PHYSIOLOGY AND NUTRI-GENOMICS
Underpinning Animal Production

DEPARTMENT OF VETERINARY PHYSIOLOGY
College of Veterinary Science and Animal Husbandry
U. P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya
Evam Go-Anusandhan Sansathan, Mathura, 281001
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FOREWORD

Field of Veterinary Physiology stems to enhance the animal health and production. Physiology has always been a bridge to reduce the gap between Production and Health. Scientific studies conducted in this field has always been efficiently utilized for advance researches in veterinary sciences. In this present era of climate change and emerging issues like food security, the status of Veterinary Physiology needs reform and this XXII Annual conference of Society of Animal Physiologist of India and National Symposium can be a platform deliberate on the issue which is a need of the hour.

In the above context, I feel proud to state that this Department since its inception has conducted pioneer research in field of Physiology directed to promote health and Production and now is in direction to conduct need based research. This department has always been a visible platform for the eminent scientists and researchers and has contributed a lot for physiological arena in the country.

With an objective to disseminate the knowledge of recent researches conducted in field of Veterinary Physiology and Allied sciences, the faculty in the Department of Physiology has compiled the book ‘Physiology and Nutri-genomics: Underpinning Animal Production’ comprising Lead papers, Invited lecture on current topics of urgent need and abstracts submitted by the researchers, scientists and post graduate students associated with Veterinary Physiology throughout the country. I am pleased to go through the content of this book, which has covered diverse issues like environment physiology in preview of climate change, studies on reproduction, lead papers on recent researches digestive physiology, cardiovascular physiology, neurophysiology etc. This book will be of utmost importance for those engaged in advance studies to enhance animal production and shall generate new ideas and open newer prospects in field of advance physiological research.

I congratulate the editors and contributors for bringing out this useful publication and wish symposium a great success.

(A.C. Varshney)
Vice-Chancellor
PROLOGUE

It is a matter of great privilege that the Organisers of XXII Annual Conference of Society of Animal Physiologist of India and National Symposium on “Physiological and nutri-genomic interventions to augment food security and animal welfare” have compiled all the invited and theme lectures of the eminent scientists and speakers on various aspects of Veterinary Physiology especially nutri-genomic interventions for augmenting nutritional security and the compilation of entitled “Physiology and Nutri-genomics: Underpinning Animal Production”. Contributors have made an effort to address the challenges of nutritional security and augmented animal production through application of nutri-genomics. The book also covers various aspects of nutri-genomics, animal-human interaction and understanding of nutri-genomic basis of diseases.

I am sure this compilation of the invited lectures will prove to be a good study material for young scientists, teachers and will help researchers in area of physiology, biotechnology, animal production and animal reproduction.

I congratulate the Organisers of this Symposium for compiling the manuscript.

(Satish K. Garg)
Dean, COVSc & AH
Preface

Globally the livestock sector is highly dynamic. In developing countries, it is evolving in response to rapidly increasing demand for livestock products. Currently, livestock is one of the fastest growing agricultural subsectors in developing countries. Its share of agricultural GDP is already 33 per cent and is quickly increasing. This growth is driven by the rapidly increasing demand for livestock products and this demand is being driven by population growth, urbanization and increasing incomes in developing countries. Climate change projections for India suggest that temperature is expected to increase between 2.3 and 4.8°C and this rise in the environmental temperature may impair production through reduced growth, meat, milk and egg, impaired reproductive performance, imbalanced biochemical and physiological process of metabolism and immune response.

Efforts to combat the contemporary challenges of livestock production in present scenario are made by academicians, researchers and policy makers in the field of animal physiology, biotechnology and animal health. The coherent approaches from all the stakeholders of livestock production have been able to solve the impending problems of the end users i.e. livestock farmers. The challenges in livestock production system have compelled the animal scientists to explore the various avenues to ensure food security to Indian human populace.

The present book is aimed to bring together various views to design strategies to solve some of the problems associated with livestock farming in the present scenario. The selected theme is very much pertinent to the present day context and we hope that this information will upgrade the researchers and scientist.

The editors are thankful to all the teachers, scientists and policy makers for communicating their research findings. The publication brought out is undoubtedly a result of untiring efforts of all members of editorial group. We thank Dr. A. C. Varshney, Honourable Vice Chancellor, DUVASU, Mathura and Dr. S.K. Garg, Dean, College of Veterinary Science and Animal Husbandry for their timely help, moral support and critical suggestions in this endeavour.

Sarvajeet Yadav
Jitender Kumar
A. K. Madan
Brijesh Yadav
Mukul Anand
## CONTENTS

<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>Authors</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reproductive interventions to augment farm animal productivity</td>
<td>K.P. Agrawal</td>
<td>1-7</td>
</tr>
<tr>
<td>2</td>
<td>Endocrinological interventions to augment nutritional security</td>
<td>B.S. Prakash</td>
<td>8-13</td>
</tr>
<tr>
<td>3</td>
<td>Current physiological approaches and future thrust areas in augmenting sheep production</td>
<td>S.M.K. Naqvi, Davendra Kumar and Kalyan De</td>
<td>14-29</td>
</tr>
<tr>
<td>4</td>
<td>Poultry production: New milestone to achieve nutritional security</td>
<td>P.K. Shukla, Sujit Nayak and A. Arun Kumar</td>
<td>30-39</td>
</tr>
<tr>
<td>5</td>
<td>Applications of electrocardiography in physiological and clinical research</td>
<td>J.P. Varshney</td>
<td>40-44</td>
</tr>
<tr>
<td>6</td>
<td>Advances in Renal Dynamics associated with nutrition in chronic kidney diseases</td>
<td>H. S. Singh</td>
<td>45-50</td>
</tr>
<tr>
<td>7</td>
<td>Cardiovascular and hemodynamic changes in endotoxemia and physiological interventions for animal welfare</td>
<td>D.V. Singh</td>
<td>51-55</td>
</tr>
<tr>
<td>8</td>
<td>Scopes and limitations in stem cell therapy: IVRI experience</td>
<td>G. Taru Sharma</td>
<td>56-58</td>
</tr>
<tr>
<td>9</td>
<td>Future prospects of stem cells in intervention in livestock reproduction</td>
<td>Kumar Dharmendra and Yadav P. S.</td>
<td>59-65</td>
</tr>
<tr>
<td>10</td>
<td>Assisted Reproductive biotechnologies in camel</td>
<td>Sajjan Singh and S. K. Ravi</td>
<td>66-71</td>
</tr>
<tr>
<td>12</td>
<td>Climate change: Bovine productive, reproductive and adaptive performance and mitigation strategies</td>
<td>S.V. Singh, R.C. Upadhyay, O.K. Hooda, Beenam and A.K. Singh</td>
<td>85-95</td>
</tr>
<tr>
<td>13</td>
<td>Physiological adaptability of goats of arid and semi-arid climate of India in hot and cool periods of the year in shelters</td>
<td>Puneet Kumar</td>
<td>96-100</td>
</tr>
<tr>
<td>14</td>
<td>Cellular thermo-tolerance and gene expression in livestock</td>
<td>K S Roy and C S Prasad</td>
<td>101-105</td>
</tr>
<tr>
<td>15</td>
<td>Microbial manipulation for sustainable livestock production</td>
<td>J.P. Puri</td>
<td>106-110</td>
</tr>
<tr>
<td>16</td>
<td>Free radicals and phyto antioxidant in metabolic diseases- current status and future prospects</td>
<td>V. Leela</td>
<td>111-113</td>
</tr>
<tr>
<td>17</td>
<td>Nutrigenomics: Animal Science perspectives</td>
<td>J.P. Ravindra and C.G. David</td>
<td>114-122</td>
</tr>
<tr>
<td></td>
<td>Title</td>
<td>Authors</td>
<td>Page Range</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>18</td>
<td>Nutraceutical concept for gut health in poultry</td>
<td>A. V. Elangovan</td>
<td>123-128</td>
</tr>
<tr>
<td>19</td>
<td>Functionality of essential fatty acids in animal reproduction</td>
<td>S. Jayachandran and P. Selvaraj</td>
<td>129-132</td>
</tr>
<tr>
<td>20</td>
<td>Heat stress: As it affects nutrient digestibility and methane emission in ruminants</td>
<td>A. K. Madan and Brijesh Yadav</td>
<td>133-138</td>
</tr>
<tr>
<td>21</td>
<td>Physiology of muscular stress</td>
<td>S. K. Rastogi and R. Huozha</td>
<td>139-148</td>
</tr>
<tr>
<td>22</td>
<td>Regulation of corpus luteum function by locally produced growth factors in buffalo</td>
<td>Mahesh Gupta, S S Dangi, Gyanendra Singh, V P Maurya, Mihir Sarkar</td>
<td>149-156</td>
</tr>
<tr>
<td>23</td>
<td>Melatonin and female reproduction- Emerging concepts</td>
<td>J. Kumar and D.K. Swain</td>
<td>157-162</td>
</tr>
<tr>
<td>24</td>
<td>Status and prospects of meat production and export potential</td>
<td>M.K. Agnihotri and N. Rana</td>
<td>163-176</td>
</tr>
<tr>
<td>25</td>
<td>Manipulating the postpartum physiological and metabolic adaptation to augment milk production in bovines</td>
<td>Mahendra Singh</td>
<td>177-185</td>
</tr>
<tr>
<td>26</td>
<td>Stress and lactation : An overview</td>
<td>Sarvajeet Yadav and Mukul Anand</td>
<td>186-196</td>
</tr>
<tr>
<td>27</td>
<td>Recent advances in induced pluripotent stem cells (iPSC) for health management</td>
<td>Sadhan Bag, P S Mahapatra and Bhabesh Mili</td>
<td>197-203</td>
</tr>
<tr>
<td>28</td>
<td>Advances in Innate Immunity</td>
<td>Rashmi Singh and Ajay Pratap Singh</td>
<td>204-208</td>
</tr>
<tr>
<td>29</td>
<td>Physiological stress in poultry production</td>
<td>P.K. Shukla, Sujit Nayak and A. Arun Kumar</td>
<td>209-218</td>
</tr>
<tr>
<td>30</td>
<td>Time demands re-tuning of veterinary education (Special reference to Veterinary Physiology)</td>
<td>Jitendra Singh Bhatia</td>
<td>219-222</td>
</tr>
<tr>
<td>31</td>
<td>Veterinary Physiology – Paradigms of undergraduate and postgraduate teaching</td>
<td>R Kumar</td>
<td>223-224</td>
</tr>
<tr>
<td>32</td>
<td>Strategies for effective teaching and learning veterinary physiology</td>
<td>Sandhya S. Chaudhary</td>
<td>225-227</td>
</tr>
</tbody>
</table>
Livestock are a vital natural resource of livelihood security for hundreds of millions of people and the most economically important sub-sector of agriculture throughout the developing world. India had 218.18 million cattle, 93.77 million buffaloes, 57.96 million sheep, 123 million goats, 16 million pigs and 402 million poultry (year 2000). It ranks 1st in cattle and buffaloes, 2nd in goats, 3rd in sheep and 7th in poultry. 600 million rural people rely on livestock related activities for their livelihoods. Livestock not only provide poor people with work, food, income, traction, fertilizer and fuel but also act as catalysts that transform subsistence farming into income-generating enterprises, allowing poor households to join the market economy. Livestock also play a major role in disaster management. Livestock, poverty reduction, livelihood security and sustainable development are complementary to each other. Livestock systems, if managed properly, play an important role in alleviating hunger and counteracting environmental degradation. Well managed domestic animals can make agricultural systems in the developing world more productive and more sustainable.

The productive and reproductive efficiency especially of animals are complimentary to each other. There is great potential for increasing fertility and productivity of farm animals. According to a survey, fertility/productivity can be enhanced to 100% with three pronged approach, viz: 30% by improving reproductive management, 40% by use of modern reproductive tools and 30% by control of reproductive diseases. This paper describes different reproductive interventions to augment farm animal productivity.

**Reproductive Management:** Reproductive efficiency is determined by many different processes which results from interactions among genetic and environmental factors. The processes involved singly or in concert, include age of puberty/maturity, pattern of oestrous cycle and oestrous behavior, length of breeding season, ovulation rate/litter size, lactational anoestrus period/post-partum anoestrus, inter-calving period and reproductive lifespan. These traits are combined to measure breeding efficiency. The intercalving interval comprises of two components: 1) the service period and 2) gestation period. Where there is no room to reduce the latter, there is ample scope to cut down the former. Improved feeding and management can reduce the length of service period in animals.

**Adverse Climatic Conditions:** Environment is one of the determining factor in the production and reproduction of farm animals all over the world. Inborn production potentialities can be severely affected by exposure to adverse climatic conditions. Season affects the breeding efficiency in animals. Season of calving affects lactation performance, lactation length and calving interval. Winter calvers produce more milk in a lactation as compared to those calve in rainy season. Those who calve in rainy season have least calving interval. Oestrogen activity of some fodders also influences seasonal activity. Measures to ameliorate climatic stress help in improving reproductive efficiency. Such measures are very important in exotic animals.

**Effect of Nutrition on Reproduction:** Adequate nutrition is the prerequisite for proper functioning of the reproductive system in animals. Under feeding, over feeding, protein and vitamin deficiencies, imbalance of trace elements result in various reproductive abnormalities both in male and female. Poor nutrition during early life in female retards the onset of puberty. In parous cows, it leads to anoestrus, anovulation and metabolic disorders. During early pregnancy, moderate to severe under nutrition may lead to embryonic mortality and/or abortion. During late pregnancy it has an adverse effect on calf birth weight. A poor body condition score (BCS) at calving adversely affects milk production and fertility characterized by prolonged post-partum oestrus interval, reduced conception rate and more services per conception. The nutrient deficiencies, excesses and imbalances
during initial period of lactation can lead to several disorders, which affect the post-partum reproduction. A very low protein diet can cause cessation of oestrus, if fertilization occurs, foetal death or the birth of premature and weak offsprings results. The other common reproductive problem in lactating cows is cystic ovaries. High levels of grain feeding and plant oestrogens are associated with increased frequencies of cystic follicles. The plane of nutrition also has an influence on the performance of bulls. Ration containing low protein affects adversely semen volume quality. Continued feeding of low protein ration, besides affecting quality and quantity of semen also affects sex libido.

**Lack of Forces of Selection:** The main factor responsible for poor production is lack of forces of selection as a result of which the poor yielders do not get out of the population and hence contribute offsprings from generation to generation. Slaughter is being used throughout the world to restrict an increase in population of undesirable livestock. The need for slaughter is also important if there is a programme of genetic transformation of the population through crossbreeding. In view of the religious taboo and legislative restrictions on the slaughter of the cows in many states of India, the poor yielders do not get out of the population, and contributes offsprings from generation to generation. These low yielding cows compete with better stock in respect of feeding, housing and other managerial necessities.

**Other Factors:** Other factors which adversely affect oestrous exhibition after calving include almost total lack of weaning practice (even in most organized farms) and inadequate supply of nutrients to the dam. Suckling is one exteroceptive stimulus that leads to extended and variable period of anoestrus and anovulation following calving. It also increases the incidence of spontaneous short cycles, associated with short duration and reduced intensity of oestrus. The adverse effects of suckling are further aggravated during summer months.

**Reproduction Tools:** During the last two decades, farm animal reproduction has entered into an era of a new biotechnology revolution which includes artificial insemination, induction and synchronization of oestrus (manipulation of breeding cycle), superovulation, embryo transfer, cryopreservation, embryo resource development, sexing, transgenesis, cloning, chimera production and transgenesis. Some of these technologies have immense potential to revolutionize world animal agriculture in the twenty first century.

**Embryo Transfer:** Application of embryo transfer includes its use in genetic improvement through selection of parents, multiplication and mechanical manipulation of superior germplasm using genetically unreliable mothers as foster mothers for embryos of superior genetic make up, preservation/conservation of threatened breeds, more rapid propagation where a limited number of individuals exist, production of specific pathogen free (SPF) population and movement of germplasm. Successful embryo transfer involves several steps, viz: selection of donor parents and recipients, superovulation of donors, breeding of donors, synchronization of oestrus in recipients, embryo collection, embryo evaluation, embryo processing, embryo storage, embryo transfer, post-transfer care of recipients and early detection of pregnancy. Failure to comply strictly with any one of these steps will lead to disastrous results.

**Induction and Synchronization of Oestrus:** For satisfactory pregnancy rate in an embryo transfer programme, the embryo must be placed in an environment that simulate the one from which it is removed. Therefore, synchronization of oestrus in donor and recipient in each set of experiment is considered essential to obtain the best results. Two alternate approaches are used for induction and synchronization of oestrus. The first is by artificially extending the luteal phase (using progestational compounds) and second by inducing the demise of the corpus luteum (using prostaglandins and their analogues). Progestogen and prostaglandin in combination are also used. During the non-breeding and anoestrus season, treatment of the females to induce oestrus and ovulation is similar to that described for the breeding season. Progestogens with some possible adjustment in PMSG and/or FSH
are used for anticipated lower response. PGF$_2$ alpha in double dose schedule (10-11 days interval) can be used to cover those animals who do not have functional CL at first injection.

**Superovulation:** Superovulation is one of the major reproductive technologies for rapid genetic improvement of the Livestock. Unfortunately, high variability in the ovarian follicular response to gonadotropin treatment continues to be the major problem. Considerable inconsistency in follicular response after superovulation treatment has been observed in Zebu cattle and buffaloes as compared to results observed in Bos taurus. Regulation of reproduction by immune intervention is fairly a recent development. Immunization by raising antibodies against reproductive hormones has been used to augment reproductive efficiency in livestock. Passive immunization against PMSG to prevent ovarian over-stimulation in superovulated animals has yielded good results. Immunization against inhibin has shown promising results for increasing ovulation and litter size. Possible application of immunomodulators and/or cytokines for increasing uterine defence in case of microbial invasions and to monitor embryonic and fetal development are currently under intensive investigation. Immunomodulation may be considered as an alternate therapeutic agent in clinical and sub-clinical uterine infection. Trophoblastic protein(s) or interferon purified recently in some of the species has potential application in regulation of fertility.

**Embryo Resource Development:** In vitro production (IVP) of embryos has considerable potential value in disseminating genetic improvement and shortening the generation interval (6.28 to 3.25 yrs in cattle) as compared to programme based on progeny testing. Identification of various factors that could affect oocytes yield and quality, in-vitro maturation (IVM) and in-vitro fertilization (IVF) is important for producing blastocysts of high genetic merit.

**Oocytes yield and quality:** The recovery of large number of oocytes with high developmental competence remains an ultimate goal for the mass production of embryos. Harvesting techniques affect the oocyte recovery rate. Higher oocyte recovery rate is possible by slicing of ovary compared with follicle puncture or aspiration. Few studies recorded higher recovery rate with individual follicle isolation when compared with aspiration or slicing.

**In-vitro oocyte maturation (IVM):** The culture conditions employed in IVM not only affect the proportion of oocytes that reach metaphase II (M II) and are capable of in vitro fertilization but can also influence subsequent embryo development. The culture conditions influencing oocytes maturation include maturation medium, source of serum, supplementation with hormones, growth factors and follicular fluid etc.

**In-vitro fertilization (IVF):** The medium employed in IVF system must provide suitable conditions which is congenial for sperm penetration. Relative to cattle, buffalo sperms have poor fertilizing capacity and low viability after freezing. Addition of caffeine and heparin in medium used in IVF systems helps in inducing capacitation and acrosome reaction.

**Cryo-Preservation:** Cryo-preservation of embryos has become a routine procedure for success of embryo transfer in livestock species. The methodology is used to freeze and store surplus embryos recovered from elite animals, IVF derived, transgenic and cloned embryos. The methodology is also used to freeze and store oocytes for in-vitro fertilization at a later date. Several methods have been developed to cryo-preserve embryos such as slow cooling, fast cooling, slow warming, fast warming and vitrification etc. Vitrification because of its low cost and simple approach may ultimately take ET into field. Cryo-preservation of embryos has multifarious application, viz: relieves for having simultaneous synchronization of oestrus in donor and recipient, easy and safe transport of valuable germplasm around the World, conservation of superior genetic material, protection of valuable strains of experimental animals against possible loss through disease, accident or genetic drift, possibility of shortening the generation interval for progeny testing programme.

**Sexing:** A sex ratio favouring males is more desirable in meat breeds because of the faster growth and lean carcass and females in dairy breeds. There are two approaches for the control of sex
of offsprings: 1) regulation of sex of embryos and 2) identification of sex of embryos. Regulation of sex of embryos are based on i) sperm separation, ii) parthenogenesis iii) nuclear transplantation and iv) removal of pronucleus. Identification of sex of embryo involves removal of trophoblastic cells by biopsy/bisection of embryos. The sex of the biopsied cells is determined by i) karyotyping, ii) identification of sex chromatin, iii) assay of sex-linked enzymes, glucose – 6 phosphate dehydrogenase, iv) identification by DNA probes or Y chromosomes and v) serological determination of H-Y antigen.

**Cloning:** The ability to produce multiple copies (clones) of outstanding animals has potential significance to the livestock industry. Clones can be produced by 1) embryo splitting, 2) blastomere separation and 3) nuclear transfer. Identical twins have been produced using approach 1 and 2 but production of more than 2 clones is difficult due to low cell numbers at the blastocyst stage. Third approach is through transfer of embryonic cell nuclei. In this approach, the single blastomere of preimplantation embryo is fused with the zygote whose pronucleus has been removed. A number of sheep, bovine, pig, mice, rabbit and rhesus monkey have been produced with this method. The number of clones produced, however, is limited to less than ten. The other approach is the somatic cell nuclear transfer. A large number of cloned sheep, calves, goats, pigs and mice have been produced after nuclear transfer of various somatic cells from mammary gland, cumulus, oviduct, skin, ear, muscle, liver, tail or sertoli cells during last 4-5 years.

**Chimera:** Chimera is a composite animal having cells from more than one cell line. The main objective is to combine the best genetic traits of each donor in the chimeric animal. Sheep-goat chimera has successfully been produced. Successful chimera production requires perfection of several micromanipulation techniques, viz: i) separation of blastomeres, ii) aggregation of blastomeres of two different sources, iii) separation of inner cell mass from trophectoderm, iv) micromjection of ICM into blastocoele and v) study of development and physical behaviour of chimeric animals.

**Transgenesis:** An animal whose genetic composition has been altered by addition of foreign DNA is said to be transgenic. The DNA that is introduced is called a transgene and the overall process is called transgenic technology. Transgene may be cloned genes, composed of deoxyribonucleic acid (NDA) from microbes, animals, or plants. The technology affords methods that allow the transfer of genes between different species.

Transgenic animals are of particular interest in relation to growth, production traits, reproduction and disease resistance. The production of pharmaceutical proteins and nutraceuticals (genetically engineered whole milk) promises immediate application. A variety of blood-borne clotting and anticoagulating products (tissue plasminogen activator, Factors VII, IX and X, Protein C, Anthrombin III. And fibrinogen) have been manufactured in the mammary glands of transgenic bioreactors that includes goats, pigs and sheep. Other products for transgenic bioreactors include human hemoglobin (pigs) human α-anti trypsin (sheep), human lactoferrin and human lactalbumin (bovine), α-glucosidase, antibodies for anticancer agents, collagen for rheumaitoid arthritis treatment cystic fibrosis transmembrane conductance regulator for treatment of cystic fibrosis, and serum albumin to assist with control of blood pressure and as burn treatments. Cattle are difficult species to use for production of transgenic products because limitations of zygotes availability. The species monotocuous, also limits the number of embryos that can be transferred to a surrogate mother to carry to term.

**Production of Transgenic Animals:** The three principal methods used for the creation of transgenic animals are DNA microinjection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer. The steps of transgenic animal production are identification of genes of desired characters, construction of gene construct, laboratory production of zygotes/embryos at pronuclear stage, nuclear/gene transfer and culture of zygote/embryo after gene transfer and assessment of DNA incorporation using dot blot hybridization techniques. Mice because of their small size, low maintenance cost, short generation time and their fairly well defined genetics, have become
the main species used in the field of transgenics in comparison to that for larger vertebrates. Methods for transfer of genes into the genome of cattle, sheep, swine, rabbits and rats are similar to those for mice. The principal differences, however are: 1) the eggs and recipient animals are more expansive, 2) zona pellucida is difficult to penetrate and 3) eggs of cattle and swine are opaque and must be centrifuged to clear the cytoplasm.

**DNA Microinjection:** The method is widely used for producing transgenic mice. The method involves the direct microinjection of a chosen gene construct (a single gene or a combination of genes) from another member of the same species or from a different species, into pronucleus of a fertilized ovum. Micromanipulators on a specially equipped microscope are used for this purpose. A glass pipette drawn or pulled to a fine point is used to immobilize the embryo on one side. On the opposite side, the foreign DNA (cloned gene) with a second finely drawn injection needle is injected into either of two pronuclei of the embryo. After the injection, the embryos are transferred back into the pseudopregnant recipient females or foster mothers. About 1 to 4 percent of the injected embryos result in transgenic offsprings. A major disadvantage of this method is lack of applicability to a wide variety of species.

**Embryonic stem cell-mediated gene transfer:** The method involves microinjection of embryonic stem (ES) cells derived from the inner cell mass of blastocyst-stage embryos (about 7 days postfertilization) into embryos to produce "hybrid" embryos of two or more distinct cell types (chimeric animals). Once isolated, ES cells may be grown in the lab for many generations to produce an unlimited number of identical cells capable of developing into fully formed adults. These cells may then be altered genetically before being used to produce embryos. When these transformed cells participate in the formation of sperm and eggs, the offspring that are produced will be transgenic. The method is very promising for producing transgenic mice. ES cell lines for other livestock species such as swine, cattle, and sheep.

