed resources and their use has resulted in economic benefits to farmers. Ben Salem and Znaidi (2008) noted that lambs supplemented with 500 g concentrate and those supplemented with 125 g of the same concentrate and olive cake-based FBs had similar growth rates. Recently, some FB variants have been developed and used which incorporate vitamin E (AD3E) to improve the fertility of rams or PEG as a tannin-inactivating agent to increase the utilisation of tannin-rich browses and trees (Ben Salem and Nefzaoui, 2003). Also, medicated FBs containing anthelmintic agents such as fenbendazole, pineapple leaves, nematophagous fungi and tannins to control internal parasites have been used in some countries, for example Bangladesh, Vietnam, Malaysia and Australia, and minerals such as phosphorus, copper, selenium etc. have also been incorporated in FBs to mitigate their deficiency in the diets of cattle, yak and sheep (Makkar, 2007). The success of FB technology depends mainly on proper formulations and their favourable benefit:cost ratio. In addition, this technology has been most successful in regions with strong extension services and where FBs were prepared and promoted by private industry.

#### AGIBPs-based pellets

Conserving AGIBPs in the form of pellets is another promising option. Nefzaoui and Ben Salem (unpublished data) have developed olive cake-based pellets and determined their nutritive value. The formulation of these pellets was inspired by the ingredients forming the FBs,

being composed of olive cake, wheat bran, rapeseed meal, wheat flour residue, salt and minerals. The *ad libitum* intake of these pellets by sheep averaged 2.5 kg/d, and their cost was about half that of lucerne pellets which are imported by Tunisia and subsidised and which produced almost similar weight gains. Mechanisation, however, is necessary for making pellets.

### CONCLUSIONS

Several simple approaches are available for improving livestock performance under smallholder conditions. Many of these also reduce livestock mediated environmental pollution, and some increase the environmental sustainability of livestock production systems. The possible use of natural plant products as growth promoters provides cheaper, safer and more acceptable alternatives to synthetic compounds. Successful transfer of these alternative options to farmers will require participatory approaches involving a wide variety of stakeholders.

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