**Retrovirus-mediated gene transfer:** In this method, gene transfer is mediated by means of a carrier or vector, generally a virus or a plasmid. Retroviruses are commonly used vectors to transfer genetic material into the cell. Offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus. Transgenesis is possible only if the retrovirus integrates into some of the germ cells.

**Sperm Mediated Gene Transfer:** This is very recent and a practical technique for obtaining a transgenic embryo. As a significant improvement over previous methods, this technique allows greater control of a desired transgenic outcome and promises to be a reproducible method of multispecies transgenesis. The method comprises two fundamental steps: 1) co-injecting exogenous DNA and a membrane disruptive sperm head into an unfertilized oocyte, and 2) facilitating the development of this fertilized oocyte to develop into a transgenic embryo. Transgenic mice have been produced following the addition of DNA to mouse spermatozoa that are subsequently used to fertilize eggs in-vitro. The technique can be used in all animal species with high accuracy, high efficiency and unparalleled ease of use. However, these results could not be repeated.

**Disease Transmission:** Diseases of livestock can cause major economic losses, both to livestock owners and the country as a whole. Inadequate health management, lack of control efforts and lapses during transport of germplasm from one country to another have resulted in large number of deaths throughout the world.

For breed improvement in livestock throughout the world, the exotic germplasm is introduced from one country to another either as live animal, frozen semen or embryo. There has been considerable concern about the semen and embryo quality and their impact on disease transmission. Advent of cryopreservation of semen and embryo as an adjunct to AI and ET and import/export of germplasm had made the users to re-think about disease transmission problems. Survival of many pathogens under cryopreservation process and after the addition of antibiotics to the media used for semen and embryo, reawakened interest in this area and led to health regulations being developed for
global movement of semen and embryos. Infectious agents transmissible through semen and embryos have been discussed in present communication.

Semen is a potential source of several agents, viz: infectious bovine rhinotracheitis virus, infectious pustular vulvo-vaginitis virus, bovine viral diarrhoea virus, mucosal disease complex virus, foot and mouth disease virus, bluetongue virus, African swine fever virus, porcine parvovirus, brucella abortus, chlamydia, trichomonas, toxoplasma spp., leptospora spp., mycoplasma spp., campylobacter spp., ureaplasma spp., mycobacterium spp. There is great potential for the spread of infectious disease to large number of females through artificial insemination because of several reasons: 1) using single infected ejaculate after dilution in large number of females, 2) allowing source of infection for a long time if infected semen is frozen for future use and 3) bypassing the natural defences of the female vaginal and cervical mucosa against invading micro-organisms. There is also possibility of semen contamination by micro-organisms present in the atmosphere, on the body of teaser and donor animal, in unsterilized equipments, in the semen extender, and in case of frozen semen, from liquid nitrogen or its containers. On the other hand, Al if carried out under strict sanitary and controlled conditions, provides an excellent means for disease control because: 1) when semen is diluted, the number of organisms per ml of inseminating dose is decreased approximately ten fold which makes the number below the minimum infective dose, 2) the technique avoids animal to animal contact, 3) collection of semen from clinically healthy and disease free donor ensures earliest and safest approach for obtaining semen that is free from infectious disease causing organisms, 4) possibility of testing the ejaculate for the presence of micro-organisms prior to use.

The disease transmission potential of embryos is much less than that of semen. A few characteristics of embryo, viz: presence of zona pellucida and secondary resistance factors such as young age, small size and limited mobility serve to reduce the potential exposure of embryos. It is difficult for bacterial and/or fungal agents to penetrate the zona pellucida to gain access to the embryonic cells. Thus infectious agents that are of concern in embryo transfer are mostly viral rather than bacterial or fungal. Pathogens through embryo transfer are transmitted either within or on the embryo or in fluids transferred with the embryo. Transmission through embryo association occurs under the following circumstances: 1) If the pathogen is in the ovum at the time of fertilization, 2) if the pathogen is carried into the ovum at fertilization either within or attached to the spermatozoa, 3) if the pathogen passes through, becomes embedded in, or adheres to the surface of the zona pellucida following fertilization and 4) if the pathogen reaches the embryo as a result of damage to the Z. P. during filtration, freezing or micro-manipulation. Other factors which help to reduce the potential of early embryos to transmit disease are inherent in the techniques that are used in the collection and processing of embryos. For instance, embryos are flushed from the uterus of the donor with a considerable volume of fluid which helps to dilute the pathogens that might be present in the uterus. In addition, freezing has been found very effective in inactivating low levels of many viruses. Washing and enzyme treatment also help to control the spread of diseases.

Farm Animals of 21st Century:

- Robust animals with higher growth by adding double muscling and growth genes to the Y-chromosome.
- With development of successful in-vitro oogenesis and spermatogenesis, the need to keep animals for breeding purposes will be eliminated.
- Animals exhibiting early puberty/maturity and shorter gestation.
- Animals with higher rate of ovulation to produce twins in cattle, sheep and goats, large litters in swine and daily ovulation in chickens.
- Animals resistant to diseases and other stresses.
- Animals producing high protein milk, low fat meat and poultry producing low cholesterol eggs.
- Animals with change in metabolic pattern. A sounder strategy would be to start milking a cow at 1 ½ years of age and continue for five years without the annual cycle of reproduction including dry periods.
- Animals with changes in biologic characteristics, e.g.: circumventing seasonal breeding, change in gestation lengths improving fertility etc. may be evolved. Other useful characteristics include production of males that produce only X-bearing sperm. Analogously, chickens may be available that produce Z- or W- chromosome-bearing eggs.
- Creation of new species/hybrids. In future, new species will be created, possibly extinct ones recreated and probably new hybrids will come into use.

It is expected that by 2050, animals genetically engineered for rapid growth, lean carcasses, high milk production, improved reproduction performances and increased disease and stress resistance will be commercially available. In addition, transgenic animals capable of producing appreciable quantities of specialized pharmaceutical products, including drugs, hormones, enzymes and growth factors will also be available.
Endocrinological interventions to augment nutritional security

B.S. Prakash
ADG (Animal Nutrition and Physiology), ICAR, Krishi Bhawan, New Delhi

Livestock serves as one of the main pillars of India’s agrarian economy, food and nutritional security and livelihood. Its ownership is highly egalitarian and the growth potential is highly pro-poor. India possesses the highest cattle population of around 199 million in the world (15% of the total world’s cattle population). Buffaloes with 105 million population form a third of the total cattle and buffalo livestock bovine population and contribute more than 55% of the milk. Farmers prefer buffaloes over cattle, particularly in Northern and North-West India as it is a triple purpose animal contributing milk, draught and meat as compared to the dual purpose native cattle. The buffalo milk also fetches higher price to the farmer due to higher fat content. It can utilize poor quality roughages and is capable of adjusting to wet conditions better than cattle. Sheep and goat are also important livestock species in India, especially in the arid/semi-arid and mountainous areas. While sheep are mostly reared for wool and meat, goat provide both milk and meat. Backyard pig farming systems is also practiced as part of the mainstream farming in Kerala, Goa, North-Eastern States and by socially weaker sections and tribals in Jharkhand solely for meat. The domestic demand for livestock products is going to increase substantially in the years to come. Additionally, there is a good export potential for livestock products. In order to meet the domestic and export demand the production from livestock sector need to be targeted for rapid growth. Good reproductive performance is essential for efficient livestock production. Livestock improvement programs should aim to increase reproductive efficiency to the extent that this can be justified economically. The females must grow rapidly to attain sexual maturity, initiate estrous cycles, ovulate and be mated by fertile males or inseminated with viable semen at the proper time for producing offspring. Improved buffalo and zebu cattle production in particular, could significantly enhance the economy and living standards of farmers in India. There are at least 30 different zebu breeds in India. Sahiwal, Red Sindhi, Gir, Kankrej and Tharparkar are predominant dairy breeds. The average lactation yield of these breeds is around 1800 litres in a 305 days lactation period. However, most of the cattle in India are of the nondescript types which yield very little milk. In an effort to increase milk production, cross breeding of zebu with exotic breeds has been carried out. The average milk production over a 305 days lactation period of milch buffaloes ranges from 1500 – 2500 litres. Better adaptation of buffalo and zebu to tropical climates and disease resistance ensures their place in the future of world agriculture facing the challenge of global warming due to climate change. Perusal of livestock census of 1992, 1997 and 2003 reveals that there has been a significant decline in cattle population from 204.58 million in 1992 to 198.88 million in 1997 and 185.13 million in 2003. Interestingly, there has been a consistent increase in buffalo population from 84.21 million in 1992 to 105.34 million in 2007 indicating preference for buffalo rearing among farmers. With an overall 127 million tonnes of milk production in 2011-12 from cattle, buffaloes and goats and a per capita milk availability of 290 g/day the Indian Dairy scenario is constantly looking ahead and promises to take greater strides in making Dairying more remunerative to the farmer. However, there are serious bottlenecks in our quest for making livestock rearing a profitable venture. An important issue is flagged under. Anestrous and repeat breeding in buffaloes and bovines are two of the most serious reproductive problems affecting 30-40% of the total cattle and buffalo population. On a conservative estimate the country is losing 20-30 million tonnes of milk annually on account of anestrus and repeat breeding in cattle and buffaloes which translates to a loss of nearly Rs. 50000 crores annually. At a micro level, each missed heat is a missed opportunity. For each heat missed the farmer incurs a loss of milk production of 21 days, in addition to bearing the feeding cost for animal maintenance. This tantamounts to about Rs. 3300. Artificial insemination (AI), which is a normal practice in cattle, is not as successful in buffalo, especially in hot summer months, because of the weakness of oestrus symptoms and the variability of oestrus length, which make oestrus detection
very difficult. The usual weak symptoms of estrus in the normal breeding season (September to February) become even weaker during hot months of summer (Prakash 2002). The incidence of silent heat among buffaloes was lowest in December (10.5%) while the peak was seen in the hot summer month of April (70%; Prakash et al. 2005). Failure to detect estrus and time of onset of estrus in buffalo considerable percentage of oestrous cycles are left uncovered resulting in increase of unproductive period which adversely affect economics of livestock production. Several studies have attempted to understand the reproductive physiology of buffalo and the factors affecting its behaviour. These have been adequately reviewed (Madan and Prakash, 2007). During the last two decades, considerable attention has been focussed on reproductive endocrinology, with the aim of developing models to improve reproductive efficiency, particularly when using controlled breeding techniques.

More than 80% of our bovine population is still non-descript and the breed improvement programs have hardly taken off. Conception rate for artificial insemination (A.I.) is very poor. A.I. facilities are not available at the farmers’ doorsteps. Semen available for A.I. is not of required quality. There is shortage of qualified para-veterinarians. We have learnt a lot from our past failures. Farmers must be provided incentives to use A.I. program. An A.I. program should always be an essential part of an integrated livestock development programme for obtaining high yielding livestock, and hence, should never be implemented on its own.

**Reproductive Technologies:** The various potential reproductive technologies which hold promise are listed below.

**Field application of progesterone determination for fertility augmentation:** Since progesterone is secreted from the corpus luteum, determination of progesterone in body fluids such as plasma and milk is a good marker for determining the functional status of tissue. Of the steroid hormones known to be synthesized by the bovine ovary, it is the varying concentrations of progesterone in body fluids which has up to present yielded most information of ovarian functions. Progesterone determination in plasma or milk can therefore serve as a valuable diagnostic tool in buffaloes for:

i) Accurate estrus confirmation and hence correct timing of A.I.

ii) Diagnosis of pregnancy/non-pregnancy 20-24 days post A.I.

iii) Identifying ovarian conditions such as acyclicity, silent heat, and cystic ovarian disorders.

iv) Control of certain biotechnological manipulations like embryo transfer, estrus synchronization, parturition induction etc.

Suitable treatment can be administered for anestrum, repeat breeding and cystic ovarian conditions, if the animals are systematically monitored for ovarian activity by milk or blood plasma progesterone determinations.
Pregnancy confirmation through oestrone sulphate determination: Oestrone sulphate has been found to be quantitatively one of the major oestrogens in the milk and blood plasma of pregnant, lactating cows and buffaloes. During the first half of pregnancy its concentration increases gradually in these animals so that after 110 days of pregnancy it is present in all milk samples taken from pregnant cows and buffaloes, whereas it is low or undetectable in non-pregnant animals. These results adequately suggest the practical applicability of using estrone sulphate estimations in body fluids (milk or blood) for confirmation of pregnancy and fetal viability in bovines after 110 days post-insemination since it is low or undetectable in non-pregnant animals. Under normal circumstances a pregnant cow exhibits an exponential increase in estrone sulphate levels in blood and milk with advancing pregnancy which is indicative of fetal viability. Undetectable estrone sulphate concentrations in milk during second trimester or advanced pregnancy could indicate embryonic loss, fetal death or mummification of fetus.

Estrous behaviour: Reproductive efficiency among large ruminants is greatly dependent upon the detection of estrus. This is even more important with reference to small herds managed under tropical or subtropical environments because high air temperatures shorten the duration of estrus and lower its intensity as demonstrated under controlled environments in cattle. The intensity of estrous behavior in tropical buffaloes has been found to be much less than cows. The usual weak symptoms of estrus in the normal breeding season (September to February) become still weaker during the hot months of summer. Among Murrah buffaloes we have recorded diurnal patterns of estrous behavior with 59% of estruses detected between 10pm and 6am. The maximum occurrences of various heat symptoms were seen in the winter months of November to February while the lowest occurrences were during March to August in a selected group of buffaloes observed throughout the year. Out of the 8 major symptoms of estrus, 5 symptoms that are, vulval engorgement, frequent urination, bellowing, bull mounting and restlessness contributed to 85 percent of the total observations. Mucus discharge, licking of the female by the bull and chin resting by the bull were minor symptoms. During the summer months frequent urination was the most prominent heat symptom recorded.

Season of calving had a profound influence on the service period. We observed that the mean service period of animals calving from December till June was more than 140 days and was significantly higher than mean service period of animals calving in the months of July to November (<110 days). The high service period of buffaloes in the former group of animals was attributed to the high incidence of silent estrus, which the animals would exhibit in the summer months once they commence cycling postpartum. The effect of different seasons on both the resumption of ovarian activity and embryo survival may be a function of temperature and/or photoperiod.
Timing of insemination, estrus synchronization and timed A.I.: We investigated the timing of ovulation following spontaneous estrus which information is essential for correct timing of AI in buffaloes.

On the basis of our observations we also suggest following A.I. timings in buffaloes exhibiting spontaneous estrus.

- Double Insemination
- First Insemination – 24 h after onset of heat.
- Second Insemination – 36 h after onset of heat.

Scientists, world over, are now working on developing new estrus synchronization protocols which can reduce the ovulation time window post synchronization so as to practice insemination at a fixed time thereby obviating the need for heat detection which is a serious problem especially in buffaloes. An estrous synchronization protocol (Ovsynch Protocol) in cattle has been developed recently; it makes the use of a combination of GnRH - PGF$_{2\alpha}$ - GnRH injections which has been reported to considerably narrow down the ovulation time to a range of 24 hours to achieve the maximum conception rate with set time artificial insemination.

Ovsynch protocol: Our innovative research work has yielded a promising technology which could help augment fertility in buffaloes by bringing anestrous animals into cyclicity and/or making them pregnant. This technology named Ovsynch protocol involves the combination of GnRH-PGF$_{2\alpha}$-GnRH injections regime to synchronize estrus and ovulation. An injection of GnRH (Receptal; 10μg intramuscularly) is administered at a random stage of estrous cycle (or anestrous or repeat breeding condition) followed by an injection of PGF$_{2\alpha}$ (Lutalyse; 25mg intramuscularly) 7 days later. Ovulation is synchronized by a second injection of GnRH (Receptal; 10μg intramuscularly) given 2 days after PGF$_{2\alpha}$. The animals are then inseminated at a fixed time of 12 h and 24 h post second GnRH injection. The first injection of GnRH induces ovulation of dominant follicle and causes emergence of a new follicular wave. The PGF$_{2\alpha}$ injection induces regression of the spontaneous and / or GnRH induced corpora lutea, and the second GnRH injection synchronizes the time of ovulation of the dominant follicle of the follicular wave that began growing after the first GnRH injection. Buffaloes are subjected to set time artificial insemination after synchronized ovulation. The animals are inseminated if they do not settle and return to estrus during the subsequent cycle.

This technology viz. the ovsynch protocol for estrus synchronization and set time A.I. was initially successfully demonstrated in cycling (Paul and Prakash, 2005) and non-cycling anestrous/repeat breeding buffaloes (Roy & Prakash, 2009a, 2009b) in the NDRI farm. Subsequently in association with the KVK of the Institute technology has also been tested on village buffaloes. Initially a limited preliminary field trial conducted in the villages, 24 buffaloes became pregnant out of 60 (40%) while another 28 anestrous buffaloes became cyclic (47%) thus making a healthy 87% positive response for augmenting fertility among anestrous buffaloes. In another exhaustive trial on village buffaloes we obtained 67 pregnancies out of 131 buffaloes while another 38 anestrous buffaloes becoming cyclic giving an overall succeed rate of 87% again (Prakash et al. 2008).

Cost of treatment: The cost of ovsynch treatment (calculated for the second trial on 147 buffaloes of which data was available from 131 buffaloes) was 520 per animal (cost of drugs 450 + 15% miscellaneous expenses which includes petrol, labour, and disposables etc). Total expenditure incurred in the project for the treatment of buffaloes was 76,440. On an average, the buffaloes which became pregnant were non-pregnant for 276 days before treatment. Assuming a feeding cost of 50 per animal per day (this study is a few years old) the total loss to the farmers was 9.2 lacs. Assuming an average production of 5 litres of milk/d, the milk production loss incurred by farmers amounts 16.65 lacs taking the total losses to the farmers to 25.9 lacs. Hence the cost benefit ratio of technology
works out to be 34 times without taking into account the loss of calves and labour costs during this period.

**Heatsynch protocol:** During the last few years the estrus synchronization protocol called heatsynch in buffaloes has been developed which makes use of a combination of GnRH-PGF$_{2\alpha}$- Estradiol benzoate injection followed by fixed time AI. Estradiol benzoate is a less expensive hormone in place of second GnRH injection of Ovsynch protocol. The major advantages of Heatsynch are reduced hormone costs and somewhat easier scheduling and implementation, since all injections and A.I. are at 24 and 48 h interval in cows. We investigated the efficacy of Heatsynch protocol in buffaloes in summers and winters and evaluated the interrelationships of hormones (progesterone, estradiol and LH) during the critical periovulatory (Mohan et al. 2009, Mohan and Prakash, 2010). After making detailed laboratory investigations we examined its potential for ameliorating infertility in buffaloes belonging to farmers’ herds. To study the feasibility of heatsynch protocol application for fertility improvement in buffaloes belonging to farmers’ herds, trials were conducted on anestrus buffaloes in 11 villages of Karnal district in collaboration with the KVK of NDRI. The buffaloes were selected on the basis of being at least six months anestrus or repeat breeders (anestrus or repeat breeding ranging from 6 months to 2 years or more). The animals were treated as per the protocol and inseminated twice. In 11 trials conducted in a total number of 285 buffaloes in different villages results obtained through Heatsynch protocol were very encouraging in terms of enhancing fertility. Success rate in terms of pregnancy establishment was 48%. It is pertinent to mention here that the heatsynch protocol has been initiated after a thorough basic research on buffaloes at the Institute level involving studies on endocrine changes, timing of ovulation and success rate in the farm animals in summer & winter subjected to heatsynch protocol on an experimental scale.

**Doublesynch protocol:** Studies on dairy cows have demonstrated that the success rate of the Ovsynch and Heatsynch protocol is dependent on the estrous cycle stage at the onset of the protocol. For example, the intiation of the Ovsynch protocol between days 13 and 17 or early in the estrous cycle (days 2–4) led to a reduced pregnancy rate. Other studies have established that GnRH induced follicular turnover or induction of a new follicular wave is the most efficient if ovulation is induced in response to the first GnRH treatment and that resetting the follicular development can produce a new dominant follicle containing an oocyte with greater potential fertility.

Several strategies employing hormonal treatment before initiating the Ovsynch protocol have been utilized to minimize the proportion of cows in the above-mentioned problematic stages and to maximize the proportion of cows in favorable stages of the estrous cycle. However, these strategies have several disadvantages:

1. either pregnancy rates are limited, or
2. the protocol requires a long period of time to complete.

Recently, Cirit et al. (2007) developed a new synchronization method that includes the administration of an additional PGF$_{2\alpha}$ injection 48 h before beginning the Ovsynch protocol. They named this new protocol the Doublesynch (the abbreviation of double synchronization) protocol, as it resulted in synchronized ovulation after both the first and second GnRH treatments. Öztürk et al. (2010) confirmed the pregnancy rate success (increased by 43% compared with the Ovsynch protocol) of the Doublesynch protocol in both cyclic and anestrus cows. In a study conducted on buffaloes Mirmahmoudi and Prakash (2012) the following results were obtained:

The pregnancy rates were 60% using TAI following doublesynch application on cycling buffaloes, 55% for anestrus buffaloes, in comparison to 27.3% for cycling buffaloes inseminated following spontaneous estrus. The overall pregnancy success rates after the Doublesynch protocol in both cycling and anestrus buffaloes increased by 30.8% compared to spontaneous estrus (58.1% vs. 27.3%). The study demonstrated that the Doublesynch protocol followed by TAI significantly (P < 0.005) enhanced the pregnancy rate in cycling and anestrus buffaloes in comparison to untreated controls during the low breeding season (summer).
Final Remarks: Scope for scientist-industry interaction for improving productivity: Although several techniques have been developed for enhancing production and reproduction in livestock unfortunately most of these have not yet been adopted at the field level in a big way which is imperative if they are to make an impact towards increasing milk production and thereby enhance farmers’ incomes. One main reason for research efforts not reaching the farmers doorsteps is the lack of interaction between farmers, scientists and the industry. Till date, there are no known indigenous manufacturers of hormonal drugs for therapeutic applications as described in this text. While the knowledge is available to produce them, effective mechanisms need to be set in motion to ensure an effective dialogue between the scientists and industry to produce them indigenously.

References

On request.

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Current physiological approaches and future thrust areas in augmenting sheep production

S.M.K. Naqvi, Davendra Kumar and Kalyan De
Central Sheep and Wool Research Institute, Avikanagar via Jaipur (Rajasthan) 304 501

The challenge in developing countries like India is to rapidly increase the agricultural production to feed their growing populations without depleting the natural resource base. Sheep with its multi facet utility play an important role in the Indian agrarian economy. The sheep production sector offer unprecedented opportunities for human development through poverty reduction and enhanced nutritional security. Physiology is the dynamic study of life. A physiologist investigates how biological structures and machines work at the molecular, cellular, organ, or organ system level. New research approaches are required in this regard as a means to meet both objectives through addressing the production constraints of small-scale or resource-poor farmers who contribute more than 70% of the food produced in developing countries. Animal production and health have probably benefitted the most from newer research approaches. But successful application of these technologies has generally been limited to developed countries. As we are progressing through development, new and new challenges and issues are coming in the field of research. Some important emerging areas of research particularly in animal physiology are described in this paper.

**Gastric hormone:** It is well established that feeding influences the release of gastrointestinal (GI) hormones, which are important for food digestion and growth of the GI tract (Bowen, 2004). The most pronounced physiological action of gastrin is stimulation of gastric acid secretion whereas cholecystokinin (CCK) reduces gastric emptying. In addition, they promote anabolic metabolism. Somatostatin, on the other hand, is a potent inhibitor of the output of gastrin and CCK, as well as of most of their effects. In addition, suckling and milking give rise to increased plasma concentrations of gastrin and CCK. Also, release of somatostatin is influenced by suckling/ milking, but in a more complex way. GI hormones gastrin and CCK is important for growth of the GI tract in early lactation. This is necessary to meet the higher nutrient requirements during lactation. Furthermore, it has been demonstrated that gastrin stimulates proliferation of bovine mammary epithelial cells in vitro, and the decrease in plasma level of somatostatin during suckling is correlated with the milk yield of lactating women. These findings indicate that GI hormones may have a direct or indirect effect on the mammary gland.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Major Activities</th>
<th>Stimuli for Release</th>
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<tbody>
<tr>
<td>Gastrin</td>
<td>Stimulates gastric acid secretion and proliferation of gastric epithelium</td>
<td>Presence of peptides and amino acids in gastric lumen</td>
</tr>
<tr>
<td>Cholecystokinin</td>
<td>Stimulates secretion of pancreatic enzymes, contraction and emptying of gall bladder</td>
<td>Presence of fatty acids and amino acids in the small intestine</td>
</tr>
<tr>
<td>Secretin</td>
<td>Stimulates secretion of water and bicarbonate from the pancreas and bile ducts</td>
<td>Acidic pH in the lumen of the small intestine</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>Appears to be a strong stimulant for appetite and feeding; also a potent stimulator of growth hormone secretion.</td>
<td>Not clear, but secretion peaks prior to feeding and diminishes with gastric filling</td>
</tr>
<tr>
<td>Motilin</td>
<td>Apparently involved in stimulating housekeeping patterns of motility in stomach and small intestine</td>
<td>Not clear, but secretion is associated with fasting</td>
</tr>
</tbody>
</table>
Gastric inhibitory polypeptide

Inhibits gastric secretion and motility and potentiates release of insulin from beta cells in response to elevated blood glucose concentration

Presence of fat and glucose in the small intestine

Rumen biogeography: Interest in symbiotic relationships between microbes and their animal and plant hosts is intensifying, and researchers from many disciplines within biology are striving to understand their functional and evolutionary significance. The microbial ecology in the gastro-intestinal tract (GIT) of various animal species is characterized by high population density, wide diversity, anaerobic nature and complexity of interactions. The major groups of microbes which inhabit the gastro-intestinal tract include bacteria, protozoa, fungi, yeasts and bacteriophages (Kamra, 2005). The rumen eco-system contains bacteria ($10^{10} - 10^{11}$ cells/ml, comprising 200 phenotypically different species), ciliate protozoa ($10^3 - 10^6$/ml, from 25 genera), anaerobic fungi ($10^3 - 10^5$ zoospores/ml, representing 5 genera) and bacterio-phages ($10^8 - 10^9$/ml. These numbers might even be larger as majority of them are non-culturable. Recent efforts to reevaluate microbial richness in the rumen, for example, by analyzing 16S rRNA gene sequences suggest that the typical rumen contains 300-400 bacterial "species," (Yunhong Kong et al., 2010) on the basis of operational taxonomic units-a figure that is about 10 times higher than culture-based estimates. It is estimated that 10% or less of the total viable bacteria in the rumen of forage-fed animals can only be cultivated. The use of molecular techniques based on nucleic acid probes is likely to revolutionize the approach to enumerate and characterize gastrointestinal microbial ecosystem. Diet is the major factor influencing the structure and function of the rumen microbial population (Petri et al. 2013). Over the years, diet diversity have drastically altered which might have a profound effect on rumen microbial ecology and needs methodological investigation for identifying and typifying the altered rumen microbes vis-à-vis ecological diversity. In this line, climate change and altered physical environment has impacted the internal rumen environment including its microbial ecology. So the study rumen biogeography is thus intended to explore the increasing characterization of diversity and function of microbial communities that allows more opportunities to account for variation among diets, animals, and environment along with that better prediction of rumen digestibility of nutrients and efficiency of microbial growth, accounting for increasing societal demands for improved efficiency of nutrient usage and more desirable animal products.

Metagenomics: The emerging field of metagenomics presents the greatest opportunity—perhaps since invention of microscope-to revolutionize understanding of the living world. Through metagenomics, scientists can apply the power of genomic analysis (analysis of all of DNA in an organism) to entire communities of microbes, bypassing the need to isolate and culture individual community members (Camera, 2007). The global microbial diversity of microorganisms is an important area of scientific research and presents an enormous, largely untapped genetic and biological pool that could be exploited for recovery of novel genes, metabolic pathways and valuable products. Unfortunately scientists are able to grow in vitro only less than 1% of all microorganism present in nature (Walsh et al., 2013). This leaves more than 99% of microbial diversity unexploited. The apparent underestimation of true microbial diversity is largely due to reliance on culture based microbiological studies (Hugenholtz, 2002). Recent years have seen a remarkable evolution in the development and application of molecular tools that are allowing microbiologists to characterize and understand microbial communities in unprecedented ways (Rastogi et al., 2011). By creatively leveraging these new data sources, microbial ecology has potential to have a transition form a purely descriptive to a predictive frame work, in which ecological principles are integrated and exploited to engineer the systems that biotechniques are based on 16S ribosomal DNA (rDNA) sequences and exploit either hybridization or PCR techniques.
Meat: Strategies for modifying

Meat is a major source of saturated fatty acids (SFA) in human diet (Valsta et al., 2005), especially in developed countries, and has been implicated in diseases such as cardiovascular diseases and some types of cancer (Nieto and Ros, 2012). The incidence is associated with the amount and the type of fat consumed in the diet. In recent years, consumers’ pressure to reduce the composition and quality of fat in meat has led to attempts to modify meat by dietary means. Breeds / genetic group with low concentration of total lipid in muscle (high proportion of phospholipid), will have higher proportions of PUFA in total lipid as there is inverse relationship between the proportion of 18:2 (n – 6) in subcutaneous adipose tissue and the amount of fat. Several studies have shown that dietary n - 6 and n - 3 PUFA can be incorporated into adipose tissue and muscle of ruminants despite the biohydrogenation of dietary fatty acids in the rumen (Wood et al., 2008). Higher levels of 18:2n - 6 than 18:3n - 3 in tissues are not only due to a higher affinity for incorporation into phospholipid molecules but also due to reduced biohydrogenation in the rumen. Levels of n -3 PUFA in ruminant tissues can be increased by feeding dietary lipid which is ‘protected’ from biohydrogenation in the rumen using formaldehyde treatment of linseed. Feeding ewes with pasture increases the PUFA content of intramuscular fat of the lamb compared with diets consisting of concentrate. Similarly, feeding lambs with diets rich in fish oil can modify the fatty acid profile of meat (increasing the level of PUFA) and linseed in diet increase the content of long-chain n-3 fatty acids in lamb meat.

GIS and animal health: Recent advances in information technology—including hardware, software, and the Internet—have provided capabilities to potentially enhance problem solving in areas that require information processing. Among several information technologies that have been incorporated with other areas, Geographic information systems (GIS) are one of the most popular tools to be utilized in decision making (Eldrandaly, 2007). GIS have had a profound effect on decision support system development, especially environmental modeling and model development, because GIS can supply functionality for dealing with spatial information that is required in most decision-making processes (Eldrandaly, 2007). The reason for using a GIS in an animal health information system is to enable the spatial component of animal health to be incorporated in the reporting and analysis of animal health data. Spatial data collection and database construction is important to ensure the GIS provide appropriate information to decision makers, because the data quality in the database affects secondary information quality. A well-prepared database can make analysis fast and efficient and provides versatile support in decision making.

Nanotechnology to study cell and organ function: Living cells can sense mechanical forces and convert them into biological responses. Similarly, biological and biochemical signals are known to influence the abilities of cells to sense, generate and bear mechanical forces (Bao et al., 2003). Studies into the mechanics of single cells, subcellular components and biological molecules have rapidly evolved during the past decade with significant implications for biotechnology and human health. This progress has been facilitated by new capabilities for measuring forces and displacements with piconewton and nanometre resolutions, respectively, and by improvements in bio-imaging (Bao et al., 2003). Details of mechanical, chemical and biological interactions in cells remain elusive. However, the mechanical deformation of proteins and nucleic acids may provide key insights for understanding the changes in cellular structure, response and function under force, and offer new opportunities for the diagnosis and treatment of disease (Schaller et al., 2008).

Many efforts are currently underway to try and mimic properties of single cells with the aim of designing chips that are as efficient as cells. However, cells are nature's nanotechnology engineering at the scale of atoms and molecules, and it might be better to envision a microchip that utilizes a single cell as an experimentation platform (Andersson et al., 2004). A novel, so-called laboratory-in-a-cell concept has been described, where advantage is taken of micro- and nanotechnological tools to enable precise control of the biochemical cellular environment; these tools also offer the possibility to analyse the composition of single cells (Andersson et al., 2004).
Nanotechnology is no more being a concept or theory of new world, but has turned into a new enabling technology over years, with tremendous potential to revolutionize agriculture and livestock sector in India as well all over globe (Patil et al., 2009). Nanotechnology can provide new tools for molecular and cellular biology, biotechnology, veterinary physiology, reproduction and many more (Patil, et al. 2009). Biochips, which are created with the use of nanotechnology, can be used for disease diagnosis. It also can trace and locate the presence of pathogens, and harmful chemicals (Info tech4you, 1 Dec 2012). This gives veterinarians more information on their clients. It also helps in the reproduction of animals. Livestock can be implanted with a bio-chip that detects certain genetic traits. Allowing breeders to select “trophy winning” gene carriers, and breed them for a desired animal.

Reproductive Technologies

Oestrus synchronization: Oestrus synchronization in sheep offer three major advantages i) synchronizing oestrus in donor and recipient in ET programme, ii) reducing inter-lambing period and accelerated lambing, and iii) performing large scale AI programme. Oestrus synchronization may be achieved through the use of either progesterone or prostaglandin F2α (PGF2α). Progesterone impregnated intra-vaginal sponges or CIDRs are inserted intra-vaginally for 12–14 days (Abecia et al., 2011). Ewes exhibit oestrus approximately 48 h after device removal. The cost-effective and efficient indigenous intra-vaginal sponges, suitable for Indian sheep and goat, have been developed at our institute (Naqvi et al., 2001). Attempts have been made to reduce the exposure period of progesterone from 12 to 6 days without affecting response (Letelier et al., 2009). Recently, it has also been shown that the application of the equine chorionic gonadotrophin (eCG), 48 h prior to sponge removal, gives better results than the application of eCG on the day of sponge removal (Haristova et al., 2011).

Prostaglandin F2α and its analogues have luteolytic action and two injections administered 10 days apart in cycling ewes give good results because CL is responsive to PGF2α from day 3 of the oestrous cycle (Rubianes et al., 2003) to the day of natural luteolysis. This protocol can be applied throughout the entire year in tropical breeds, where continuous breeding season and no seasonal anoestrus prevail (Godfrey et al., 1999), but it can only be used during the breeding season in the temperate areas (Acritopoulou and Haresign, 1980). Attempts have been made to reduce the period between two injections of PGF2α from 10 to 7 days (Menchaca et al., 2004). The variability in the timing of ovulation after treatment may be diminished by applying the ‘male effect’ coincidentally with the second PGF2α injection (Contreras-Solis et al., 2009). During the breeding season, two GnRH injections given 7 days apart, and PGF2α is administered on the fifth day resulted in desirable oestrous synchronisation rates (Amiridis et al., 2005). However, in a recent study Olivera-Muzante et al. (2013) observed that reproductive performance of ewes was impaired by GnRH at 24 h and not improved by GnRH administered at 36 h after the second PG injection. A single cloprostenol injection administered during the mid-luteal phase of the oestrous cycle as synchronization agent resulted in acceptable embryo production (Mayorga et al., 2011).

Artificial insemination: Artificial insemination maximizes the safe (minimal STDs) use of outstanding males of superior genetic material in minimum time and cost. AI has substantial impact on genetic gain and market value. The main obstacles for success of AI programme are lack of awareness among farmers, less number of high pedigreed breeding rams, poor estrus detection, lack of skilled AI workers, poor maintenance and delivery of AI services especially in remote areas and lack of proper breeding policies. The success of AI depends upon the route of insemination and how the semen is processed and stored (Tsakmakidis, 2010). Tris-based diluents are recommended for the routine use of ram semen [Tris–glucose–egg yolk, Tris–citrate–fructose–egg yolk (Salamon and Maxwell, 2000)]. Newly developed diluents, mainly used for cryopreservation, are based on disaccharides, trisaccharides, complex polysaccharides or other complex molecules (Cseh et al., 2012). Over the past decades, there was an increasing interest in developing chemically better defined
semen extenders (CDE), particularly diluents free from additives of animal origin (Moustacasa et al., 2011). Recently vegetable oils components (casein, palm or coconut oil), biologically safer media were used as substitutes for egg yolk in diluents for the cryopreservation of ram, and maintained the function of ram spermatozoa after freeze-thawing better than S-containing egg yolk (Del Vallea et al., 2013). These investigations achieved a double target: improvement of biosafety of semen and upgrade of standardization of semen processing.

By using fresh semen, vaginal (peri-cervical deposition of semen) or cervical (intra-cervical deposition of semen) insemination techniques result in acceptable pregnancy rates. However, by using frozen semen, laparoscopic or trans-cervical intrauterine insemination (TCAI) techniques are the only means to achieve acceptable pregnancy rates because of the necessity of cryo-damaged sperm to deposit directly into the uterine lumen (Kershaw et al., 2005, Naqvi et al., 1997, 1998, 2005). The application of oxytocin (King et al., 2004), epidosin (Naqvi et al., 2004), carazalol (Bademkiran et al., 2007), PGE$_2$ (Candappa et al., 2009), ketamine (DeRossi et. al., 2009) and hyaluronan (Perry et al., 2010) improves cervical relaxation, increases cervical penetration and supports TCAI in ewes.

Multiple ovulation and embryo transfer: MOET is considered to be the most frustrating of all ART, since the results can vary from complete failure to total success without any variation in the procedure. The main factors contributing to the unpredictability of technique are the variability of the superovulatory response, the poor fertilization associated with high ovulatory responses, early regression of corpora lutea and improper estrus synchronization of recipients. This is attributed to a number of endogenous (genetics, nutritional status, follicular status, season of the year) and exogenous (superovulatory treatment, nature and possible ‘contamination’ of the gonadotrophin) factors. However, the contribution of each factor is almost impossible to assess (Cognie et al., 2003; Gonzalez-Bulnes et al., 2004). These unpredictable results, combined with high costs and the use of surgical procedures for collecting and transferring embryos, have prevented large-scale use of MOET. Growing scepticism is emerging about the safety of pituitary gonadotrophins as potential risk factor for human food safety. Recombinant gonadotrophins may be an effective alternative, due to high purity, stable composition between batches, pathogen-free nature and potency (Adams and Boime, 2008).

Ovum pickup and IVF: Applicability of In vitro fertilization is limited because of lower ability to recover oocytes from elite animals. OPU-IVEP is extensively used in many developed countries in commercial scale (Cognie et al., 2004). The technology also extends its application in collecting oocytes from pre pubertal (Morton et al., 2005a,b; Valasi et al., 2006, 2007a,b, 2009) and pregnant animals. Oocytes can be collected by aspiration of follicles in ewe lambs around six to eight weeks of age. Oocyte collection can be performed during breeding and the seasonal anoestrous period; at anoestrus, priming with melatonin improves number of available follicles and developmental competence of collected oocytes (Tsiligianni et al., 2009). However high costs of the instrument and need of skilled personal limits its practicability in developing countries like India.

Intra cytoplasmic sperm injection: In addition to its clinical use in infertility in humans it can be used for production of transgenic animal and to study the mechanism of fertilization. The technique is preferably used in species like equines where IVF is difficult. Despite of having high fertilization rate ICSI is no more effective in terms of clinical pregnancy rate than in IVF. In 1995, an Australian research group published the first report on intra cytoplasmic sperm injection (ICSI) in sheep (Catt and Rhodes, 1995), exploring the potential of ICSI for IVF. Later on, Catt et al. (1996) used ICSI with sexed sperm to obtain a live offspring, transferring the presumptive embryo shortly after injection. This first offspring was a male that corresponded to the type of sexed sperm used. The ICSI, compared to AI and IVF, is also the only technique that can produce ovine transgenic embryos using sperm-mediated gene transfer (Pereyra-Bonnet et al., 2011). There were no differences in blastocyst production by IVF and ICSI (Catala et al., 2012; Hosseini et al., 2012). However, ICSI was superior regarding embryo cleavage and blastocyst development using vitrified oocytes.
Cryopreservation of semen and embryo: Concerted research efforts are going on worldwide to improve the methods of cryopreservation for obtaining good post-thaw recovery and fertility with frozen semen. The combinations of storage temperature, chemical composition of extender and the hygienic control are the key factors that affect the life span of spermatozoa. Spermatozoa reach to a state of suspended quiescence for an indefinite period on cryopreservation but can be resuscitated on thawing. Assessment of semen status after thawing is of practical relevance to test the functional integrity of spermatozoa. However, the extent of cryoinjury to spermatozoa during cryopreservation is not clear even under controlled freezing conditions because observations on sperm viability are assessed prior or after the freeze-thaw process is over. It has become apparent that preservation greatly affects many sperm attributes, such as motility, respiratory activity, membrane state and DNA quality. Consequently, the viability is reduced leading to poor fertility (Leahy and Gadella, 2011).

Research in the area of embryo freezing has led to the increase of embryo survival through the adoption of low toxicity cryoprotectants such as ethylene glycol and to incorporation of non-permeating osmotic buffer, sucrose, into the freezing medium, which allows direct transfer after thawing (Loi et al., 1998). Further research allowed the development of cryopreservation protocols like vitrification, has been adapted for many species including sheep, and the results in terms of survival rate and lambs born are continuously progressing (Shiraji et al., 2010). Success in vitrification has been improved by increasing the cooling rate to approximately 20,000 °C/min (Vajta, 2000). Various so-called ‘carriers’ have been developed, in order to decrease the volume of the freezing solution (in which the embryo is frozen) and this has led to a dramatically increased cooling speed. Sheep embryos have been vitrified successfully (e.g. by using straws [Baril et al., 2001], OPS [Green et al., 2009], and plastic micropipette tips – Cryo-tips [Gibbons et al., 2011]). Comparing the ultra-structure lesions of slowly frozen and vitrified ovine embryos, no difference was found independently of the embryo developmental stage (Bettencourt et al., 2009). Recently simplified techniques (direct transfer of embryos) were described for transfer of vitrified small ruminant embryos in practical conditions (Green et al., 2009).

Semen and embryo sexing: In every sector of commercial animal breeding there is clear preference of one sex over the other. Hence, semen sexing by flow cytometry has been one of the most significant new technologies for artificial breeding of livestock development in 20th century. The main application of this technology is faster multiplication of flock size of desired sex, increased weaning weight of lamb thereby maximizing productivity, profitability and genetic potential. Sexing of embryo- PCR based, boost up the ET programme because more ewe lambs per ET programme can be produced. Thus both the programme with further refining can improve the genetic potential of flock in short interval time.

The development of sperm sexing technique has added new dimensions on semen processing. Live offspring born from fresh sex-sorted semen have been reported in sheep (Hollinshead et al., 2002). Ram sperm have to be diluted 20- fold before sorting and a further 200-fold dilution takes place when it passes through the cell sorter resulting in capacitation-like changes that lead to its reduced fertility (Hollinshead et al., 2003). Further, freezing of sorted sperm require an additional step of reconcentration by centrifugation to enable packaging thereby imposing additional stress to the sperm. However, ram sperm appears to exhibit an inherent tolerance to sorting process, greater than that of bull or boar semen (De Graaf et al., 2007).

Cloning: Cloning makes the animal breeder idol as breeding needs genetic variation (Zero breeding). And this is fast developing technology (SCNT to Advanced HMC) and far from being optimized. Major limitation includes high cost, low efficiency due to incomplete reprogramming, developmental defects and acceptability by consumers is questionable. However this technology with other technologies like Transgenesis or vice-versa can boost pharmaceuticals. In 1996 Keith Campbell and Ian Wilmut reported first somatic cell nuclear transfer (SCNT) in sheep and provided the platform
for this new revolution in animal biotechnology (Campbell et al. 1996). The first cloned sheep, named Dolly, has been produced through nuclear transfer using a mammary gland cell from a 4-year old sheep as a cell donating genetic material to the oocyte from another sheep. In addition to producing Dolly from an adult mammary gland cell, the same researchers produced lambs from fetal cells (Wilmut et al., 1997). Research efforts have been intensified toward improving the efficiency of reprogramming cells such as adult mammary gland cells and fibroblasts for starting life all over again.

**Transgenesis:** Transgenic animal production to produce valuable recombinant (rc) proteins in the milk has become an important research tool in pharma industries than in breeding (disease resistance and improved quantitative and qualitative traits). Small ruminants like Goats are efficient means of producing worthy rc-proteins since they produce considerable amounts of milk, and incur lower investment and maintenance costs than other domestic species. Studies have also been made on xenotransplant in pig. It involves the microinjection of a DNA construct into the pronuclei of in vivo produced zygotes however the method is inefficient due to random integration, resulting in unpredictable results in terms of transgenesis rate (< 10%) and expression. Recently pronuclear microinjection has been reported by using in vitro produced zygotes from Laproscopic OPU-derived oocytes with better success rate. The application of transgenic technology to improve other production traits of economic value, such as increased milk, meat and/or wool production has not been studied. While the opportunity for such applications is envisioned in the near future, however widespread implementation will occur unless efficiencies and costs of producing transgenic improve significantly. Cloning techniques are a great hope to improve transgenic animal production (McCreath et al., 2000; Westhusin et al., 2001). Genes of these animals are altered, so the animals can secrete rare and expensive drugs in their milk (i.e., transgenic sheep which produced human antihemophylic factor IX in milk; Niemann et al., 1999; McCreath et al., 2000). Sheep-derived protein has now entered clinical trials for cystic fibrosis (UK and USA) and congenital emphysema (UK), and the utilization of cloning and transgenic technology is making inroads into more traditional ways of making biopharmaceuticals (Colman 1999).

**Stem cell:** Embryonic stem cells, umbilical cord cells, adult stem cells and spermatognial stem cells are the major types of stem cells used in Reproductive biotechnology. Stem cells are having various applications like, model for developmental biology, organ transplantation, gene therapy, drug development, chimera production and in the field of regenerative medicines. Embryonic stem cells are important to provide a method to introduce precise genetic modification into animals by homologous recombination of ES cells followed by blastocyst injection for chimera derivation and breeding cell therapy of various tissues. Spermatogonial stem cell transfer is commonly used in rodents to study Spermatogenesis. In cattle SSC transfer has potential use in commercial breeding system so that elite animal genetics is disseminated more widely. Germ cell transplantation has been successfully reported in sheep (Rodriguez-Sosa et al., 2006). Present knowledge on application of SSCs is limited in research field and farm animal reproduction. But research is still continuing across the globe for betterment.

**Kisspeptin:** Kisspeptins, the peptide products of Kiss-1 gene have been substantially associated with the initiation of puberty. Kisspeptin consists of 54 amino acids and its biological activity can be localized to the C-terminal segment which is cleaved into C-10, C-13, and C-14 segments. Binding of kisspeptin to its cognate receptor, GPR-54 in the hypothalamic neurons stimulates GnRH secretion and has been shown to be essential for the initiation of the pubertal LH surge. The major C-terminal fragments appear to be Kisspeptin-54 (metastin) but other derivatives like Kisspeptin -14, Kisspeptin -13 and Kisspeptin -10 have also been identified. Kiss1 gene is expressed mainly in two hypothalamic sites in rodents, the arcuate nucleus (ARC) and anteroventro periventricual nucleus (AVPV) as well as in pre-optic area in sheep. The reproductive function of these molecules have been identified in late 2003 by virtue of inactivating mutations of GPR-54 leading to human patients suffering from idiopathic form of hypogonadotrophic hypogonadism. Thereafter, a number of studies have firmly substantiated the role of Kisspeptin in the regulation of
GnRH secretion in sheep as well as in other animals. Kisspeptin conformational properties have been studied by us and now its mechanism of action is being studied for its wider use as a therapeutic regimen for early initiation of puberty.

**Melatonin:** Seasonality in sheep is mediated by photoperiod, which is conveyed to the reproductive neuroendocrine axis by melatonin. Melatonin is released under conditions of darkness and acts in the mediobasal hypothalamus to modulate the pulsatile secretion of GnRH (Robinson et al., 1985). Subcutaneous implants of melatonin are widely used to advance the breeding season and to improve reproductive performance during anestrus both in highly seasonal (Haresign et al., 1990) and in Mediterranean ewes (Zuniga et al., 2002). Subcutaneous implants cause a short-day length-like response without suppressing endogenous secretion (Malpaux et al., 1997). In general, melatonin treatment increases fertility and prolificacy in ewes (Palacin et al., 2011); but particularly, it can have a positive effect on embryo survival in ewes (Vazquez et al., 2010) even after superovulation (Forcada et al., 2006) due to its luteotrophic effects (Forcada et al., 2006). Treatment with exogenous melatonin improves the viability of ovine embryos from undernourished ewes during the anoestrous period (Abecia et al., 2008; Vazquez et al., 2010) as well as breeding season (Vazquez et al., 2013), although this effect seems not to be mediated at the oocyte competence level. Melatonin regulates the circadian rhythmic cycles and reproductive changes in seasonally reproductive animals by virtue of its binding to high affinity melatonin receptors which are G-protein coupled receptors prominently located in the pars tuberalis (PT) region and pre-maxillary hypothalamus (PMH). Pars tuberalis region of the hypothalamus where the highest density of melatonin receptor (MTNR1A) has been documented was found to be associated with prolactin secretion whereas the PMH region is the one which is closely associated with the control of GnRH secretion. MTNR1A is a high affinity receptor. There are other melatonin receptor subtypes also (MTNR1B & 1C) but only the first (MTNR1A) seems to be involved in the regulation of seasonal reproductive activity. Polymorphic variants of MTNR1A are being studied in our laboratory for selection of animals having out of breeding capacity.

**Pregnancy diagnosis kit:** Early pregnancy diagnosis remains a key factor in improving reproductive performance. It has been noticed that nearly 20-30% of non-pregnant animals have irregular or extended estrous cycle and did not return to estrus on 21st post-insemination day but may return to heat anywhere between 25-40th post-insemination day and give a false impression of pregnancy, but routine pregnancy test after post-insemination will clear this confusion. Some test was optimized to measure the level of hormone progesterone in milk, between 19-25 days after insemination. In pregnancy this level is > 20 ng./ml (Shemesh et al.,1979). In non-pregnancy the hormone level is <2.5 ng./ml (Alwan et al., 2010). The test determines the exact level of progesterone in milk. A serum based sandwich ELISA kit for pregnancy diagnosis (PD) in mares is available. Pregnancy can be diagnosed between 35 to 120 days of gestation (Purohit, 2010). This kit detects concentration of PMSG or eCG hormone in serum. Recently available tests detect so called early conception factor (ECF) or pregnancy-associated glycoprotein in blood samples. They are reported to detect the pregnancy-associated glycoprotein within 48 hours of conception (Cordoba et al., 2001). Because of the high incidence of embryonic mortality this test should be treated solely as an indication of conception. Pregnancy should be confirmed later by rectal or ultrasound examination.

**Emerging reproductive technologies:** Emerging reproductive technologies include transforming somatic cells into personalised stem cells, creating viable sperm and eggs from stem cells or somatic cells and In vitro gametogenesis for continues supply of superior germplasm for regenerative embryo biotechnological work. However, many questions about safety and ethics will have to be answered first.

**Medicinal plants and reproduction:** The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbs (Kala et al., 2006). Theses traditional knowledge systems have started to disappear with the passage of time due to scarcity of written documents and relatively low income in these traditions. However, the medicinal
plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects. India has a rich biodiversity of medicine plants. Many medicinal plants have been claimed to be effective in modulating reproduction but the systematic scientific evidence regarding their mechanism of action, dosage or clinical efficacy is lacking. Though incorporation of cheap, efficacious and scientifically proven indigenous plant based medicines is needed, their safety needs to be established. But there lies huge scope of research in the aspect of medicinal plants and reproductions. Feeding of maca (*Lepidium meyenii*) and khat (*Catha edulis*) has been shown to positively affect sperm production and quality in animals. Some evidence points to favourable effects of leucaena (*Leucaena leucocephala* and *Leucaena leucoaphylla*), sesbania (*Sesbania sesban*), pomegranate (*Punica granatum*), tomato (*Solanum lycopersicum*) and amaranth (*Amaranthus hypochondriacus*) as well, but studies are either superficial or results are partially contradictory (Clement et al., 2012).

**Climate Change and Reproduction: Experimental Tools from Evolutionary Biology:** It is well known fact that climate change has wide-ranging biological consequences, potentially leading to impacts on biodiversity. Environmental factors can have diverse and often strong effects on reproduction (Lamy et al., 2012), with obvious ramifications for population fitness. Nevertheless, reproductive traits are often neglected in conservation considerations. Focusing on animals, recent progress in sexual selection and sexual conflict research suggests that reproductive costs may pose an underestimated hurdle during rapid climate change (Naqvi et al., 2011), potentially lowering adaptive potential and increasing extinction risk of certain populations. Nevertheless, regime shifts may have both negative and positive effects on reproduction, so it is important to acquire detailed experimental data. From the enormous diversity of findings, it is concluded that climate change research could benefit greatly from more coordinated efforts incorporating evolutionary approaches in order to obtain cross-comparable data on how individual and population reproductive fitness respond in the long term. Therefore, the ideas and methods concerning future efforts dealing with reproductive consequences of climate change, in particular by highlighting the advantages of multi-generational experimental evolution experiments.

**Climatic change and effects on brain:** Climate Change Science Program reports that we can expect more extreme weather, including droughts, strong storms, floods, and heat waves. Now it is proved that environmental change is a very real stressor, affecting brain and behavior of the animals. In some songbirds, climate change may impact breeding seasons, which could affect species survival. During mating season, male songbirds sing to attract mates and to mark their territories. Song-related brain regions show seasonal plasticity - they expand during mating season compared to other times of the year. For most songbirds, changes in day length induce these brain changes and indicate that it is time for breeding. However, research has shown that songbirds that live near the equator, where day length does not vary much seasonally, still show seasonal behavioral changes. These changes occur in response to other local environmental cues, potentially including temperature, rainfall, and food availability.

Some species may be able to adapt to ongoing change in the ecosystem. Recent research suggests that some birds can influence the social behaviors of their offspring, potentially as a response to environmental conditions. Recent research showed that women who reported chronically high stress levels over many years had a smaller hippocampus, a brain region important for learning and memory (Bremner, 2008). Other research data suggest that the brain is sensitive to many aspects of the environment, and that climate change could have effects on species survival. But how environmental change may affect brain is still unclear and more research is needed.

**Multiple stresses amelioration:** Under the changing climatic scenario, the concept of multiple stresses emerges as a potential threat to small and large ruminant production. Hence research needs to be prioritized to tackle multiple stresses simultaneously. Generally when animals are exposed to one stress at a time, they can effectively counter them based on their stored body reserves without altering the normal body functions. However, if they are exposed to more than one stress at a time, the
summated effects of the different stressors might prove detrimental to these animals. This is because of their inability to cope with the combined effects of different stressors simultaneously (Sejian et al., 2012). In such a case, the animal’s body reserves are not sufficient to effectively counter such environmental extremes. As a result their adaptive capability are hampered and the animals struggle to maintain normal homeothermy.

**Conclusion:** A critical and integrated analysis of the future challenges to meet the ever increasing demand of animal products in the wake of climate change, depletion of natural and energy resource base (arable land, water, fossil fuel) and heightened food safety concerns will be crucial for strategic research and development priorities that primarily benefit the needy and small holder farmers. When designing effective strategies and interventions for future research, we must take multidisciplinary approach and it should focus on the physiological mechanisms of animals to respond to varied environmental conditions in an area relevant to animal production, health, or well-being for heightened food safety. With advancement of new collaborative, multidisciplinary approach in research, it is important to balance the concerns and constraints with the remarkable opportunities, in scientific capabilities to answer scientific inquiries in all fields with greater accuracy and precision.

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♦ ♦ ♦
Poultry Production: New milestone to achieve nutritional security

P.K.Shukla, Sujit Nayak and A. Arun Kumar

Professor and Head, Poultry Science and Dean (PGS), DUVASU, Mathura

Introduction: Food security exists when people have access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. If nutrition improves food security will be achieved more efficiently and specially when people have access to an optimal combination of food from both animal and plant sources. The animal source foods are rich in energy, protein, and micronutrients that have greater bioavailability than plant sources. The food security as well as poverty reduction can be achieved simultaneously through various livestock production system.

One of the great challenges currently posing us is how to feed an estimated 9 billion people by the year 2050, 40% more than presently inhabit the planet; even more formidable is the challenge to achieve this without damaging the environment. The challenge is how to increase the food supply, particularly food of animal origin, in light of increasing global demand from predominantly urban populations with increased purchasing capacity (FAO, 2009).

Importantly, in coming years the rural backyard poultry systems plays a pivotal role in achieving nutritional security of the country in rural areas. In village poultry systems the production of poultry meat and eggs is extremely efficient in terms of feed and water inputs. These nutritious products can supplement household grain-based diets. Family poultry have a special place as they are under the control of women, require low investment, assist in pest control and provide manure for fertilizer. Improvements in their production can meet the nutritional demand in the household and in the community by increasing their social standing and financial autonomy.

Major objective of livestock/poultry production is to provide safe and healthy animal food/protein for the growing population. However there are many serious challenges cropping up on its sustainability. This paper examines areas of current challenges to meet the nutritional demand globally by assessing the sustainable rural livestock/poultry production systems and explore a linkage between poultry sector development and human nutrition.

Global requirement of livestock products including poultry: The demand for livestock products will continue to grow, and it will become increasingly challenging to meet that demand. It is estimated that by around 2050 there will be 9.15 billion people to feed, 1.3 times more than in 2010 and most of the new population will be in urban areas (UNFPA, 2010). Based on estimates published in 2006, the expanded population is expected to consume almost twice as much animal protein as today (FAO, 2011).

Table 1: Projected Total Consumption of Meat Products Globally (In Million tonnes)

<table>
<thead>
<tr>
<th>Year/ Meat type</th>
<th>2010</th>
<th>2020</th>
<th>2030</th>
<th>2050</th>
<th>2050/2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>All meat</td>
<td>268.7</td>
<td>319.3</td>
<td>380.8</td>
<td>463.8</td>
<td>173%</td>
</tr>
<tr>
<td>Beef</td>
<td>67.3</td>
<td>77.3</td>
<td>88.9</td>
<td>106.3</td>
<td>158%</td>
</tr>
<tr>
<td>Mutton</td>
<td>13.2</td>
<td>15.7</td>
<td>18.5</td>
<td>23.5</td>
<td>178%</td>
</tr>
<tr>
<td>Pork</td>
<td>102.3</td>
<td>115.3</td>
<td>129.9</td>
<td>140.7</td>
<td>137%</td>
</tr>
<tr>
<td>Chicken</td>
<td>85.9</td>
<td>111.0</td>
<td>143.5</td>
<td>193.3</td>
<td>225%</td>
</tr>
</tbody>
</table>

Source: FAO, 2011

Consumption growth and production costs: FAO, 2006 projections suggest that in 2050, 2.3 times as much poultry meat and between 1.4 and 1.8 times as much of other livestock products
will be consumed compared to 2010. The additional demand besides that from population growth will result from increases in income encouraging a higher consumption per person. The 2007–08 economic crisis temporarily reduced the growth rate of GDP and therefore the purchasing power for livestock products, but expectations are that the effect will not be prolonged and that average long-term growth will be as expected. Purchasing power is also influenced significantly by the price of livestock products. In turn price of livestock products will depend on the cost of production which is likely to keep increasing as feed and fuel energy become more expensive, water availability is becoming difficult and/or livestock value chains have to bear the costs of the externalities/concerns of environmental pollution, welfare, food safety etc. Both food and feed crop prices are likely to increase (Thornton, 2010), since increased yields will depend in part on fossil fuels and scarce minerals. Competition for bio-energy also may also escalate prices, although new technology is exploring a wider range of non-food inputs to produce biofuel. Water availability is also a serious concern, since population in water-stressed regions is expected to rise to 64 percent in 2025 compared to 38 percent in 2002 (Rosegrant et al., 2002) and there would be a great stress on livestock as they are a major consumer of fresh water, estimated at 20 percent of green water flow (Deutsch et al., 2010). Livestock production creates externalities through water pollution and greenhouse gases emission (mainly large ruminants) – costs for which may have to be paid in future. The relative price of livestock protein and substitute proteins also affects the demand for livestock products. In the past 20 years, fish consumption per person has remained fairly stable (FAO, 2008) while consumption of livestock products has grown, but this could change if relative prices change.

India’s current production capacities and future requirement of livestock products including poultry

India is the third highest producer of egg but still the per capita availability is around 55 per person per year which is far from the recommendation of National Institute of Nutrition of 180 eggs per capita (around half an egg a day). The present egg and poultry meat production is 69.73 billion numbers and 2.69 million metric tonnes respectively (BAHS, revised 2012-13). Animal husbandry contributes 3.37 per cent to India’s GDP (NIAFP, 2013). Whereas share of agriculture to total GDP at current prices has declined from 28.3 in 1990-91 to 12.34% in 2011-12, the share of livestock sector to agriculture has increased from 20 to 27.28 % during the same period. Livestock and poultry are significantly contributing towards providing employment opportunities, livelihood, subsidiary income and socioeconomic and nutritional security to small holder livestock farmers and other socially backward sections of the society. Besides, in the industrial farming sector, especially poultry, the exports are also increasing inspite of challenges of avian influenza outbreaks etc. By 2050, it is expected that the population in India would increase by 34% and to fulfill the dietary recommended levels of the livestock products by Indian Council for Medical Research (ICMR) for a population of 1.7 billion people, the livestock sector should produce 186.2 million tons of milk, 18.7 million tons of meat and 306 billion eggs per annum. From the current level of production, the milk, meat and eggs have to increase by 1.5, 3.7 and 4.7 folds respectively. Fulfilling the feed demand for this huge livestock from same resource base of land and water is going to be a huge challenge (NIAFP, 2013).

Table 2: Projection comparisons of World and India with respect to production, consumption and per capita consumption of Poultry Meat (Ready to Cook equivalent):

<table>
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<tbody>
<tr>
<td>India Production, MT</td>
<td>2.88</td>
<td>3.06</td>
<td>3.16</td>
<td>3.30</td>
<td>3.37</td>
<td>3.62</td>
<td>3.83</td>
<td>4.03</td>
<td>4.20</td>
<td>4.40</td>
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<tr>
<td>World Production, MT</td>
<td>104.1</td>
<td>106.7</td>
<td>109.3</td>
<td>111.7</td>
<td>114.2</td>
<td>116.8</td>
<td>119.3</td>
<td>122.0</td>
<td>124.3</td>
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31
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<tr>
<td>India Consumption, MT</td>
<td>2.87</td>
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<td>3.62</td>
<td>3.83</td>
<td>4.03</td>
<td>4.19</td>
<td>4.40</td>
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<tr>
<td>World Consumption, MT</td>
<td>103.9</td>
<td>106.5</td>
<td>109.0</td>
<td>111.5</td>
<td>114.0</td>
<td>116.6</td>
<td>119.1</td>
<td>121.8</td>
<td>124.1</td>
<td>127.0</td>
<td></td>
</tr>
<tr>
<td>India Consumption per capita, kg</td>
<td>2.01</td>
<td>2.11</td>
<td>2.15</td>
<td>2.22</td>
<td>2.24</td>
<td>2.37</td>
<td>2.48</td>
<td>2.58</td>
<td>2.66</td>
<td>2.76</td>
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</tr>
</tbody>
</table>

**Source:** OECD-FAO Agricultural Outlook 2012-2021

A steep rise in increase of wholesale prices of food and meat products, Figure 1 suggests that the livestock value chains are increasingly required to bear the costs of the negative externalities they create.

**Figure 1: Wholesale prices for selected foods, India (2005-2010)**

Small animal holding systems are increasingly becoming unsustainable due to high input costs alongwith meeting environment, food safety regulations etc. and non remunerative prices for animal products. However increase in the demand for milk, meat and eggs, on the other hand, is creating high expectations from animal husbandry sector to meet the increased demands of the ever growing population. Either there would be emphasis on transformation into an industrialized production system or there alternative integrated farming predominantly comprising of small holders. This type of production is considered to be incredibly important in terms of providing nutrition as well as to have a low carbon foot print (World Dairy Summit, 2012).

**Nutritive value of eggs and poultry meat:** Egg is a wholesome, nutritious food with high nutrient density because, in proportion to their calorie count, they provide 12% of the Daily Value for protein and a wide variety of other nutrients like vitamins, essential amino acids and minerals such as vitamin A, B₆, B₁₂, folate, iron, phosphorus, Selenium, Choline and zinc etc. alongwith various other
important ingredients so crucial for growth and good health. Protein in the nutrition is one of the most important health indices that affect children’s growth and development. Lutein and zeaxanthin are two newly-recognized nutrients that have put eggs in the “functional foods” category. A functional food is one that provides health benefits beyond its basic nutrient content. Recent studies have shown that consuming lutein and zeaxanthin can significantly lower risk of age-related macular degeneration (AMD), a leading cause of blindness affecting people over the age of 65. In addition, there is a less likelihood of cataracts (IEC, 2013).

Poultry meat is a good source of protein and vitamins and minerals, such as iron, selenium, zinc, and B vitamins. It is also one of the main sources of vitamin B₁₂. It has several advantages as half of the fat from chicken meat is made up of the desirable monounsaturated fats, and only one-third of the less healthy saturated fats. There are much higher proportions of saturated fats in most cuts of red meat, which also vary considerably in total fat. Chicken meat is therefore seen as a healthy meat. Chicken meat does not contain the trans-fats that contribute to coronary heart disease (Heart Foundation, 2013).

Poultry meat is rich in the omega-3 fats and is an important provider of the essential Poly Unsaturated Fatty Acids (PUFAs), especially the omega (n)-3 fatty acids. Scavenging chickens are a particularly good source because of their varied diet. The amounts of these important fatty acids can be increased more easily in chicken meat than in other livestock meats; so too can some trace minerals and vitamins.

Poultry meat can be enriched with several of the important dietary nutrients like Selenium whose deficiency is becoming more widespread in humans because soils are becoming depleted and the foods grown on them are therefore lower in selenium.

Artificial Meat and Eggs: In vitro artificially produced Meat offers a possible future competition as well as alternative solution to meat from food animals, initially for those having concerns about animal welfare and in long term consume meat sustainably. It has the potential advantages of using less water, energy and for being more welfare-friendly, but the technology is yet to cross some hurdles. Current techniques involve growing cultures from stem cells of farm animals into 3-dimensional muscle/ fibre structures. However the texture and flavor etc will take some time before being standardized.

As per World Poultry, 2013 report a plant-based egg product was recently introduced in US supermarket shelves. It is stated that producing emulsifying plant product cuts out the need for grain to be transported, fed to birds for them to then produce eggs, and thereby reduces carbon and waste. It is also stated that 92% of the world's plant species' properties are yet to be explored, and that many could replicate the taste, texture and nutritional value of meat or eggs – and in combinations, create equivalent products. Artificial egg is a mixture of different plants, including canola, peas and sunflower lecithin, and claims to cost 18% less than real eggs. Two lines – an egg-free mayonnaise and a powdered egg product for baking – are being sold initially through Wholefoods stores in the USA as per the news report. The firm (Hampton Creek Foods) is also developing a scrambled egg substitute that, although not ready for commercial production, is almost indistinguishable from real eggs in both taste and texture, according to testers.

Overall challenges to meet overall food security globally: Major challenges for the future has been enumerated to deal with food security (Foresight, 2011) viz. future demand and sustainable supply balance to ensure that food supplies are affordable, ensuring adequate stability in food supplies and protecting the most vulnerable from the price and availability fluctuations; achieving global access to food and ending hunger. This recognizes that producing enough food in the world so that everyone can potentially be fed is not the same thing as ensuring food security for all or to simplify, per capita availability is not same as per capita consumption; managing the contribution of the food system to the mitigation of climate change and maintaining biodiversity and the ecosystem while feeding the world.
Areas for gearing up to challenges for meeting the demand for food of animal origin:
There are many areas (as per NIANPs vision 2050), the majority of which have been listed as to
counter low productivity, reproductive efficiency and poor efficiency in nutrient utilization (as direct
livestock productivity/ production level intervention); shortage of quality feed and fodder and costs,
conflict between food and feed crop security, imbalanced feeding, water quality and quantity for
livestock, diversion of feed resources - biofuels, export etc. (managing resources); degradation of
natural resources and impact of climate change (environmental factors); high cost of feed and fodder,
changing global trade policies, meeting consumer preferences in animal products, feed and livestock
product safety (to address market requirements).

Accordingly, research areas in poultry where our premier institutes are concentrating
are: Enhancement in the productivity of diversified poultry species and development of their
management practices- biotechnological interventions such as functional genomics for genes
influencing disease resistance, and tropical adaptability; *in-vivo* techniques of gene silencing of target
genes e.g. TGFβ1, ACC, PPARγ etc. Genome-Wide Marker Assisted Selection (GWMAS) using high
density Single Nucleotide Polymorphism array, transgenic approaches for pharmaceutical protein and
metagenomic approaches to break the productivity barriers are proposed to be employed.

Updating nutrient requirement for different classes of poultry species alongwith identification
and utilization of new and non-conventional feed resources and technologies- Micro-nutrient
requirement determination, precision feeding alongwith development of melioration techniques for
incriminating and toxic substances in poultry feed and products will be undertaken;

Database creation on gut metagenome of poultry species and identification of active clones for
application as prebiotics, developing stocks with better immunocompetency and tropical adaptability,
and climate resilient poultry production systems using Thermal Humidity Index and modulation of
Heat Shock Proteins (HSPs) alongwith exploring epigenesis under extreme climatic and production
stress.

It is proposed to revisit the management practices to make them more eco-friendly, have a low
carbon footprint, and to address welfare concerns of both poultry and also of workers by reducing
pollution etc. Value-addition, food safety, quality assurance areas will be given due emphasis by
paying attention to crucial areas like developing areas of *Salmonella* and *Campylobacter* serotypes of
zoonotic importance using real-time Polymerase Chain Reaction (rt-PCR) techniques, developing
thermal inactivation model to decontaminate by assessing thermal death time and studying thermo-
tolerance gene expression profiling e.g. in *S. typhimurium* isolates from broilers, assessing efficacy of
Generally Recognized As Safe (GRAS) antimicrobials, develop database for residues of chemical
contaminants in poultry feed and products, thus helping in developing quality standard. New product
development like low cholesterol and Omega-3 rich designer eggs are already in vogue in private
sector. Further development of value-added novel egg and functional poultry meat products with
longer shelf-life is contemplated.

On the marketing side HRD, market intelligence, technology dissemination and economic
implications of emerging issues in poultry value chain are going to be analysed. Efficient utilization of
poultry waste through integrated production systems and alternative usage is also a focus area.

**Special emphasis on reducing waste:** Doubling supply would put a considerable burden on
already depleting natural resources and in turn, would drive up the prices of livestock products and
threaten access to food by the poor. However, there is a great deal of waste in food systems. Natural
resources are not always converted efficiently into meat, milk or eggs, and a great deal of the food
currently produced does not reach the plate. Improving efficiency and minimizing waste throughout
livestock value chains could go a long way towards meeting increased demand.

FAO has noted that the growth in production during the livestock revolution may largely be
attributed to an increase in the number of animals. Demand grew very fast that it was difficult to have
commensurate productivity improvements. It is difficult to cater to the projected demand by keeping twice as many poultry, 80 percent more small ruminants, 50 percent more cattle and 40 percent more pigs. On top of it, keeping these using the same level of natural resources that we currently use is not feasible. In other words, efficiency needs to increase drastically or as a corollary, there is a need to reduce waste of natural resources.

Throughout the livestock food systems wastage is rampant due to production inefficiency resulting from disease or poor feeding or from loss of food between production and till it reaches the consumers’ plate, amounting to as much as 33 percent for all global food production (Stuart, 2009).

Three food security situations – livestock dependent societies, small-scale mixed farmers, and city dwellers – with their associated livestock production and marketing chains, have to be examined for critical areas of inefficiency and ways to mitigate the same:.

In case of Livestock-dependent societies Pasture management, Animal health, Transportation infrastructure and Markets are identified as important areas for improvement. In case of Small-scale mixed farmers, again, poor animal health, poor feeding practices, and post harvest losses are the major concerns. In case of Feeding cities from large-scale intensive production a large part of the loss is at the retail end of the value chain, to meet the demands placed on supermarkets and fast-food retailers for quality and freshness (Stuart, 2009). Animal welfare standards, which are becoming more demanding in developed countries, may increasingly influence the limits on feed conversion and other productivity improvements. For instance, there may be no battery cage production of eggs in the European Union after 2015. It is feasible to recycle livestock waste through large-scale anaerobic digesters that turn solid food waste into biogas, or large-scale composters to convert food waste into compost which may be used as farm fertilizer (Harvey, 2010). India has also laid emphasis biogas production.

Initiatives of Government of India and State Governments

**Food Security Act:** The entitlements under the same presently restrict entitlement of priority households foodgrains. *Children’s Entitlement includes* age group of 6 months to 6 years: an age-appropriate meal, free of charge, through the local anganwadi. For children aged 6-14 years, one free mid-day meal every day (except on school holidays) in all government and government-aided schools, up to Class VIII. For children below six months, “exclusive breastfeeding shall be promoted”. For children who suffer from malnutrition, meals will be provided to them free of charge “through the local anganwadi”. Every pregnant and lactating mother is entitled to a free meal at the local anganwadi (during pregnancy and six months after child birth) as well as maternity benefits. The Act provides for creation of State Food Commissions. The main function of the State Commission is to monitor the implementation of the Act, give advice to the states governments and their agencies, and inquire into violations of entitlements. The Act has schedules including prescribing “nutritional standards” for midday meals, take-home rations and related entitlements.

**Specific provisions to link food of animal origin with human protein supplementation-**

**National Mission for Protein Supplements:** To increase the supply of the protein supplements, government had launched a National Mission for Protein supplements in 2011-12 and continued currently. The Mission has taken up activities to promote animal based protein production through livestock development, dairy farming and fisheries in selected blocks. Government has also attempted to further strengthen the mission in following years.

**Mid-Day-Meal Scheme- Inclusion of Eggs:** In India the largest majority of pre-school children experience protein-energy malnutrition and micro nutrient deficiency (World Bank, 2005). With a view to enhancing enrollment, retention and attendance and simultaneously improving nutritional levels among children, the National Programme of Nutritional Support to Primary Education (NP-NSPE) was launched as a Centrally Sponsored Scheme by the GoI. The inclusion of eggs in Mid-day meal schemes provides a wholesome nutrition to the growing school going children.
The States which include eggs, frequency in the scheme is given in the following table:

Table 3: Information of the States on Distribution of Eggs in Mid Day Meal scheme

<table>
<thead>
<tr>
<th>S No.</th>
<th>States</th>
<th>Eggs Per Kid/Week or per fortnight as mentioned</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Andhra Pradesh</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Assam</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Bihar</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Jharkhand</td>
<td>1/fortnight</td>
</tr>
<tr>
<td>5</td>
<td>Karnataka</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Mizoram</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Nagaland</td>
<td>1/fortnight</td>
</tr>
<tr>
<td>8</td>
<td>Odisha</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Sikkim</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>Tamil Nadu</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>Tripura</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>West Bengal</td>
<td>3</td>
</tr>
</tbody>
</table>

Source: Collected by the 4 regional Central Poultry Development Organizations as per available information

Direct intervention for ensuring nutrition besides supplementary income generation-

Rural Backyard Poultry: In 2003-04, a study commissioned by Department of Animal Husbandry, Dairying & Fisheries (DADF) through National Productivity Council, on ‘Aspects of Rural Production and Consumption with special emphasis on Marketing problems of small/marginal farmers’ concluded that there is need to take up specific rural poultry production programmes to meet requirements of rural sector where poultry farming constitute a source of subsistence income, as a subsidiary occupation by taking up colour- bird units ranging from 20 to 100 birds in their backyard as these require very little hand-feeding and manage to give handsome return, with bare minimum night shelter. In 2003-04, a ‘Prospective Plan for Food Security, Employment and Income Generation through Poultry by 2010’ also recommended rural development through central assistance to state poultry farms by providing low-input technology birds for beneficiaries who are below poverty line with minimal input cost. During May 2004, a workshop on Rural Backyard Poultry Development was organised and as a spinoff to the recommendations, a serious thought was given to the rural backyard sector.

Government of India planned a long term strengthening program by first strengthening the infrastructure required for producing the low-input technology birds which is conventionally not produced by the commercial sector. Therefore GoI launched a scheme in 1999-2000: Assistance to State Poultry/Duck Farms and one time assistance is provided to strengthen farms in terms of hatchery, brooding and rearing houses, laying houses for birds with provision for feed mill and their quality monitoring and in-house disease diagnostic facilities. The assistance provided is 100% in the case of the North Eastern States, including Sikkim and 80% in respect of other States. The limit of assistance provided is Rs. 85.00 lakhs for each farm.

Till date, 236 farms have been assisted under the scheme since inception. An evaluation study was conducted by NABCONS (NABARD Consultancy Services) and it has also been observed that this is the only scheme supporting backyard poultry sector and that if it is withdrawn the state poultry farms will become redundant again or will become commercial units.

Accordingly, basic approach of the erstwhile ‘Assistance to State Poultry Farms’ scheme has been to increase egg and meat production especially in rural areas of country with use of improved low input technology birds without putting substantial strain on feed resources and other inputs which small farmers are not able to absorb and also to meet specific rural consumer preference. Farms maintaining poultry species other than chicken are also assisted on priority basis. Diversification with
species like turkey, Japanese quail, guinea fowl, ducks etc. is encouraged. The facility is also extended for farms in States, which may be run in collaboration with Co-operatives/private sector/NGOs etc. as before.

This scheme during XI Five Year Plan has been subsumed under Centrally Sponsored Scheme, ‘Poultry Development’ as a component.

DAFD also took up, in association with ICAR, a targeted programme for up-gradation of low-input technology birds through their All India Coordinated Research Project on Poultry, Project Directorate on Poultry and Central Avian Research Institute activities and have since developed and released birds like Vanaraja, Krishibro, Krishilayer, Caribro, Cari Red Naked neck broiler, Dwarf broiler etc., by making available these stock for further multiplication and distribution among farmers. Besides, some State Agriculture/ Veterinary Universities have also taken initiative in developing such breeds/strains.

The Department has established suitable infrastructure of which presently four large scale Central Poultry Development Organizations (CPDOs) located in Mumbai, Bangalore, Bhubaneswar and Chandigarh and one Central Poultry Performance Testing Center (CPPTC) at Gurgaon exist. The four centers of the Central Poultry Development Organizations are promoting the development of poultry by making available quality chicks of Identified low-input technology poultry stocks, diversification of alternate species like Duck, Japanese Quail and Guinea fowl etc., training of trainers, farmers, women beneficiaries, various public and private sector poultry organizations, NGOs, Banks, Cooperatives and foreign trainees etc. and testing of various stocks available in the country to assess their performance.

Breeding program by these Central Farms has been re-oriented around 2003 towards goal of making improved rural poultry a viable supplementary income generation venture. Stocks like Nirbheek and Hitcari etc. are found particularly suitable for backyard farming and CPDOs are maintaining parent stocks for distribution to state Poultry Farms for onward multiplication and distribution to farmers. CPDOs are the major sourcing agencies for the seed of chicks required for Rural Backyard Poultry Development. CPPTC is also imparting valuable information on stocks available in the country and in fact some facilities in this Center needs further strengthening.

A ‘Poultry Development’ scheme was launched in 2009-10 subsuming the above-mention scheme and component ‘Rural Backyard Poultry Development’ envisaged to cover beneficiaries from BPL families to enable them to gain supplementary income and nutritional support.

The idea of implementing a large scale rural backyard poultry development program also took a set-back as many issues on bio-security particularly due to avian influenza outbreaks, and other risks were discussed. The State Government ensures health and proper biosecurity coverage to the birds and will also keep account of the units under the scheme. The Government is already implementing an IEC (Information, Education and Communication) campaign for awareness creation with respect to notifiable avian influenza and information sharing. This will be a continuing exercise and may be intensified wherever needed. The State laboratories are also being adequately geared up to deal with the surveillance and monitoring programs. However, as stated earlier, in States like West Bengal, this scheme is proving to be a remedy against the depopulation through culling.

Therefore, the activities of GOI at the apex level towards rural backyard poultry development can be summarized as follows: The Indian Poultry Sector is broadly divided into organized and unorganized sub-sectors. The needs of the organized and unorganized sectors are very different. Central idea of this scheme component revolves around providing higher potential birds to help increase their income from their enterprise similar to small agriculture farmers being provided with improved seeds to increase productivity & their income from same land holding. Further, the scheme component has created an intermediate step ‘mother units’ for rearing chicks upto 4 weeks of age prior to distribution to avoid high mortality when introduced as day-old chicks in the field directly.
Since launch of the scheme, till date, nearly Rs. 135 crore have been released covering more than 5.8 BPL beneficiary families.

**Egg Day Celebrations on 11th October, 2013:** International Egg Commission has declared the second Friday of October every year as World Egg Day. This is celebrated in countries all around the world, and is a unique opportunity to help raise awareness of the nutritional benefits of eggs. The Department of Animal Husbandry, Dairying and Fisheries, Government of India facilitated celebration of the “World Egg Day” on 11th October, 2013 to increase awareness on the nutritive value of eggs and highlight its importance in human nutrition. This was celebrated at New Delhi for Northern Region, Pune for Western Region, and Bengaluru for Southern Region.

Department of Animal Husbandry, Dairying and Fisheries, Government of India has decided to coordinate and facilitate organizing this event by involving stakeholders and Poultry Associations like National Egg Coordination Committee, Poultry Federation of India etc. to spread valuable information and knowledge about the nutritive value of egg.

The program was organized at Tihar Jail in New Delhi with jail inmates, personnel etc. while in Pune, Raipur and Bengaluru with school children along with accompanying parents and teachers, etc. and information on the importance of eggs was given. In some places, specific organizations, like 8000 women workers in garment factory at Bengaluru were distributed eggs and mementos, pamphlets, brochure, and Recipe Book etc. The literature prepared and distributed on this occasion helped them understand the goodness of egg for better health. The contents of the literature are very basic, easily understandable and in multiple languages depending on the place/ region where it is celebrated. The egg recipes include both indigenous and exotic preparations like egg biryani, egg roll, egg curry, egg noodles, egg frittata, Malaysian Sweet and sour eggs, egg club sandwiches etc. which elicited a lot of interest.

In India the level of awareness about eggs is low and this event of celebrating egg day was a stepping stone to not only spread the knowledge of value of eggs in human nutrition, but also to encourage the private poultry farmers to pledge support to schools, hospitals and orphanages, supplying eggs to help provide the essential nutrition required and improve people’s overall health and diet. However, this is only voluntary and a continual effort is required to enable dissemination of information through various fora involving doctors, nutritionists, educationists, women & child institutions, concerned policy makers, and of course, layer & egg processing industry.

**Conclusion:** The global food system will experience an unprecedented confluence of pressures over the next 40 years. On the demand side, global population size will increase to over nine billion by 2050. Further, the main issue of food security is not currently one of supply but of demand. The 925 million undernourished people are not due to deficit in global food supply, but because distribution is skewed. Either they cannot afford to buy food or they live in inaccessible places or societies. There is a need for urgent action in the global food system as a plethora of factors converge to impact demand, production and distribution of food over the next 4 decades. These drivers for change are the expected growth in the world human population; increase in incomes globally; urbanization, competition for land, water and energy resources; the need to reduce greenhouse gas emissions; and adaptation to a changing climate (Foresight, 2011).

The significance of sustainability and agro-ecological approaches are highly significant not for current situation only but to plan for the long-term future as well. Further these approaches are multi-inter-disciplinary in nature and cross over many specialized domains. Awareness creation among the masses about nutritive value of eggs and poultry also would help in an equitable uptake of the products.

Reduction of wastes requires a balancing act among animal welfare (suggesting extensive farming), productivity (more intensive farming), emission reduction and safety (certified biosecure farming and no recycling of animal products through livestock). However, this would be a major area
to recoup the apparent waste for eventual feeding the mushrooming populace.

References


♣♣♣
Applications of Electrocardiography in Physiological and Clinical Research

J.P. Varshney
Sr. Consultant Medicine, Nandini Veterinary Hospital, Surat

Electrocardiography is a non-invasive diagnostic tool that is used to record the activity of the heart in man and animals. It provides a graphic record of the voltage produced by cardiac muscle cells during atrial and ventricular depolarization and repolarization plotted against time. The instrument is being used most commonly in routine practice in human medicine and has gained popularity in veterinary medicine also for health check up and diagnosis of cardiac problems. A normal electrocardiogram does not rule out cardiac malformation or other cardiac changes. An abnormal ECG may suggest side of the heart affected, disturbance of rhythm or rate. The present deliberation will cover development of electrocardiography, its basics, electrode placement, different lead systems, normal wave forms, uses of electrocardiography, its application in physiological and clinical research, ECG in health and diseases in different species of animals.

Mile Stones in the Development of Electrocardiography

1616  Harvey  Blood circulation is due to heart beat
1786  Dr. Galvani, Italian Physiologist  Noted electrical current from skeletal Muscle
1842  Dr. Carlo, Prof. Physics  Recorded electrical current associated with heart beat in frog.
1887  Dr. Waller, Br. Physiologist  Published 1st human ECG using capillary electric meter. Demonstrated electrical circuity preceded ventricular contraction. Later shown similar activity in cat heart.
1891  Dr. W. Bayliss & Edward Starling, Br. Physiologists  Demonstrated triphasic cardiac electrical activity in each heart beat using improved capillary electrometer.
1893  Dr. Einthoven, Nobel Laurete Dutch Physiologist  Refined Capillary electrometer and demonstrated 5 deflections and coined P,Q,R,S,T terminology
1911  Dr. Einthoven  Developed new string Galvanometer. Beginning of clinical Electrocardiography.
1922  Dr. Nurr, Veterinarian  Clinical use of ECG in dogs

Uses of Electrocardiography: Electrocardiography can be used for routine health checkup, cardiac monitoring during anesthesia and surgery, evaluation of trauma, evaluation of heart size, shape, rhythm and rate, evaluation of electrolyte disorders, routine presurgical examination and for preventive health check up in animals, and creation of physiological data base.

Utility of Electrocardiography: It adds a new dimension to the diagnosis and treatment of disease states. It is non-invasive and quick easy tool in clinics and its results are immediately available. It can provide differential diagnosis of arrhythmias in all class of animals. Cardiac patients can be evaluated in a better way. ECG provides visual records of cardiac status.

Different Lead Systems

A. Bipolar Standard lead system—consists of lead I, II and III. These leads compare potential difference of two limbs’ and evaluate activation of the heart in the frontal plane.
B. Augmented unipolar limb lead system- The augmented unipolar limb leads (aVR, aVL and aVF) are the additional leads. An augmented lead compares the electrical potential at the reference limb to the sum of electrical activity at the other two limbs.

C. Special Lead System-Unipolar precordial chest leads (CV5RL, CV6LL, CV6LU and V10) view the heart from the transverse plane and provides important information for the diagnosis of heart enlargement (right or left), bundle branch blocks, ischaemic heart changes and arrhythmias.

D. Base-Apex Lead system – LA, RA and RL leads are used.

**Placement of electrode in different Lead systems**

Before putting the electrodes both electrode and skin are moistened with electrocardiographic gel, paste, or alcohol. Electrodes are attached directly to the skin.

RA & LA - Electrodes are attached proximal to the olecranon on the caudal aspect of the respective forelimbs.

RL & LL - Electrodes are attached over patellar ligament on the anterior aspect of the respective hind limbs.

V leads or unipolar precordial chest leads are attached as follows:

- Lead CV5RL (rV2) - at 5th right intercostals space near edge of sternum
- Lead CV6LL (V2) - at 6th left intercostals space near edge of sternum.
- Lead CV6LU (V4) - at 6th left intercostals space at costo-chondral junction.
- Lead V10 - over dorsal spinous process of 7th thoracic vertebra.

**Base- Apex Lead System**

Lead RA - lower one third left or right jugular furrow

Lead LA - Left chest just caudal to point of elbow on 5th intercostals space.

Lead RL - Wither or just cranial to right scapula.

**Lead Systems being used in different Animals**

- Hexaxial Lead System - Dog, Cat, Birds, Pigeon, Snake, Tortoise, Rabbits, Mice, Squirrel
- Base-Apex - Cattle, Buffalo, Goat, Sheep, Horse, Donkeys

**Recording of ECG in Different Lead systems**

- Hexaxial Lead System - All six leads + special precordial 4 leads also (6 or 10 lead recording)
- Base-Apex Lead System - Lead I

**Electrocardiogram:** It is a graphic representation of the voltage and direction of electrical activity produced during depolarization and repolarization of cardiac muscle cells plotted against time. ECG provides an information about heart rate, its rhythm, enlargement of chambers, conduction defects, myocardial diseases or ischaemia, certain electrolyte imbalances (hypocalcaemia, hypopotassaemia, hypercalcaemia or hyperkalaemia) and some drug toxicities.

**The shape of the ECG:** In ECG atrium depolarization is represented by ‘P’ wave. The ventricular mass is large, so there is a large deflection of the stylus during ventricular contraction leading to formation of ‘QRS’ complex. Repolarization of ventricular mass leads to formation of ‘T’ wave.
**What to expect from an ECG:** ECG is a tool, and not as an end in itself. It is essential for the diagnosis and treatment of cardiac arrhythmias. Since treatment of arrhythmias is specific, error in diagnosis may result in fatalities. ECG is no panacea as it has its own limitations. It can not detect mechanical status of the heart; pathology of valves, coronary arteries, endocardium and pericardium; and predict prognosis always. Therefore, always consider ECG as a part of clinical findings and interpretate in conjunction with history, clinical findings and other laboratory investigations.

**Normal Cardiac Waveforms:**

<table>
<thead>
<tr>
<th>Wave Forms</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘P’</td>
<td>Atrial depolarization. Normally positive in lead II and aVF.</td>
</tr>
<tr>
<td>‘P-R’ interval</td>
<td>Time from onset of atrial muscle depolarization through conduction over AV node, Bundle of His and Purkinje fibers.</td>
</tr>
<tr>
<td>‘QRS’ complex</td>
<td>Depolarization of ventricular muscle. Q is first negative deflection, ‘R’ the first positive deflection and ‘S’ the second negative deflection.</td>
</tr>
<tr>
<td>‘J’ Point</td>
<td>End of QRS complex</td>
</tr>
<tr>
<td>‘S-T’ segment</td>
<td>Period of phase-2 of action potential.</td>
</tr>
<tr>
<td>‘T’</td>
<td>Ventricular muscle repolarization.</td>
</tr>
<tr>
<td>‘Q-T’ interval</td>
<td>Total time of ventricular depolarization and repolarization.</td>
</tr>
</tbody>
</table>

**Future Applications of Electrocardiography in physiological Research**

- Myocardial cellular electro-physiology of different species of animals
- ECG signals reflect the electrical activity of the heart. Though it has been analysed for clinical application, research around its feasibility in identification frame work is rather very new and several model studies are in vogue in humans. Inter individual variability of ECG is due to differences in size and location of the heart and physiological factors as gender, habits, age, height, weight and very many factors. Now it is being focused to use highly distinctive features from ECG signals for biometric application.
- Exercise ECG
- Heart rate variability (technique- vaso vagal tonus index-a valuable tool in analyzing heart rate variability).
- ECG averaging function
- R heights
- QT corrected (technique Bazett’s formula). Standard formulas may be developed for increase or decrease in heart rate.So that correct interval of QT may be worked out for exact interpretation.
- To develop and standardize wireless ECG for freely moving and restrained farrocious animals.
- Creation of dynamical models for generating ECG signals.Synthetic ECG can be generated with different sampling frequencies and different noise levels.Such models have application in 1. Simulating abnormal beats,2. To produce multi lead ECG signals, 3. To assess the effectiveness of different techniques for noise and artifact removal, 4. To generate corrected QT independent of HR and 5. To generate realistic ECG despite noise and other artifacts.
- Development of high resolution surface ECG mapping for assessing local myocardial repolarization to detect minor myocardial lesions.
- ECG data under different physiological states of the animals.
- Development of dry electrodes for prolonged use without skin preparation.
- Development of MRI smart amplifiers to improve advanced signal processing circuitry

**ECG in Clinical Research and Investigations**
- Arrhythmias (Tachycardia, or Bradycardia or irregular heart beat)
- Cardiomyopathy
- Assessing cardiac injury in systemic diseases.
- Shock
- Sudden onset of Dyspnoea, syncope or seizures.
- Cardiac murmurs
- Increased auscultation area of the heart.
- Renal disease.
- Endocrinopathies (Addition’s disease, Cuchings syndrome, thyroid dysfunctions).
- Systemic diseases (Pyometra, pancreatitis, uremia, neoplasms) affecting heart.
- Acid-base and or ionic imbalances.

**Limitations of electrocardiogram**
- ECG serves as a rough guide for evaluating the heart and should, therefore, be interpreted in conjunction with clinical state of the ailing animals as its interpretation in isolation may be misleading.
- It reflects functional status of the heart only and not of mechanical status.
- Pathology of valves (mitral, tricuspid, pulmonary, and aortic), coronary arteries, endocardium or pericardium cannot be diagnosed by ECG.
- Significant cardiac disease may sometimes produce only minor changes or no change at all in electrocardiogram. Hence it should be used in conjunction with complete clinical history, clinical examination and other diagnostic procedures.
- Species, body conformation and breeds of the animals may alter mean accepted measurements. Hence standard measurements for different species and breeds are to be worked out.

**References**


Changkija,B. (2007).Electrocardiographic studies in dogs with reference to management of cardiac tachyarrhythmia by alternate drugs. Ph.D. Thesis in Veterinary Medicine, submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar (Bareilly)


♦ ♦ ♦
One of the most interesting facts about the renal circulation is that during marked changes in renal blood flow (adrenalin ischemia and pyrogenic hyperemia) the rate of glomerular filtration typically remains unchanged. This fact has been attributed to the circumstance that the changes in renal blood flow are mediated primarily by changes in the tonus of the efferent arterioles; consequently, any increase or decrease in blood flow is accompanied by a reciprocal change in glomerular filtration pressure, with the result that the filtration rate remains unchanged (Diaz and Wood, 2006).

Most, if not all vascular beds display active stabilization of their blood flow when blood pressure fluctuates. This process can be demonstrated in an isolated preparation perfused with a defined medium, thus precluding any central neural or hormonal input. It is termed autoregulation because the entire process of sensation, transduction, and actuation occurs within the organ or tissue in question. The kidney displays highly efficient autoregulation so that under steady-state conditions, renal blood flow (RBF) is independent of blood pressure over a wide range of pressure. In many organs, flow is regulated and autoregulated to satisfy the metabolic needs of the parenchyma. In the kidney, however, the situation is reversed; parenchymal metabolic work is a function of RBF. More specifically, it depends on glomerular filtration rate (GFR) and thus the amount of sodium that is to be reabsorbed. This concordance of RBF and metabolic rate makes putative metabolic mechanisms of autoregulation unlikely. Two different rationales have been proposed for the importance of autoregulation i.e. regulation of body salt content and fluid balance on the one hand and preservation of glomerular structure on the other (Levey et al. 2005).

Chronic kidney disease (CKD) is a very real and growing problem, as indicated by demographic trends. An increasing number of diabetic patients is also an important factor for manifestation of such disease. CKD is characterized by progressive deterioration of kidney function, which develops eventually into a terminal stage of chronic kidney failure (CKF). CKF has traditionally been categorized as mild, moderate, or severe. Other poorly defined terms like uremia and end-stage renal disease (ESRD) have commonly been applied. During the last few years, an international consensus has emerged categorizing CKF into five stages according to the glomerular filtration rate (GFR) and presence of signs of kidney damage:

Stage 1: GFR > 90 ml/min and signs of kidney damage
Stage 2: GFR = 60-89 ml/min and signs of kidney damage
Stage 3: GFR = 30-59 ml/min
Stage 4: GFR = 15-29 ml/min
Stage 5: GFR < 15 ml/min (Levey et al. 2005).

Stage 5 represents the total inability of kidneys to maintain homeostasis, and this metabolic state is incompatible with life. Thus, at this stage, it is necessary to use methods that substitute for kidney function to ensure patient survival; these methods include peritoneal dialysis, hemodialysis, and other extracorporeal purifying procedures, or kidney transplantation. CKF is associated with many kinds of metabolic changes caused by the kidney disease and also attributable to dialysis treatment (Mehta et al. 2007).
RIFLE - Risk, Injury, Failure, Loss, End-stage kidney disease, AKIN - Acute Kidney Injury Network.

Phenomena such as accumulation or deficit of various substances and dysregulation of metabolic pathways combine in the pathogenesis of these changes. In the process of accumulation, decreased urinary excretion plays a crucial role and leads to retention of metabolites in the organism (e.g. creatinine, urea, electrolytes, water). The increased formation of metabolites through catabolic processes and alternative metabolic pathways also exerts an influence. Regular dialysis treatment partly decreases this accumulation, but cannot avert the overall deficit (Cibulka et al. 2005).

<table>
<thead>
<tr>
<th>Table 1. The RIFLE and AKIN Criteria for the Diagnosis of AKI (adapted from references 6,7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RIFLE Class</strong></td>
</tr>
<tr>
<td>Risk</td>
</tr>
<tr>
<td>Injury</td>
</tr>
<tr>
<td>or serum creatinine &gt;4 mg/dL with an increase of at least 0.5 mg/dL</td>
</tr>
<tr>
<td><strong>AKIN Stage</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

**Acid-base balance:** Acid–base disorder is commonly observed in the course of chronic kidney failure. Metabolic acidosis is noted in a majority of patients when GFR decreases to less than 20 to 25 % of normal. The degree of acidosis approximately correlates with the severity of CKF and usually is more severe at a lower GFR (Kavacic et al., 2003). Metabolic acidosis can be of the high-anion-gap type, although the anion gap can be normal or only moderately increased even with stages 4 or 5 of CKF. In mild chronic renal insufficiency, metabolic acidosis is the result of a reduced ability to reabsorb bicarbonate, to excrete ammonia, and to eliminate titratable acid excretion (hyperchloremic, normal anion gap acidosis). In more severe renal insufficiency, organic and other conjugate anions of acids (nonvolatile acids) cannot be sufficiently excreted, and elevated anion gap acidosis appears (Kraut and Kurtz 2005).

**Protein metabolism:** A strict low-protein diet can have a negative effect on nitrogen balance in the predialysis period. A safe low-protein diet should contain a minimum 0.6 g of protein/kg/day. Disorders in protein metabolism in the dialysis period are usually caused by combined (protein and energy) malnutrition that can be termed uremic malnutrition. It is present in approximately 20-50 % of patients on dialysis and is characterized by insidious loss of somatic protein stores (reflected in lean body mass and serum creatinine) and visceral protein concentrations (reflected in serum albumin and prealbumin concentrations) (Franch and Mitch, 2009).

Urinary losses of protein and losses of amino acids during a dialysis session may also play a role. Metabolic acidosis is an important factor that markedly contributes to negative nitrogen and total body protein balance in CKF (Mehrotra et al. 2003). It has been demonstrated that the presence of uremic malnutrition increases mortality and morbidity in chronic dialysis patients. It is very often combined with a chronic inflammation state in the malnutrition inflammation complex syndrome (MICS).

**Carbohydrate metabolism:** Disorders of carbohydrate metabolism are also very frequent in CKD. Diabetics represent about 35 % of all patients on dialysis therapy. Furthermore, non-diabetic CKD patients often have glucose intolerance, probably because of peripheral insulin resistance. Insulin resistance is primarily detectable when the GFR is below 50 ml/min. Reduced insulin-mediated nonoxidative glucose disposal is the most evident defect of glucose metabolism, but impairments of glucose oxidation, the defective suppression of endogenous glucose production, and abnormal insulin secretion also contribute to uremic glucose intolerance (Rigalleau and Gin 2005).
Accumulating nitrogenous uremic toxins seem to be the dominant cause of a specific defect in insulin action, and identification of these toxins is progressing, particularly in the field of carbamoylated amino acids. The consequences of CKF, such as exercise intolerance, anemia, metabolic acidosis, secondary hyperparathyroidism, or vitamin D deficiency, also indirectly play a role. It has been reported that insulin resistance may be related to arterial hypertension and may contribute to high cardiovascular morbidity and mortality in patients with CKF. The underlying mechanism can be an impaired synthesis of nitric oxide (NO) in the endothelium of patients with CKD. It was reported that appropriately functioning endothelial NO synthase (eNOS) is important for the control not only of arterial pressure but also of glucose and lipid homeostasis (Rigalleau and Gin 2005).

**Lipid metabolism:** Serum triglycerides (TG) are elevated in CKF because of enhanced production of TG-rich lipoproteins such as very-low-density lipoproteins (VLDL) in the liver and also because of dysfunction of TG degradation resulting from insufficient mitochondrial beta-oxidation of fatty acids. It can be caused by a deficit of L-carnitine, which is frequently present, especially in hemodialysis patients (Cibulka et al. 2005). Hyperinsulinemia is the main factor increasing synthesis of TG and also directly decreasing the activity of lipoprotein lipase. The most prominent changes in lipid metabolism found in many patients with CKF are increased serum TG levels and low levels of high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol levels are often normal, but the cholesterol may originate from the atherogenic small and dense LDL subclass (Cibulka et al. 2005).

The apolipoprotein B-containing part of the lipoprotein may undergo modifications (peptide modification of the enzymatic and advanced glycation end-products, oxidation or glycation). Modifications contribute to impaired LDL receptor mediated clearance from the plasma and promote prolonged circulation. HDL particles are structurally altered during the states of inflammation. The contribution of this complex atherogenic form of dyslipidemia to cardiovascular disease (CVD) in patients with renal disease is at present not clear. Therefore, hypercholesterolemia, obesity, and increased blood levels of creatinine and homocysteine appear to be protective and paradoxically associated with a better outcome in patients with CKF (Zhao, 2013).

**Water and electrolytes:** The fluid adjustment should be made according to edema and dehydration in the patient. In hemodialysis (HD) patients, if conditions such as swelling of the eyes, hands or feet, fluid weight gain, shortness of breath, increased blood pressure or tachycardia are observed, fluid consumption should be restricted. Hemodialysis patients should reduce fluid intake and should limit food consumption such as tea, coffee, soda, water, fruit juices, ice cream, sherbet, gelatin, soups and heavy sauces. Dietitians, especially renal dietitians, are most often cited as the trusted source on providing information on fluid management and delivering dietary advice (Smith 2010).

Controlling sodium and fluid intake are important components of the HD diet. Extracellular volume expansion is the main pathophysiologic determinant of hypertension in HD patients. Water and sodium intake in hemodialysis patients are adjusted according to the amount of urine, fluid balance and blood pressure. With hemodialysis, potassium restriction is often necessary, but the measure of restriction depends on residual renal function (Stark 2011). Sodium restriction should be based on the amount of urine. A mild salt restriction as 3-4 g / day is sufficient in oliguric patients that have an amount of urine totaling more than 1 liter per day. Anuric hemodialysis patients may consume up to 1 liter of liquid and 1.5-2 g / day of salt

Potassium levels are affected by hemodialysis therapy with the degree of residual renal function and net tissue breakdown (e.g. due to infections) and acid-base status. In HD patients, serum potassium concentrations may change to net intestinal potassium absorption or excretion. An example of this change or excretion is diarrhea. Serum potassium is impressed by dietary potassium intake. It
is thought this relationship is stronger when the potassium intake is very low or very high in diets of HD patients. Potassium restriction is often required because hemodialysis patients are usually anuric (Thakar et al., 2009).

**Effect of sodium chloride Intake on urine volume and urinary urea excretion:** Spek et al. (2012) found out that Milk urea nitrogen (MUN; mg of N/dL) has been shown to be related to excretion of urinary urea N (UUN; g of N/d) and total excretion of urinary N (UN; g of N/d) in dairy cows. In the present experiment, it was hypothesized that MUN and the relationship between MUN and UUN or UN is affected by urine volume as a result of dietary sodium chloride intake. Twelve lactating Holstein-Friesian dairy cows (mean ± SD: milk production 28.1 ± 3.23 kg/d and 190 ± 41 d in milk), of which 4 were fitted with catheters in the urine bladder and jugular vein, were randomly assigned to 4 dietary levels of sodium chloride (3, 9, 14, and 19 g of Na/kg of DM) according to a triple 4 × 4 Latin square design. Cows were fed at 95% of ad libitum intake, excluding salt addition.

Milk was analyzed for MUN and protein content; urine was analyzed for total N, urea, and creatinine content; feces were analyzed for total N and DM content; and blood plasma was analyzed for urea and creatinine content. Creatinine clearance rate (CCR; L/min) and renal urea reabsorption ratio were estimated based on plasma concentrations of urea and creatinine, and total excretion of urea and creatinine in urine. Intake of DM and N, milk production, and milk protein content were (mean ± SD), on average, 21.4 ± 1.24 kg/d, 522 ± 32.0 g/d, 25.4 ± 2.53 kg/d, and 3.64 ± 0.186%, respectively. A linear relationship was found between Na intake and urine production [urine (kg/d; mean ± SE) = 7.5 ± 4.33 + 0.136 ± 0.0143 × Na intake (g/d)] and between Na intake and MUN [MUN (mg/dL; mean ± SE) = 13.5 ± 0.35 - 0.0068 ± 0.00104 × Na intake (g/d)]. Despite the decrease in MUN with increased Na intake, UN excretion increased linearly with Na intake. Excretion of UUN was not affected by dietary Na content. A linear plateau relationship was observed between CCR and renal urea reabsorption. An increase in CCR coincided with an increase in calculated renal urea reabsorption until a CCR breakpoint value (mean ± SD) of 1.56 ± 0.063 L/min was reached. We conclude that Na intake is negatively related to MUN, whereas UUN is not affected. Variation in mineral intake levels that affect urine volume should, therefore, be taken into account when using MUN as an indicator of UUN in dairy cattle.

**Vitamin and Minerals:** Some studies demonstrate vitamin and mineral supplements for the long-term hemodialysis (HD) patients. Hemodialysis patients are potentially at risk of deficiency and excess of trace elements. Given that essential trace elements play key roles in multiple biological systems, including immunological defense against oxidation and infection. It has been hypothesized that the increased morbidity and mortality seen in hemodialysis patients may in part be due to the imbalance of trace elements that has not yet been recognized In HD patients, there are many problems associated with the lack of food intake. Poor nutrition, restriction of foods that are rich in water-soluble vitamins, foods that are rich in potassium, metabolic disorders caused by uremia, infection and diseases such as gastrointestinal diseases or complications associated with reduced intake of foods are some of the possible scenarios. The lack of foods containing vitamins leads to vitamin and deficiencies that can lead to further possible complications in dialysis patients (Coombes, 2012).

**Table 1: Recommended dietary nutrient intake for Chronic Kidney Disease patients**

<table>
<thead>
<tr>
<th>Polyunsaturated fatty acids</th>
<th>Up to 10% of total calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monounsaturated fatty acids</td>
<td>Up to 20% of total calories</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Rest of calories (complex carbohydrates preferred)</td>
</tr>
<tr>
<td>Total fiber</td>
<td>&quot;/&gt;20–25 g/d</td>
</tr>
</tbody>
</table>

**Minerals and Water (Range of Intake)**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>750–2000 mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>2000–2750 mg/d</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>800–1000 mg/d</td>
</tr>
</tbody>
</table>
Calcium | <1000 mg/d
---|---
Magnesium | 200–300 mg/d
Iron | 10-18 mg/d
Zinc | 15 mg/d
Selenium | 55 μg/d
Water Usually | 750–1500 mL/d

**Vitamins (Including Dietary Supplements)**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 (thiamin)</td>
<td>1.1–1.2 mg/d</td>
</tr>
<tr>
<td>B2 (riboflavin)</td>
<td>1.1–1.3 mg/d</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>5 mg/d</td>
</tr>
<tr>
<td>Biotin</td>
<td>30 μg/d</td>
</tr>
<tr>
<td>Niacin</td>
<td>14–16 mg/d</td>
</tr>
<tr>
<td>B6 (pyridoxine)</td>
<td>10 mg/d</td>
</tr>
<tr>
<td>B12</td>
<td>2.4 μg/d</td>
</tr>
<tr>
<td>C</td>
<td>75–90 mg/d</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>1–5 mg/d</td>
</tr>
<tr>
<td>A</td>
<td>800-1000 μg/d</td>
</tr>
<tr>
<td>D</td>
<td>1000-1500 IU</td>
</tr>
<tr>
<td>E</td>
<td>400–800 IU</td>
</tr>
</tbody>
</table>

Chronic kidney disease leading to chronic kidney failure is an urgent medical problem in the context of demographic trends. In addition to the basic kidney disease, many metabolic disorders develop in the course of CKF. Particularly, patients in the terminal stage of CKF are endangered. Regular dialysis treatment decreases the accumulation of metabolites; however, it contributes to a deficit of some important metabolic regulators and to the development of a chronic inflammation state. These factors can lead to serious secondary complications in CKF, including atherosclerosis and related cardiovascular disease, malnutrition, anemia, renal bone disease, and other problems. These complications markedly and negatively affect the prognosis and quality of life of patients with CKF and increase costs for their treatment. The prognosis of CKD patients can be improved if kidney disease is diagnosed early and properly cured, including secondary complications. Appropriate treatment encompasses consistent control of blood pressure, prevention of malnutrition, anemia, and hyperparathyroidism, and treatment of metabolic disorders. It is necessary to search actively for kidney disease and to develop new therapeutic methods to improve the quality of life of CKD patients.

**References**


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Cardiovascular and hemodynamic changes in endotoxemia and physiological interventions for animal welfare

D.V. Singh
Dept. of Veterinary Physiology & Biochemistry, COVS, GADVASU, Ludhiana

Endotoxic shock is an acute circulatory failure occurring in the presence of severe infection and represents an imbalance between the body’s oxygen demand and supply. It is principally of the distributive type, although cardiogenic and hypovolumic components may also be involved (Vincent and Backer 2001). Endotoxin is an integral component of the outer membrane of gram-negative bacteria and the effects of exposure of host cells to it include, the uncontrolled release of cytokines and eicosanoids, kinins and other short, medium and long-term reactants that upset the balance between pro-inflammatory and anti-inflammatory pathways, causing hypotension, disseminated intravascular coagulation, abortion and death (Hodgson 2006). Endotoxemia is a life threatening inflammatory condition, which can lead to shock, multiple organ failure, suppression of immune system and interferes with wound-healing processes (Ng et al. 2008). Infections with gram-negative bacteria and resultant endotoxemia are a common cause of morbidity and mortality in cattle of all ages. Neonatal calves deprived of adequate amounts of colostrums are among the most susceptible to gram negative bacterial infections. Even among calves with adequate passive immunity, gram negative bacterial sepsis remains a substantial problem (Morris et al., 1986). Shock is a progressive deterioration in the microcirculation due to inability of the cardiovascular system to maintain blood pressure and flow (Allen 1998).

During shock there is substantial morbidity and mortality in cattle especially neonates (Gerros et al 1993). Endotoxemia is a potentially devastating complication of several diseases of cattle and buffalo like enteric diseases, neonatal septicemia, metritis, coliform mastitis, colibacillosis and pneumonia (Semrad 1993). Endotoxemia is a state of high levels of endotoxin in blood circulation. The prevalence of septicemic shock in domestic animals has not been as well enumerated but this form of circulatory and metabolic dysfunction doubtlessly represents an important problem in veterinary internal medicine (Bottoms et al 1992).

Cows develop depression, shivering, salivation, anorexia, hyperthermia, tachycardia, ruminal stasis and diarrhea on i/v injection of E. coli LPS (@ 0.01, 0.1 and 1 μg / kg consecutively) at three weeks interval. LPS is implicated in several common bovine diseases, particularly in gram negative infections such as coliform mastitis, neonatal coliform septicemia, pastuerellosis, salmonellosis. It has also been linked to the development of the non-infectious diseases such as ruminal acidosis, laminitis and displaced abomasum. Its potential to cause harm is particularly important in cattle which appears to be several thousand times more sensitive to LPS than common laboratory species. Even low dose exposure may therefore have a severe effect on dairy cows( Jacobsen et al., 2005). Therefore, the consequences of endotoxemia are either a considerable morbidity or mortality of animals leading to severe economic losses to the dairy farmers due to decrease in production or death of the productive animals or both.

Despite many limitations, animal models remain essential for the development of new therapeutic regimens of endotoxic shock, which can’t be replicated by in-vitro studies (Garrido et al 2004).

Cardiovascular Hemodynamics: Infusion of endotoxin leads to a decrease in ‘T’ wave, its reversal and 2nd degree AV conduction block which could not be removed after i/v infusion of Flunixin meglumine or Ketanov alone or in combination (Singh and Bansal 2013). A highly significant fall in mean systolic, diastolic, pulse, MAP, CVP, haemoglobin was observed till end of the endotoxin infusion while respiratory rate was significantly elevated along with a non significant alteration in rectal temperature and hematocrit during the infusion of endotoxin (Singh and Bansal 2008).
Immediately on endotoxin infusion the systolic and diastolic pressure decreased sharply and remained so not only till end of infusion but the hypotension persisted till end of observation.

Endotoxemia increases ventilation to perfusion ratio in humans while in animals it causes pulmonary vasoconstriction and impairs hypoxic pulmonary vasoconstriction. Because endotoxemia frequently complicates the acute respiratory distress syndrome (ARDS), the pulmonary vascular response to endotoxin may, in part, be responsible for the increased ventilation to perfusion ratio heterogeneity and intrapulmonary shunt associated with ARDS (Gerbino et al., 2001).

Septic or endotoxic shock results from rapid liberation of endotoxin into circulation that results into cardiovascular collapse accompanied by severe peripheral vasodilatation, pallor of mucosa, cool skin and extremities, diarrhoea, decreased systemic blood pressure and muscle weakness (Radostits et al 2000). Perkowski et al (1996), reported that prolonged endotoxemia without volume support cause systemic hypotension associated with reduced cardiac output and increased systemic vascular resistance, pulmonary hypertension, and acute lung injury with progressive respiratory failure. Cardiovascular function, intravascular volume, vascular tone, integrity and patency are critical for normal blood circulation in body. Any abnormality in one or more of these components of circulation leads to compensatory changes to maintain normal perfusion. The haemodynamic and cellular changes that develop because of these abnormalities is called shock. As the shock progresses the oxygen and substrate delivery to the tissues become insufficient.

Garnier et. al., (2001) concluded that endotoxin severely impaired foetal cardiovascular control during normoxia and asphyxia, resulting in a considerable decrease in cerebral oxygen delivery. These effects might have important effects in the development in the foetal brain damage associated with intrauterine infections.

According to Adams et. al., (1990), organ damage is a consequence of peripheral vascular alterations, in conjunction with compromised cardiac function and direct tissue damage from localized inflammatory reactions. Peter et. al., (1990), found that endotoxin administration had profound effect on pulmonary function with severe hypoxemia accompanied by significant increase in partial oxygen levels and alveolar dead space to tidal volume ratio (Vd/Vt). Endotoxin also decreases oxygen consumption which was accompanied by an increase in systemic oxygen extraction ratio. Body temperature was not significantly altered by endotoxin administration.

The renal response to endotoxemia has been poorly characterized. Moderate decrease in renal blood flow has been observed in endotoxemic calves and goats. In the study conducted by Endotoxin was observed to cause significant reduction in GFR and urine production. These changes probably result from hypovolumia, systemic arterial hypotension and vasopressin release(Warner et. al., 1988).

Early changes in cardiopulmonary function occurred concomitantly with an elevation in tumor necrosis factor and thromboxane B2 in arterial blood. The sheep presented a persistent hyperdynamic state characterized by a significant increase in cardiac index. The pulmonary hypertension was maintained for the duration of the LPS infusion. On the other hand, the pulmonary vascular resistance had returned to near the baseline value by 16 h after the endotoxin infusion (Noda et. al., 1994).

An increased stroke volume and cardiac contractility (dp/dt) in endotoxemic calves was recorded following infusion of 4 ml/kg of 7% Nacl (acq.) which also contributed to the increase in cardiac output. The effects on stroke volume and cardiac contractility were maximal at 10 and 15 min, respectively after infusion. The cardiac contractility remained above baseline (i.e values measured before challenge) for 1 hr after resuscitation, but the stroke volume decreased immediately after peaking (Constable et. al., 1991). Sugi et. al., (1991) found an increase in the cardiac index between 8 and 12 h after infusion of LPS. The maximum elastance of Left Ventricle end-systolic pressure-volume relations significantly decreased (2.88 +/- 0.27 mmHg/ml) as compared with baseline (3.89 +/- 0.50 mmHg/ml). Other indices of the LV contractility (maximum pressure development and
ejection fraction) were also reduced. There was a simultaneous increase in the LV end-systolic and diastolic volumes, thereby confirming that there is a myocardial depression during LPS in the ovine model.

Singh and Sodhi (1996) studied, endotoxic shock particularly in buffalo calves and reported a prominent bradycardia with A-V block accompanied by sudden fall in CVP to zero or even below.

Perkowski et al., (1996), studied changes in cardiovascular and pulmonary function during prolonged endotoxia in conscious sheep and found prolonged endotoxia without volume support caused systemic hypotension associated with reduced cardiac output and increased systemic vascular resistance, pulmonary hypertension, and acute lung injury with progressive respiratory failure. Plasma tumor necrosis factor-alpha (TNF-alpha) concentrations also found to increased transiently and concluded that vascular changes in endotoxia include increased vascular permeability, changes in vascular tone, and microvascular obstruction. Increased capillary permeability promotes transmural movement of albumin and other colloids, which carry water to the interstitial space. The result is interstitial edema, pulmonary edema, hypovolemia, decreased return to the heart, and further decreases in cardiac output. Arterial and arteriolar vasoconstriction develops in the systemic pulmonary circulations. Prolonged infusion of endotoxin into sheep causes systemic hypotension, pulmonary hypertension and acute lung injury with progressive respiratory failure.

Spath et al., (1994), examined the hemodynamic response of awake sheep to prolonged endotoxin infusion (0.1 μg / kg / min for 12 h) and the in vitro endothelium-dependent relaxation of pulmonary arterial vessels excised 12 h after the end of endotoxin infusion and found that in seven of nine sheep, there was a maintained increase (4-68% of baseline) in pulmonary arterial pressure 24 h after the beginning of endotoxin infusion.

According to Constable et al., (1991), endotoxin induced considerable decrease in cardiac index, mean aortic pressure and maximal change of left ventricular pressure, severe pulmonary arterial hypertension and increased pulmonary vascular resistance was evident at the end of endotoxin infusion.

Fenner, (1991), reported that during endotoxic shock, an immediate decline in blood pressure accompanies a considerable redistribution of blood flow. Central volume is reduced, resulting in inadequate delivery of oxygen and nutrients to tissues.

Peter et al., (1990) were of the view that administration of endotoxin induces significant decrease in cardiac index, stroke volume and GFR. Femoral and mesenteric artery blood flow and urine flow also show marked reduction. Peak effects were observed at the end of endotoxin infusion (30 min) followed by a gradual return towards baseline values after 2 hours. Endotoxin administration also induces significant decrease in MAP and transient increase in systemic vascular resistance (SVR).

Singh et al., (1982) were of the view that septic shock induced by strangulating a segment of the bowel in seven healthy buffalo calves aged between 6 months and one year altered the cardiac dynamics causing a peaked T-wave along with progressive increase in the amplitude, ventricular tachycardia and elevation of S-T segment consistently in all calves. In terminal stage, there was prolongation of the QRS complex

**Hematology**

Singh et al., (2005) recorded 23.68% decrease in plasma volume and corresponding 29.13% decrease in blood volume after 3 hours of infusion of endotoxin. hematocrit and hemoglobin values showed increase up to 60 minutes of endotoxin infusion in both treated and untreated groups, which decreased to much below the normal levels through out the rest of observation

Singh & Sodhi, (1991), found significant decrease in hematocrit, haemoglobin which was attributed to the excessive loss of blood during haemorrhagic shock and haemolysis with pooling of
blood in various organs like liver and intestine during endotoxic shock and TEC & TLC in buffalo and cow calves under the effect of hemorrhagic & endotoxic shock along with highly significant neutropenia due to shifting of circulating neutrophils into the marginal pool.

Stewart (1983), found that hypertonic saline-dextran solution (HSDS, 2400 mosm of NaCl with 6 % dextran) is superior to HSS in resuscitating haemorrhaged sheep. Beneficial effects of HSDS have been attributed to synergism between the hypertonic property of NaCl and hyperoncotic property of dextran. Dextran maintains the mobilized fluid from intravascular space in the intravascular compartment, though increasing colloidal osmotic pressure.

Olson & Brown (1985), observed that endotoxin decreased central plasma volume by 1 h and cardiac index by 3 h; hematocrit and plasma protein concentration were increased by 0.5 and 1.5 h, respectively, indicating a loss of plasma volume. These changes were also blocked or attenuated by Flunixin meglumine.

Broke et. al., (1989) compared the effects of I/V administration of dextran-70 and dextran-40 and suggested that Dextran-40 can be recommended as a plasma substitute due to its higher initial increase in circulating plasma volume, its moderate duration of effect and its low incidence of anaphylactic reaction. Administration of Flunixin meglumine, (potent inhibitor of eicosanoid synthesis), completely abolishes the effects of endotoxin on reticulo-rumen motility, thereby implicating eicosanoids as mediators of effects of endotoxemia on reticulo- rumen motility(Abramson et. al., 1990).

Walsh and Kramer, (1991) found that haematocrit and total plasma protein levels were decreased after infusion of hypertonic saline (7-7.5% NaCl) in conscious or anaesthetized sheep.

Constable et. al., (1991), found that following infusion of hypertonic saline in endotoxemic calves, their haematocrit and total plasma protein concentrations dropped. The values obtained before and after endotoxin administration and 1 h after resuscitation were respectively 30%, 35% and 32% for haematocrit and 5.2, 5.5 and 4.9 g/dl for the total plasma protein, remained up to 10% higher than pre-values in the Dextran-40 group throughout the experiment, infusion.

Eades, (1993) found that Flunixin meglumine abolished endotoxin-induced reticulorumen stasis, tachycardia, and synthesis of arachidonic acid metabolites. Reticulorumen stasis during bovine endotoxemia is caused either by enhanced synthesis of an arachidonic acid metabolite other than prostaglandin E2 or by local synthesis of prostaglandin E2. Flunixin meglumine administered before the infusion of endotoxin reduced the effects of endotoxin on both respiratory rate and body temperature. However it did not affect respiratory rate, heart rate and body temperature when administered after endotoxin administration.

Walker et. al., (1998), reported that intravenous infusion of a small volume of 10% dextran-40 in saline or 7.2% hypertonic saline solutions to normal, 3 months old Holstein calves were found to be effective in increasing plasma volume. Although the increase in relative plasma volume (rPV) of Dextran-40 group was lower than that of HSS group at the end of the fluid infusion, the increases in rPV remained up to 10% higher than pre-values in the Dextran-40 group throughout the experiment. Singh et. al., (1999), reviewed shock in bovines with special reference to buffalo calves and suggested three main types of solutions available for fluid therapy for hemorrhagic & endotoxic shock are isotonic, hypertonic crystalloid and colloidal solutions. It was suggested by Berchtold, (1999), that Dextran-40 infusion should be explored as a treatment for dehydrated calves since rapid infusion of Dextran-40 may be safer and more beneficial for rehydrating calves than HSS treatment. According to Oliviera et. al., (2002), the hemodynamic effects of HSS infusion in sepsis includes a rapid and significant increase in oxygen delivery, elevated cardiac output and increased oxygen extraction but these effects were transient.

Endotoxemic buffalo calves which were infused with hypertonic saline followed by Plasmex D-40 showed the near normal or pre endotoxic infusion levels being obtained by both systolic and
diastolic pressure. While the pulse pressure tended to show minor alterations, the mean arterial pressure (MAP) exhibited a severe decline till end of endotoxin infusion but in the treated group, the MAP attained a level close to pre-infusion values subsequent to hypertonic saline and Plasmex D-40 (Singh et al., 2003).

Asakura et al., (2005) found that glomerular fibrin deposition was significantly suppressed in the groups receiving urokinase (UK) when compared with the LPS group. No manifestations of bleeding were observed in any of the groups. Enhanced fibrinolysis and depressed plasma endothelin levels induced by Urokinase appear to play an important role in preventing the development of organ failure in the LPS-induced DIC model.

Treatment with a combination of HSS, plasmex-D-40 & blood, successfully alleviated hypovolumes, raised systolic, diastolic pressure, pulse pressure, mean arterial pressure and central venous pressure. The one time blood transfusion did not evoke any cross reaction & was helpful in raising hematocrit & haemoglobin values close to preinfusion values. The general symptoms of restlessness, respiratory distress, profuse salivation violent movement of the ears, snoaring & intermittent struggle were markedly reduced. All the treated animals become quiet and lay with eyes open (Singh et al. 2005).

Suzuki et al., (2005) showed that HSS and Dextran-40 infusions induced a significant increase in relative plasma volume at the end of fluid infusion. In the HSS group, CO, cardiac index (CI) and stroke volume (SV) remained constant at low levels after 90 min despite the maximal values of CO, CI and SV at the end of infusion. CI and SV in the D40 group showed significant increases at the end of fluid infusion.

Intravenous administration of Flunixin meglumine @ 1.1 mg/kg BW resulted in increase in systolic, diastolic, pulse, CVP and MAP close to preinfusion normal levels. No improvement in haemoglobin and respiratory rate was observed consequence to Flunixin meglumine administration (Singh and Bansal (2008) while a combination of hypertonic saline solution with Dexamethasone effectively restored various hemodynamic parameters to normal preinfusion values except respiration rate which remained high indicating respiratory distress(Ghuman and Singh 2009). Flunixin meglumine in combination with hypertonic saline solution has been reported to effectively restore various hemodynamic parameters close to normal pre-infusion values and it can be used as immediate resuscitation measure to provide the clinician valuable time to plan further long term treatment according to the needs of an individual animal (Singh et al., 2011).
Scopes and limitations in stem cell therapy: IVRI experience

G.Taru Sharma
Director CAFT, Head Physiology and Climatology Division, Indian Veterinary Research Institute, Izatnagar, India

Stem cells, that have remarkable potential to give rise to different cell types from the early life to the adult form, are termed as pluripotent stem cells. These cells are derived from the late stage embryos and serve a good reference model to understand important molecular signaling pathways which control cell fate choice and organ differentiation. However, these pluripotent cells show teratogenic quality, thus limiting its therapeutic application.

This information does not limit the scope of stem cell application as there are other sources and embryos are not the only available source of stem cells. Tissue derived stem cells are there in almost every organ. Undifferentiated cell found in a differentiated tissue in animals that can renew it self with certain limitations, differentiate to yield all of the specialized cell types of the tissue from which it originated. These cells reside in small pouches termed as stem cell niche; it protects stem cells which helps maintain the homeostasis of the cellular damage to the tissue due to wear and tear. These tissue based or adult stem cells exist throughout the body after embryonic development and a lot of different tissues representing potential sources of adult stem cells have been identified in different types of tissues such as bone marrow, umbilical cord, peripheral blood, brain, cornea, salivary gland, blood vessels, skin, liver, kidney, dental pulp, blood, and skeletal muscles etc. They remain in a quiescent or non-dividing state for years until activated by disease or tissue injury.

Autologous bone marrow/other adult tissue derived mesenchymal stem cells are being used for therapy, not only in livestock but also in equines and companion animals. Adult stem cells mediate repair via providing an anti-inflammatory effect, homing to damage tissues and recruiting other cells, such as endothelial progenitor cells, necessarily required for tissue growth, by supporting tissue remodeling over scar formation, through inhibiting apoptosis leading to differentiation. Transplanted site microenvironment significantly alters both the speed and capacity of engrafted stem cells for recovery and repair. Stem cell population efficacy could also be affected by delivery method for example to regenerate bone, stem cells are often introduced using a scaffold where they produce the minerals necessary for generation of functional bone. Potential uses of these adult stem cells in veterinary sciences are slower compared to the human counterpart for disease modeling, biomarker development and cell-based therapies for regenerative medicine.

Indian Veterinary Research Institute, Izatnagar has started clinical applications using autologus stem cells with pre-clinical experiments through small animal experimentations. Clinical trials on application of different stem cells such as in non-union fracture repair, spinal cord injury, wound and bone healing etc. are done at IVRI. Physiology and Climatology Division is working for more than a decade on different aspects of embryonic and adult stem cells (Sharma et. al.2012, Gade et. al. 2012, Puri et. al. 2012, Naresh et. al, 2013)), after proper characterization, both in vitro (Sharma et. al. 2012, Rajesh et. al, 2013) and in vivo (Verma et. al.,2012), presently we are trying to understand the molecular signaling mechanism for these cells. However, teratoma forming character of embryonic stem cells has the limitation for their use in the livestock and pet regenerative medicine, hence for the clinical applications, involving tissue repair and regeneration only adult mesenchymal stem cells are being used. These plastic adherent multipotent cells are capable of differentiating into osteogenic, chondrogenic and adipogenic lineages and can easily be isolated from several adult tissue types hence they are potentially stronger candidates for therapeutics and to study the cellular differentiation.

Depending upon different clinical conditions of livestock and pets, the choice of stem cells varies; the need of this hour is to compare stem cells from various sources for a particular clinical condition to find out one of those that provide the best replacement. Our experiments intended to
compare the xenogenic transplantation of these cells and its comparison with the allogenic transplantation. Stem cells from different origins (bone marrow, adipose tissue, Wharton’s jelly and amniotic fluid) could be expanded ex-vivo, characterized through different surface markers and multi-lineage differentiation potential, they were evaluated and compared for the wound healing potential with the xenogenic, allogenic and autogenic transplantations using rabbit as an experimental model (Udehiya et al. 2013). The healing was evaluated and compared on the basis of percent wound contraction, fibrosis, neo vascularization and density, thickness and arrangement of collagen fiber. Outcome was suggestive that the xenogenic stem cells are not rejected, results of transplantation studies will be discussed in details sharing cellular, molecular characterization with the healing detail. Off late we have started working on the secretomics, using the conditioned media, instead of stem cells. For the same experiments were designed using diabetic rat model for wound healing (Ansari et al. 2013), results were suggestive of its use in the clinical conditions with the comparable impact on the recovery. In general, any translational research has various phases, likewise the stem cell research too promises to provide the long term benefits for livestock and pets and we may divide it in to four phases; i) basic and fundamental research, ii) preclinical data generation, iii) clinical trials and iv) validation of the clinical work. Last two phases are very crucial from ethical point of view as well but to address them we essentially need to have the conceptual framework through first two phases. During the talk this presentation will focus on the issues related to the clinical trails and its validation for the commercialization of this technology.

References


Future prospects of stem cells in intervention in livestock reproduction

Kumar Dharmendra and Yadav P. S.

Buffalo Physiology and Reproduction Division, Central Institute for Research on Buffaloes, Hisar, Haryana

Abstract: Stem cells have long been of interest to biologists interested in reproductive systems, with the spermatogonial stem cell providing one of the best understood systems and niches in mammals in vivo. Although highly controversial, experiments which could be interpreted as indicating the existence of ovarian stem cells capable of regenerating the oocyte pool postnatal, have also rekindled interest in the search for stem cells in the female gonad. The idea of generating gametes in vitro has tremendous applications in treatment of infertility and understanding gametogenesis and it could also be a source for therapeutic cloning and regenerative medicine. In this review, we focus on the recent advancement of stem cells in reproduction vis-a-vis spermatogonial, ovarian and mammary gland stem cells and its future prospective in reproduction of domestic animals.

Introduction: Stem cells are those master cells that have two important characteristics that distinguish them from other cells. First, they are unspecialized cells capable of their own renewal for long periods through cell division and second, under certain physiological and experimental conditions, they can be induced to become cells with specialized functions such as the beating cells of the heart muscle, insulin-producing cells of the pancreas, epithelial cells, neurons, macrophages, fat producing adipose cells, germ cells etc. The stem cells can form any specific kind of cells depending upon the signal provided which is done by changing the chemical composition of the culture medium, by altering the surface of culture dishes or by inserting specific genes. These properties of stem cells opens new opportunities for regenerative medicine, provides new tools for drug discovery and toxicology, and creates new possibilities for the understanding of cancer biology. Besides these uses, it also provide a powerful tool for the studies of complex biological processes such as genomic imprinting and early embryonic development, gene targeting, cloning, chimera formation, transgenesis and gametogenesis, (Kumar et al. 2009, Kumar et al. 2011, Yadav et al. 2012). The availability of stem cells from various sources have excited our community of reproductive biologists, reflected in the number of their laboratories with a growing interest in gamete or germ cell-derived stem cells to inform new directions in this most fundamental area of biology.

In this direction, spermatogonial stem cells (SSC) provide a unique mammalian system where the entire process of stem cell self-renewal, progenitor formation and all stages of differentiation can be followed in a single organ, in situ. The idea of generating gametes in vitro has tremendous applications in treatment of infertility and understanding gametogenesis and it could also be a source for therapeutic cloning and regenerative medicine. Beside these, stem cells derived from female germ cells may play an important role in normal uterine and ovarian physiology. They likely are involved in the response of these tissues to injury and disease. The potential for these processes to be exploited for medical treatment is of great promise. Additionally, stem cells likely play a role in pathology of the reproductive tract. Stem cells give rise to cancers and endometriosis. A better understanding of stem cell biology may prove helpful in the treatment of these conditions. Finally the fetus, placenta and even the endometrium are all sources of stem cells. Endometrial-derived stem cells may provide an immunologically matched source of multipotent stem cells for tissue engineering and regenerative medicine (Du and Taylor, 2010). But, till now there are no widespread uses of stem cell-based therapies in reproductive medicine. However, the potential utility of such approaches makes them subjects of intensive research. In this review, we focus on the recent advancement of stem cells in reproduction vis-a-vis spermatogonial, ovarian and mammary gland stem cells and its future prospective in reproduction of domestic animals.
Prospective of stem cells in male reproduction: From a reproduction point of view, one of the most exciting derivate cell types from stem cells has been SSCs also known as testicular stem cells, providing one of the best implicit systems and niches in mammals in vivo. SSCs are specific undifferentiated cells germ cells that have ability to maintain by self-renew and differentiate into mature spermatozoa. Although critically important for the production of sperm, SSCs have been difficult to study because of their small number in the testis and challenges associated with identifying, culturing, and assaying their biological activity (McLean, 2005). But, the potential to produce gametes as derivatives of mouse and human embryonic stem cells (Hubner et al. 2003, Clark et al. 2004) has also generated much excitement over the utility of these cells as tools in basic developmental research, as well as in more practical applications as alternative sources of gametes for fertility treatments. Restoration of fertility following SSCs transplantation in rodents suggests therapeutic potential in humans. When SSCs are harvested from donor testes and transplanted into a sterilized recipient testis, morphologically and functionally normal spermatogenesis is re-established (Matzuk and Lamb, 2008). Further, rat gonocytes produced mature spermatozoa in testes of immunodeficient mice (Clouthier et al. 1996). They were able to fertilize oocytes by in vivo fertilization but with reduced fertilization and development rates in the transplanted group, where live born pups did not show anomalies, but one was observed with a lower pregnancy rate and a smaller litter size in females impregnated with transplanted male mice (Goossens et al. 2006). This difference may be due to the lower motility in the epididymal sperm of transplanted animals (Goossens et al. 2003). However, although male gametes has also generated from mouse embryonic stem cells in vitro resulted in the birth of pups, most of them suffered epigenetic defects (Nayernia et al. 2006).

The principal uses of SSC transplantation would be to preserve or manipulate the male germ line or both in companion animals, non-domestic and endangered species (Dobrinski and Travis, 2007). Cell transplantation is a technique involves isolation of a mixed germ cell population from a donor testis. The isolated cells are then injected in a retrospective fashion into the testes of a recipient animal. To increase the SSC niches that might be open for colonization, the recipients are often treated with focal testicular irradiation (Honaramooz et al. 2005; Kim et al. 2008) or systemic busulfan (Brinster and Zimmermann, 1994; Hill and Dobrinski, 2006) to reduce their endogenous SSC. After allowing for colonization, proliferation, and spermatogenesis, semen is collected and assessed for the relative percentage derived from donor origin. Although it has been performed successfully in several species, this technique has multiple steps that are technically challenging, time and labor-intensive. Therefore, it is likely to be used in the future primarily as a clinical tool to develop transgenic biomedical research models or for the production of transgenic farm animals that produce genetically engineered tissues/organisms compatible across species (Houdebine, 2009). The transplantation of SSC derived from an organism of a different species has been attempted with various donor and recipient species. But, unless the donor and recipient are closely related taxonomically like rat and mouse (Clouthier et al. 1996), as opposed to dog and mouse (Dobrinski et al. 1999), the recipient testes do not support spermatogenesis. Therefore, utilization for the conservation of threatened species would require not only the use of a suitable domestic animal recipient that would support spermatogenesis of the donor but also some method of sorting the sperm of donor origin from that of recipient origin (Fortier and Travis, 2011).

Now a day, testis xenografting also considered as a principal application for preservation of breeding potential of a genetically valuable pre-pubertal male animal (Pukazhenthi et al. 2006). For example, if neonatal or juvenile males die, testis xenografting offers a means to develop sperm from their gonocytes or SSCs, which are present from parturition. In this procedure, small pieces (1 to 2 mm³) of donor testes are surgically grafted into immunodeficient mice. In the absence of a functioning immune system, the recipient mice nurture the foreign testis tissue, which supports spermatogenesis (Honaramooz et al. 2002). By utilizing this technique, morphologically mature sperm have been produced in xenografts from a number of species, including rabbits (Shinohara et al. 2002), pigs, goats (Honaramooz et al. 2002), cats (Snedaker et al. 2004), sheep (Zeng et al. 2006), and dogs
(Abrishami et al. 2010). However, the efficiency of spermatogenesis in xenografts differs among species, where the bull (Oatley et al. 2004), cats (Kim et al. 2007) and dogs (Abrishami et al. 2010), being less efficient. But, one common finding across species is that if the donor testis tissue has germ cells actively undergoing meiosis (as in puberty or adulthood), then the xenografts lose the ability to support spermatogenesis (Kim et al. 2007; Arregui et al. 2008). The fertilizing ability of graft-derived sperm has been verified by the production of viable offspring in allografted mouse (Schlatt et al. 2003) and xenografted rabbit (Shinohara et al. 2002) and pig (Nakai et al. 2010). Because there is no epididymis in this system, the functionally immature sperm can help generate offspring only through intracytoplasmic sperm injection (ICSI), a procedure in which sperm are injected directly into an oocytes. Thus, although banking of material from genetically valuable individuals of multiple species might begin now, the ultimate production of offspring is restricted until ICSI is optimized for that species.

**Prospective of stem cells in female reproduction:** Recent findings in stem cell biology have squeezed the long held dogma of fixed numbers of oocytes in ovary; current literature suggests that human ovaries contain stem cells which form new oocytes even in adulthood and that stem cells can be cultured in vitro to develop into mature oocytes. These findings have provided new hope and broader options for fertility preservation. Recently, stem cells has been isolated from ovaries of reproductive age women and used it to generate oocytes (White et al. 2012), it could be appears as wonder if this research will find translation in the clinics. Other applications of stem cells in the field of reproductive health have also been reviewed including the treatment of reproductive diseases (Du and Taylor, 2010). The potential advantages of stem cells in reproductive biology and medicine are apparent in livestock. Despite the fact that female reproductive tract tissues undergo major remodelling events as part of the reproductive cycle, adult stem cells in these tissues had been overlooked and understudied for many years. The most of study on stem cells in the female reproductive tract has been conducted in mouse and human, however, is still in its infancy stages in livestock. There is a pressing need to identify definitive markers for both myometrial and endometrial stem cells. A thorough characterization of uterine stem cells is a prerequisite for understanding the complex mechanisms underlying the morphogenesis and physiological regeneration of the female reproductive tract (Ono et al. 2008).

For the first time, in the year 1959, DeOme discovered that murine mammary gland has an unexplainable regenerative potential, which he related to the presence of distinct cells that can divide generating both ductal and lobular components of the mammary epithelium. These cells were defined as mammary adult stem cells. Transplantation studies demonstrated that small parts of mouse mammary tissue derived from donor mice could engraft cleared fat pads and these outgrowths could be serially passaged for up to seven transplant generations of donor-derived mammary cells (Daniel, 1968). After that, Kordon and Smith (1998) showed that an entire mammary gland could be regenerated with the progeny of a single cell following transplantation of tissue fragment into cleared mammary fat pads.

Recently, mammary stem cell has been isolated and characterized in human (Lim et al. 2009), and bovine mammary epithelial stem cells (Li et al. 2009). Bovine mammary epithelial stem cells provide an opportunity for cell-based therapies aimed at altering mammary function, either by repopulating tissue with genetically modified exogenous stem cells or by modifying the function of endogenous mammary stem cell. The latter provides an opportunity for altering management strategies, whereas the former provides a basic research tool for studying mammary function. Human and mouse mammary epithelial cells have been identified as type I and type II cells (Kang et al. 1997; Kao et al. 1997), whereas, in bovine these mammary epithelial cells have been identified as small light cells (SLC), large dark cells (LDC) and large light cells (LLC) (Holland et al. 2005). Examination of the mammary gland stem cell at the light and ultrastructural level has revealed many similarities between mouse, human and bovine cells, with the type I cells consisting of undifferentiated SLCs and LLCs. Type II cells are large intermediate cells that include both
differentiated LDC and terminally differentiated cells. Mammary epithelial stem cells can generate a series of branching ducts that terminate in sac-like lobules embedded in stromal tissue. The mammary gland epithelial components are thought to arise from stem cells that undergo both self-renewal and differentiation. Self-renewal has been shown to be regulated by the MAPK, Wnt/beta-catenin, Notch, Hedgehog, transforming growth factor (TGF)-beta, PTEN and Bmi-1 signalling pathways (Liu et al. 2004; Seo et al. 2006). Wiseman and Werb (2002) suggested intrinsic factors to these stem cells as well as their interactions with stromal cells and the extracellular matrix is important for normal morphogenesis of the gland. The differentiation of mammary epithelial stem cells in vivo has been identified in most research, but Li et al (2009) shown the in vitro differentiation potential of bovine mammary epithelial stem cells into myoepithelial-like cells expressing alpha actin and epithelial-like cells that could be induced to secrete beta-casein, which indicates that they had differentiated into glandular epithelial cells. After several repetitions of subculture and induction with hormones, the cells secreted beta-casein protein, but did not differentiate into myoepithelial, and no expression of alpha-actin was detected which shows that bovine mammary epithelial stem cells share the same molecular signature as human mammary epithelial stem cells (Li et al. 2009).

Mammary gland epithelial cells are likely to be important effectors in the defence against intramammary infection (Wellnitz and Kerr, 2004). It has even been postulated that the mammary gland itself may be an extension of the innate immune system (Vorbach et al. 2006). Before now, we thought that only blood cells have neutrophils and monocytes, that migrate at the site of infections in response to invading mastitis causing pathogens and worked as innate immunity in the udder. However a study of Gray et al (2005) has reported that mammary epithelial cells may also play an important role in the innate immune response through secretion of antimicrobial peptides and then attraction of circulating immune effectors cells. Further, the interaction and function of mammary epithelial cells against bacteria has been reviewed by Stelwagen et al (2009). So all together, mammary epithelial stem cells are essential for mammary tissue regeneration with each cycle of lactation and required for net growth, renewal and turnover of mammary epithelial cells. Therefore, isolation and characterization of mammary epithelial stem cells and their progenitors is of primary interest, not only to extend our knowledge regarding the diverse regulation of mammary epithelial stem cells among livestock, but also for the dairy industry, since their activity may directly affect lactation persistency (Rauner and Barash, 2012). Moreover, a more precise study is required to identify bovine mammary gland stem/progenitor cells markers for isolation of specific cell populations for further application in udder repair. Simultaneously easy and accurate techniques for isolation of bovine mammary gland epithelial stem/progenitor cells and its long term culture methods are needed to develop. The isolation and characterization of mammary stem cells is an important step towards elucidating the hierarchy of epithelial cell development in the mammary gland and identifying the pluripotent cells in the udder along with their further application in the correction of damage. If possible then it can consider the most important research aspect of the reprogramming of adult somatic/stromal cells to differentiate into mammary epithelial, myoepithelial and cuboidal/columnar cells by using specific factors.

In conclusion, there are plenty of stem cells available in male and female reproductive system and these cells could allow the development of novel routes for the generation of transgenic livestock and the targeting for male contraception. Further, it could be provide future direction for conducting research for treatment of infertility, in vitro spermatogenesis, markers for identification of spermatogonial, ovarian and mammary epithelial stem cells and an alternative strategy for fertility preservation of endangered animals.
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Rauner, G and Barash I. 2012. Cell hierarchy and lineage commitment in the bovine


Camel is well adapted in arid and semi-arid regions worldwide and has its great significance for draft power, milk, meat, leather and fiber production as well as in racing. Both dromedary and bactrian camels are seasonal breeders during the cooler months when better pasture conditions prevail (Chen and Yuen, 1979; Wilson, 1984). The breeding season of camel in India extends from November to March (Matharu, 1966) and calving usually occur from December to April (Khanna et al., 1990). The reproductive efficiency of the camel under natural conditions is considered to be poor. Overall calving rate of around 40% for 30 herds and a mortality rate between birth and one year of age was 17% (Djellouli and Saint-Martin, 1992). Some of the reasons for this include a delay in the onset of puberty (3 to 4 years for female and 5 to 6 years for male), restricted breeding season (short day breeder), induced ovulation, longer gestation period (13 months), longer inter-calving interval (2 years), a prolonged (8 to 10 months) period of lactation-related anoestrus when nutrition is suboptimal and a high rate of early embryonic mortality (Nawito et al., 1967). There is an increase in early embryonic death during the hot summer months and a decrease in libido in the male as environmental temperatures increases. Sexual activity in female starts as early as 2 to 3 years of age (Arthur et al., 1985) but in most management systems dromedaries are not bred until she has almost reached her mature physical size at 4 years of age, resulting in an age at first calving of 5 years or more (Beniwal and Chaudhry, 1984). Therefore, it become important to understand the physiology of camel reproduction and to use assisted reproductive techniques, such as estrus synchronization, artificial insemination, superovulation and embryo transfer, in vitro embryo production, cloning etc that could offer an opportunity to improve their reproductive efficiency. However, scanty information is available on application of reproduction technologies like in vitro maturation, in vitro fertilization, intracytoplasmic sperm injection, cryopreservation of gametes, and other associated technologies in this species (Tibary and Anouassi, 1997).

Synchronization of estrus: In camels, conventional breeding practices involving detection of oestrous behaviour for determining the right time to mate the females are particularly unreliable and time consuming. This is because female camels are induced ovulators in response to copulation, and therefore they exhibit waves of follicular growth and regression (unmated female dromedary camels have follicular wave cycles) rather than regular oestrous cycles (Musa and Abusineina, 1978; Skidmore et al., 1995). There is no classical estrous cycle, divided into follicular and luteal phases and therefore do not have a corpus luteum during the non-pregnant reproductive cycle (Nawito et al., 1967; Skidmore et al., 1997). Each follicular wave is initiated by the emergence of several small follicles one of which then becomes the dominant (mature) follicle and matures to the size of 13–17 mm in diameter (Skidmore et al., 1997). Induction of ovulation either by ovulating-inducing hormones or sterile mating culminates in the formation of a corpus luteum (CL) with a functional lifespan of about 9 days after ovulation (8.5 ±0.5 days (Skidmore et al., 1997). Synchronization of oestrus is one of the ways to regulate the oestrus signs detection. It is a very effective method to increase the proportion of animals that are bred at the beginning of the breeding season. If the oestrus synchronization protocol needs to be successful then it requires to synchronize the follicular waves. Synchronization allows increased use of artificial insemination with sires having superior germplasms. Oestrus is synchronized by using PGF2a, GnRH and controlled Intravaginal Drug (Progestosterone) Releasing device (CIDR). Several methods to control the estrous cycle of other large animals have been developed but these may not be applicable to camels. For example, the simple method of estrus synchronization in cattle, based on two PGF2a injections, 11 days apart (Cooper et al., 1976), cannot be used in camels due to the lack of a functional CL during their reproductive cycles (Skidmore, 2005). In dromedary camels, treatment with progesterone releasing intravaginal
devices (PRIDs) for 7 days was found to be unreliable for controlling follicular dynamics and in some cases led to vaginal discharges (Cooper et al., 1976). Two GnRH injections, 14 days apart, may be used to synchronize follicle wave emergence in Bactrian camel (Nikjou et al., 2008) or two GnRH injections 14 days apart and PG on Day 7 after the first GnRH (Skidmore et al., 2009).

**Artificial Insemination:** Artificial Insemination (AI) is the most effective method being used for the genetic improvement of animals. Reproductive capacity and efficiency has been improved tremendously since the introduction of artificial insemination. The use of AI as a breeding technique has been reported in camelidae since the 1960s with the first camelid offspring from AI being reported in a Bactrian camel in 1961 inseminated with frozen semen collected by electro-ejaculation (Elliot, 1961). However, it has only been during the last 25 years that this technique has started to be used more frequently as interest grows in trying to improve genetic traits such as milk, meat and wool production as well as racing ability in the Middle East (Skidmore et al., 2013). A major advantage of artificial insemination is that it can be used to increase the overall reproductive efficiency in camel species; some essential prerequisites are required before an artificial insemination programme can begin. Firstly the male camel has to be trained to the use of an artificial vagina (AV) so that semen can be collected, and then it has to be diluted in a suitable extender to maximize the use of each ejaculate. Secondly, as camels are induced ovulators, ovulating only when mated, ovulation has to be induced in each female camel that is to be inseminated. It can be especially advantageous in camels because females exhibit follicular waves (Chaudhary, 1995).

Because of many inherent problems such as no adequate method of collection of semen, poor post-ejaculation sperm motility, lack of standard technique for freezing semen, difficulties in transcervical pipette passage and induced nature of ovulation, AI has not been extensively used in this species (Tibary and Anouassi, 1997). Collection of semen from camels is considered to be a difficult task because of a long copulation interval and copulation at ground level. Refusal to serve the artificial vagina (AV), incomplete ejaculation and sand contamination are other associated problems (Aminu and Sahani, 2000). The ejaculated semen is in gel form and does not mix with semen extender until the gel liquefies. In the dromedary camel several authors have studied semen preservation and insemination (Skidmore et al., 2006), but the majority of studies report low post-thaw motilities and few, if any, pregnancies with AI of chilled or frozen semen (Deen et al., 2003). The reasons behind these are explained as difficulties involved in collecting semen from male camels and that the ejaculates are of low volume, low sperm concentration and are highly viscous. The spermatozoa are entrapped within this viscous seminal plasma which makes the handling, diluting and cryopreservation difficult (Deen and Sahani, 2000). Post-thaw motility of thawed semen was observed microscopically (X 400). The presence of at least 20% progressively motile spermatozoa was considered as successful freezing (Deen et al., 2003). Skidmore et al. (2013) reported that collection of semen is best achieved using an artificial vagina, and the highest pregnancy rates are obtained if a minimum of 150 x10⁶ live spermatozoa (diluted in Green Buffer, lactose (11%), or I.N.R.A. 96) are inseminated into the body of the uterus 24 h after the GnRH injection, given to the female camel to induce ovulation.

**Superovulation:** Superovulation followed by recovery of embryos and transfer to appropriately synchronized recipients has proved to be an effective method of increasing the contribution of superior females to the gene pool of various animal species (Seidel, 1981). Ovarian response to superovulation depends on the number of gonado-sensitive follicles present at the time when superovulation is initiated (Driancourt, 2001). Several studies in cattle have shown that better superovulatory responses were achieved when superovulation was initiated at the time of follicular wave emergence (Nasser et al., 1993; Singh et al., 2004). This could be achieved using either GnRH or progestogen approaches. Superovulation in camel was induced by a single IM injection of eCG (2000 IU; Folligon1, Intervet, Holland) on Day 0, in association with twice daily (8 a.m./8 p.m.) i.m. injections of purified porcine pituitary FSH (400 mg Folltropin-V; Bioniche, Canada) for 4 days, at decreasing doses (80, 60, 40, 20 mg NIH), beginning on Day 0 (Skidmore et al., 2002; Nowshari et al., 2005).
**Embryo Transfer:** Embryo transfer has been developed in dromedary camels over the last 20 years owing to increasing demand from the camel racing industry particularly in the Arabic peninsula. Non-surgical transfer of fresh Day 7 embryos from superior racing male and female pairs has gained widespread acceptance and is practiced routinely (McKinnon et al., 1994; Tibary and Anouassi, 1997; Skidmore et al. 2002). Pregnancy rate increased to a maximum of 67% when ovulation in the recipient occurred 1 day later than that of the donor, but fell dramatically when the level of asynchrony between recipient and donor increased to +1 (9%) or −3 (10%) days (Skidmore et al. 2002). Nowshari et al. (2005) reported the birth of the first camel calf from cryopreserved embryos but the efficiency was very low. Until recently there has been very limited data on the cryopreservation of camel embryos. As per report of Nagy et al. (2013), embryos were transferred non-surgically into 111 recipients (83 single and 28 twin embryo transfers). Pregnancy rate at 21 days and 5 months was 55% (61/111) and 45% (50/111), respectively. Finally, a total of 46 recipients delivered a live calf. These results document the utility of embryo transfer using high milk producing dromedaries as donors.

**In Vitro Embryo Production:** In vitro embryo production technology (IVP) has been successfully applied to a number of animal species with transferred embryos resulting in live offspring (Gordon, 2003). In vitro maturation (IVM) of oocytes and their in vitro fertilization (IVF) are used to produce large number of embryos cheaply for transfer and for manipulations. These embryos are also beneficial for studies on pre-implantation development and for application of new technologies, such as embryo cloning by nuclear transfer and production of transgenic offspring. There are a number of factors, which play a role in successful in vitro embryo production, such as source of oocytes, source and preparation of semen, culture media, and culture conditions, which seem to differ for different species. There are a few reports on the in vitro oocyte maturation (Abdoon, 2001; Torner et al., 2003; Kafi et al., 2005; Wani and Nowshari, 2005) in camelids. However, information available on in vitro fertilization and development of IVP embryos is very limited for both dromedary (Khatir et al., 2004; Nowshari and Wani, 2005; Khatir and Anouassi, 2006) and new world camelids (Del Campo et al., 1994). Though there is limitation, oocytes can be harvested from the ovaries collected from slaughterhouse or from the pre-ovulatory follicles of live animals by an ultrasound guided transvaginal ovum pick-up. Optimization of an ultrasound guided transvaginal ovum pick-up (OPU) after ovarian superstimulation would be of tremendous help in providing and increasing the yield of oocytes for developing this technology and also for the production of embryos from elite animals over a limited interval (Wani and Skidmore, 2010). Improvements in culture conditions resulted in the first camel offspring obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir-derived oocytes (Khatir and Anouassi, 2006). Fresh ejaculated spermatozoa have been used for IVF of dromedary oocytes with a blastocyst production rate ranging from 0 to 23% (Khatir et al., 2004).

**Intra-cytoplasmic Sperm Injection:** Intracytoplasmic injection of a spermatozoa into in vitro matured oocytes has been developed in human reproduction to counteract male infertility factors (Palermo et al., 1992). The technique is important in camelids because embryos could be produced in vitro from semen samples with low concentrations or when semen collection is difficult.

**Cloning:** The development of cloning using various cells from the animal body has created opening of a fascinating scientific arena. Cloning remains inefficient compared with other assisted reproductive technologies, such as conventional embryo transfer, in vitro fertilization, or artificial insemination. Typically, only 1% to 5% of all cloned embryos transferred into surrogate mothers develop into viable offspring (Wilmut et al., 2002). A number of approaches have been shown to improve the in vitro development of NT embryos, including better sources of recipient oocytes (Hiragi and Solter, 2005); altering epigenetic marks in donor cells (Shi et al., 2003); using chromatin transfer (Sullivan et al., 2004), serial NT (Ono et al., 2001), or sperm-mediated activation (Schurmann et al., 2006); or aggregating somatic NT embryos (Boiani et al., 2003).
somatic cell nuclear transfer techniques has led to the production of the first cloned camel by Wani et al. (2010), but the efficiency for nuclear transfer in camels is between 0 and 10%.

Reference


Schurmann A, Wells DN, Oback B. Early zygotes are suitable recipients for bovine somatic nuclear transfer and result in cloned offspring.Reproduction 2006; 132:839–848.


The term “Parthenogenesis” is derived from Greek word (Parthenon, virgin + Genesis, origin) means virgin birth. The individual produced by parthenogenesis is called Parthenogenone in Britain and Parthenote in America. According to Beatty (1957) parthenogenesis means “the production of an embryo from a female gamete without the concurrence of a male gamete”.

In many invertebrates parthenogenesis occur as a natural process in the life history and called natural parthenogenesis. It may exclusively by parthenogenesis with no sexual generation that is complete parthenogenesis or it may exhibit an alternation of sexual and a parthenogenetic generation called as cyclic parthenogenesis. Natural parthenogenesis occurs frequently in certain orders of insecta e.g. hymenoptera, homoptera, rotifera, coloptera and lower crustaceans. It has been noticed in a few vertebrate also for example a lizard Lacerta saxicola Americana. In many animals which usually reproduce sexually, the egg will be activated by artificial methods to start the development without fertilization called as artificial parthenogenesis. Physical stimuli, temperature, chemical stimuli, ultra sound or stimulus provided by sperm entry are the stimulus for artificial parthenogenesis.

In most of mammalian species oocytes are formed during fetal life and are arrested at the prophase stage of the first meiotic division until around the time of ovulation. Resumption of meiosis leads to germinal vesicle breakdown and chromosomal condensation followed by progression through metaphase of the first meiosis (MI), release of the first polar body and then arrest at metaphase II (MII) stage until activated by fertilizing spermatozoon (Smith and Wilmut, 1989) or by an artificial stimulus (parthenogenetic activation).

Parthenogenetic embryo fails to develop to term and exhibit phenotypic abnormalities due either the lack of expression or to over expression of several of these developmental related genes (Kaufman et al., 1977, Barton et al., 1984, Ranjan 2013, Singh et al., 2013).

Spontaneous parthenogenesis: Spontaneously occurring cleavage division of ovarian or tubal eggs has been described in many species like mice, guinea pig (Pincus and Enzman, 1936) and human particularly in atretic follicle. Stevens and Varnum (1974) described an inbred strain of mice, in which parthenogenesis occurred in a small percentage of virgin female. Some time, the Mos-deficient oocytes fail to arrest at metaphase II and undergo parthenogenetic activation spontaneously (Choi et al., 1996).

Induced parthenogenesis: Parthenogenetic activation of oocytes aims to mimic the action of sperm cells during fertilization (Nakada and Mizuno, 1998). There are many experimental procedure (mechanical, electrical, ultrasound and chemical) which induce parthenogenetic development in mammals (Kaufman et al., 1983). Artificial activation protocols promote an increase in intracellular free calcium concentration in the oocytes by the release of calcium from cytoplasmic stores, such as strontium (Cuthbertson et al., 1981), calcium ionophores (Loi et al., 1998, Kharche et al., 2012a). Some factors promote influx of calcium from the extracellular medium, such as electrical stimulation. There are also some treatments that promote increase in intracellular free calcium concentration by the release of calcium from cytoplasmic stores as well as from the extracellular medium (Loi et al., 1998). Inositol 1, 4, 5-triphosphate induces calcium to release from non mitochondrial stores, most probably from the smooth endoplasmic reticulum by signal transduction pathway leading to parthenogenetic activation in porcine (Machaty et al., 1996). The number, frequency, amplitude and duration of calcium pulses influences the efficiency of oocyte activation (Vitullo and Ozil., 1992; Loi et al., 1998) and regulate later developmental events, such as blastocyst development (Grupen et al.,
Calcium activating treatments are associated with protein synthesis inhibitors such as Cyclohexamide (CHX) to prevent cyclin synthesis and improve the rate of activation of the young oocytes, and the phosphorylation inhibitor 6-DMAP to prevent MPF activation and enhance the formation of pronuclei in non-aged metaphase II oocyte (Loi et al., 1998; Liu and Yang, 1999a; Pathak et al., 2013). Cytochalasin B (CCB) prevent the release of second polar body by inhibiting second meiotic division, which results in diploid parthenogenetic embryo development (Niemierko, 1975) and also prevent fragmentation of embryos (Yang et al., 1993). A solution of ethanol (7%) in MM will be found to be effective for activation of cattle (Nagai, 1987) and goat (Kharche et al., 2013) follicular oocytes that were pre-incubated for more than 27 hours before ethanol treatment. High concentration of ethanol treatment lead to formation of blastomeres of different size or with out nucleus and promotes damage to the cytoskeleton and aneuploidy (Neill and Kaufman, 1989; Kharche et al., 2013). 6-DMAP the protein phosphorylation inhibitor/histone kinase inhibitor enhances and accelerates the formation of pronuclei in non aged metaphase II oocyte in mice and cattle (Leal and Liu et al., 1998). Ethanol promotes intracellular calcium increase of greater and longer amplitude than initial increase observed at fertilization (Grupen et al., 2002). Activation of buffalo and goat oocytes by ethanol and 6-DMAP significantly improved cleavage rates and blastocyst formation (Gasparini et al., 2004; Pathak et al., 2013).

There are several advantages and disadvantages of parthenogenesis.

**Advantages:**

1. The offspring developed by parthenogenesis are more alike to mother and eliminates variety in population.
2. Haploid and diploid parthenogenesis are good proof for the chromosomal theory of sex determination.
3. Parthenogenetic embryo is good tool to study the genes important for development and growth of early embryo
4. Parthenogenesis is mode of reproduction in certain invertebrate and it ensures the continuity of race when the sexual reproduction fails in absence of fertilization.
5. Development of triploid and aneuploid races and parthenogenesis is thus a means for aneuploid individuals to develop.

**Disadvantages:**

1. Parthenogenesis does not encourage the formation of new and advantageous combination of genes in a population.
2. Parthenogenetically produced embryos lack adaptability and cheek on evolution.
3. In mammals parthenogenetic embryo does not develop to viable due to lack of certain developmental genes and other factors.

**Parthenogenetic activation of oocytes**

A wide range of procedures for artificial oocyte activation have been established including mechanical, chemical, ultrasound and physical stimuli that elicit one or several Ca$^{2+}$ transients in the oocyte.

**A. Mechanical**

Mechanical disruption of the plasma membrane of oocytes with a fine needle is sufficient to generate Ca$^{2+}$ influx and to initiate development. It was done in the frog oocyte (Kawamura, 1939). Microinjection of Ca$^{2+}$ is another way to increase intracellular calcium. This is effective for the activation of pig oocytes in which CaCl$_2$ was microinjected (Machaty et al., 1996).
B. Physical

Oocytes can also be activated by physical stimulation. Electrical stimulation induces Ca\(^{2+}\) influx through the formation of pores in the plasma membrane. Periodically repeated electrical stimulation mimics the pattern of oscillations observed during fertilization. Freshly ovulated rabbit oocytes were activated parthenogenetically by repeated calcium stimuli generated by electric field pulses of 1.8kV/cm delivered every 4min for 1hr 30 min in a specially designed chamber (Ozil, 1990). The single electrical stimulation induces the production of Insosital triphosphate (IP3) that leads to intracellular Ca\(^{2+}\) release. Electroporation of IP3 in a calcium and magnesium-free medium followed by incubation in 6-Dmethlamino purine (6-DMAP) has been used to activate parthenogenetic and nuclear transfer rabbit oocytes (Mitalipov et al., 1999). Porcine embryos have been most often activated using electrical methods (Polejaeva et al., 2000) as opposed to chemical methods (Yamauchi et al., 1996). In porcine oocytes, cycloheximide improved the effects of chemical activation, whereas it did not induce activation of porcine oocytes when applied alone (Petr’ et al., 1996). Heat shock was found to activate mouse egg and induces a smaller proportion of diploid morula and blastocyst but the same treatment was ineffective for rats. Osmotic shock and ether anesthesia also causes parthenogenetic activation in rats and mouse (Ursula, 1978). In vitro matured bovine oocytes can be activated by a low temperature stimulus, and could develop to the blastocyst stage following treatment with cytochalasin D (Otoi et al., 1996). Magnetic field was also used successfully to activate cat oocytes; which resulted in 12% activation rate. When combined with ethanol or calcium ionophore it resulted about 75% and 48% activation rate in cat oocytes (Grabiec et al., 2007). Similarly, application of ultrasound enables intracellular uptake of drugs, macromolecules, DNA and fluorescent markers that are otherwise not permeable through the intact cell membrane. Ultrasound exposure is thought to induce the formation of pores in the cell membrane that permit easier inward transport of molecules or other agents in to the cells across the membrane barrier, resulting in faster and direct intracellular uptake.

C. Chemical methods

Chemical activation methods are widely used in domestic species for parthenogenetic studies. Commonly used chemicals are calcium ionophore, ethanol, ionomycin, strontium chloride (Nakada and Mizuno, 1998). Ionophore A23187 promotes the release of intracellular Ca\(^{2+}\) stores but also facilitates the influx of extracellular Ca\(^{2+}\) ions (Kline and Kline, 1992a). Ethanol treatment promotes an increase in intracellular free calcium concentrations by the release of calcium from cytoplasmic stores and also promotes influx of calcium from the extracellular medium where as ionomycin increases intracellular free calcium concentration by the release of calcium from cytoplasmic stores (Loi et al., 1998). The substances mentioned above induce a single Ca\(^{2+}\) rise in the oocyte. However, the initial Ca\(^{2+}\) rise is normally followed by Ca\(^{2+}\) oscillations during fertilization in mammals. Strontium chloride induces multiple Ca\(^{2+}\) transients probably by displacing bound Ca\(^{2+}\) in the oocyte (Whittingham and Siracussa, 1978) but also by inducing intracellular Ca\(^{2+}\) release (Kline and Kline, 1992b).

i). Ethanol: Ethanol treatment promotes in oocyte an increase in intracellular free calcium concentrations by the release of calcium from cytoplasmic stores and also promotes influx of calcium from the extracellular medium (Loi et al., 1998). It promotes single intracellular calcium increase of greater and longer amplitude than initial increase observed at fertilization (Vitullo and Ozil., 1992; Grupen et al., 2002; Nakada and Mizuno et al., 1998). When oocytes were cultured for 27-33 hrs before ethanol treatment 60-68% of the oocytes were activated. In contrast, maturation of oocytes for 24-26 hrs resulted in low activation rates (25-38%) (Nagai, 1987). Similar results were reported when 7% ethanol was used for activation of bovine oocytes where 20, 23.3, 48.4 and 66.7% activation rates were observed when oocytes were matured for 24, 26, 28 and 30 hrs respectively (Simone et al., 2004). In goat, oocytes activated by treatment with 7% ethanol followed by 4 hrs incubation in 2mM 6-DMAP had greater cleavage rate (58%) as compared to in vitro fertilized oocyte (47.2%).
Blastocyst development rate from same activation treatment was significantly greater 27.4% compared to 10.3% in vitro fertilized oocyte (Ongeri et al., 2001). In buffalo when 24 hrs in vitro matured oocytes were activated with 7% ethanol for 7 min followed by 4 hrs incubation in 6-DMAP, 71% cleavage rate was observed compared to 55% in in vitro fertilized oocyte. Also the tight morula and Blastocyst formation was 32% of total oocyte activated compared to 22% in in vitro fertilized oocyte (Bianca et al., 2004). In our earlier study on dose dependent effect of ethanol (1%, 3%, 5%, 7% and 9%) on activation of in-vitro matured oocytes, the cleavage rates were gradually higher with higher concentration of ethanol treatment. Development of the embryo up to morula stage were also follow the same trend upto 7% ethanol treatment and decreased at higher concentration i.e. at 9% ethanol. Blastomeres were also shows less compaction in all other treatments including 9% ethanol concentration. These results suggested that ethanol treatment (7% for 5 min.) is most effective for parthenogenetic caprine embryos production (Kharche et al., 2013). Furthermore, the cleavage rate following 7% ethanol activation and 4 hr of culture with 2mM DMAP, 10μg/ml CHX and 2mM DMAP + 10μg/ml CHX was 42.83%, 58.62% and 74.0%, respectively. Similarly the morula production in above groups were 24.59%, 30.58% and 31.08%, respectively (Pathak et al., 2013). Ranjan et al., (2013b) observed that the over all cleavage rate following parthenogenetic activation was 77.22±1.04, which was significantly higher (P<0.05) than IVF produced embryos. The overall percentage of 2-4 cell, 8-16 and morula stage embryos production were 22.90±1.38, 34.46±0.89 and 42.64±1.04, respectively in goats by ethanol activation.

**ii). Calcium ionophores and ionomycin:** Calcium ionophores increase intracellular free calcium concentration by the release of calcium from cytoplasmic stores. The study of calcium ionophores effect on the pig oocytes revealed that treatment of oocyte after 24 hours of culture with calcium ionophore A23187 (50µM for 10min) overcome the block at metaphase II (Linder and Wright, 1983). The addition of magnetic field to ethanol or calcium ionophore treatment resulted in increased parthenogenetic activation, but when the magnetic field will be added to ethanol and cyclohexamide treatment, activation rates decreased in cats (Grabiec et al., 2007). Calcium rise induced by ionomycin is monotonic and unable to persistently subdue MPF activity (Kubiak et al., 1993), requiring the use of protein synthesis or protein kinase inhibitors to further development. The protocol of ionomycin followed by 6-dimethylaminopurine (6-DMAP) is commonly used for activation of oocytes and reconstituted embryos. The best oocyte activation (87-95%) will be obtained when oocytes matured in vitro for 27 hours will be treated with 0.625-20 µM ionomycin for 1 min before 6 hours incubation in 2mM 6-DMAP. Duration, concentration, and timing of ionomycin and 6-DMAP treatment had marked effects on goat oocyte activation, and to obtain better activation and development, goat oocytes matured in vitro for 27 hr should be activated by 1 min exposure to 2.5 µM ionomycin followed by 2 mM 6-DMAP treatment for 4hrs (Guo-Cheng et al., 2005; Lan et al., 2005). When 28 hrs matured goat oocytes were treated with 5μM of ionomycin for 5 min followed by incubation in 6-DMAP for 4 hr resulted in 57.8% cleavage and 28% blastocyst formation as compared to 47.2% and 10.3%, respectively for in vitro fertilized oocytes (Ongeri et al., 2001). Out of different agents examined, Ca Ionophore followed by 6-DMAP resulted in the highest blastocyst rate (George et al., 2011). Furthermore, activation of oocytes by 5 μM Ca Ionomophore followed by 4 hr culture in different doses of DMAP showed use of 5mM DMAP resulted in highest cleavage rate (72.43%), morula (41.62%), blastocyst (10.66%) and hatched blastocyst (1.52%) production (Kharche et al., 2012a). In a study Ranjan et al., (2013b) used CCB in maturation medium to prevent the extrusion of first polar body for diploid parthenogenetic embryo production. Maturation of oocytes in the presence of CCB did not affect embryonic development in goats. Both ethanol and ionomycin activation can result in producing high percentage of parthenogenetic embryos with well developmental potency in caprine. CCB treatment during maturation did not have adverse effect on activation of oocytes with either ethanol or ionomycin rather enhanced cleavage rate as well as there was no constraint on further embryo development (Pankaj et al., 2012).
iii). **Strontium**: Strontium is the only parthenogenetic agent for mouse oocytes that induces repetitive intracellular calcium releases in a fashion similar to those following normal fertilization by spermatozoa (Bos-Mikich et al., 1995). Although strontium has been used often to activate normal oocytes for analytical studies of oocyte activation (O’Neill et al., 1991; Bos-Mikich et al., 1995) and to activate enucleated oocytes for cloning (Kono et al., 2004) there have been only a few systematic studies to determine the optimal concentration and duration of strontium chloride (SrCl$_2$) treatment for mouse oocytes (Otaegui et al., 1999). O’Neill et al. [128] obtained over 85% activation of mouse oocytes after treatment with 1.6 mM SrCl$_2$ for only 5 or 10 min; according to them, treatment longer than 20 min caused lysis of many oocytes. The concentration and duration of SrCl$_2$ treatment and the presence or absence of CB in activating medium and cumulus cells had marked effects on mouse oocyte activation and development. To obtain the best activation and development, cumulus-free oocytes collected 18 h post hCG should be treated for 2.5 h with 10 mM SrCl$_2$ in Ca$^{2+}$-free medium supplemented with 5 mg/mL of CB (Ma Suo-Feng et al., 2005).

**Developmental ability of parthenogenetic embryos**: A lot of studies have been done in laboratory animal in respect to developmental ability of heterozygous parthenotes. In bovines, 24-37% haploid parthenotes formed compact morula. However, the drops of developmental ability became more evident at the stage of blastocyst. In mouse, diploid parthenotes develop to blastocyst up to 84-94% (Heindryckx et al., 2001). While in bovines, diploid parthenotes develop to compact morula up to 46.9% and blastocyst up to 43% (Lagutina et al., 2004). Transfer of single diploid parthenotes can establish pregnancies that are maintained up to 48 days and aggregated parthenotes delayed estrous up to 67 days in bovines (Fukui et al., 1992, Boediono and Suzuki, 1994). When parthenogenetic sheep embryos were transferred in surrogate sheep, all parthenogenetic sheep fetuses on day 21 of pregnancy were viable, with beating hearts. Normal fetuses were slightly larger than those derived from parthenogenetic embryos. There were no difference in fetal membrane size or membrane morphology between the normal and parthenogenetically fetus (Loi et al., 1998). Several developmental defects have been identified in parthenogenetic embryos viz. delayed development, reduced total cell number, fewer cells in the inner cell mass of blastocysts compared with fertilized embryos, high number of apoptotic body. The cleavage rate of haploids are significantly lower than diploids and the development of cleaved haploids to the compacted morula stage tended to be lower compared with diploids. This difference in the developmental ability became significant at the blastocyst stage. Moreover, haploid parthenogenetic embryos are developmentally delayed compared with diploid parthenogenetic embryos in the mouse, pig and cow. The comparative studies on parthenogenetic embryos development (Haploid and Diploid) and their growth vis a vis IVF derived embryos have been done in goat (Mishra et al., 2007). The colony size developed in presence of melatonin was comparatively larger as compared to any other culture condition for all days of culture (Chaudhari et al., 2013). The morphological development, cell number and allocation of cells to trophectoderm and inner cell mass of in vitro fertilized and parthenogenetically developed goat embryos due to the effect of IGF-I have been studied (Narula et al., 1996). The death of diploid parthenogenetic mammalian embryos is determined by the absence of expression of the genes of imprinted loci of the maternal or paternal genome, which leads to significant defects in development of tissues and organs (Platonov, 2005). Co-transfer of parthenogenotes and a single embryo resulted in the successful development to full-term of the pig embryo (Kawarasaki et al., 2009). The in vivo developmental potency of parthenogenetic embryos were day 10 in mouse (Kono et al., 1996); day 21 in sheep (Loi et al., 1998); day 34 in goat (Kharche et al., 2012b); day 40 in pig (Kawarasaki et al., 2009); day 11.5 day rabbit (Ozil et al., 1990, Kure-bayashi et al., 2000) and day 67 in cattle (Boediono and Suzuki, 1994).

Parthenogenetic embryos of different stage of development have been produced in higher mammal including domestic animals viz. mice (Tada and Takagi, 1992), pig (Kure-bayashi et al., 2000; Yi and Park, 2004), sheep (Loi et al., 1998), bovine (Fukui et al., 1992), goat (Ongeri et al., 2001; Kharche et al., 2012a,b; Kharche et al., 2013; Pathak et al., 2013; Pankaj et al., 2012, Ranjan et
The transfer of parthenogenetic embryo in different species has been done and animals showed different days of non return. When mammalian oocytes were activated and transferred to surrogate mother, they were capable to surviving up to day 10 in mouse (Kono et al., 1996; Surani et al., 1986); day 21 in sheep (Loi et al., 1998); day 34 in goat (Kharche et al., 2012b); day 40 in pig (Kawarasaki et al., 2009); day 11.5 in rabbit (Ozil, 1990); day 67 in cattle (Boediono and Suzuki, 1994), marmoset monkey 10–12 days (Marshall et al., 1998) and 30-65 days in goat (Ranjan et al., 2013a). On 14 December 2001, one captive female of hammerhead shark gave birth to a normally developed, live female pup despite being held in extended absence of male. Komodo dragons, Turkey etc. are also reported to reproduce parthenogenetically. The phenomenon is rarer among plants (where it is called Parthenocarpy) as compared to animals.

There is no live birth after parthenogenetic embryo transfer in higher mammal. Recently, mammalian parthenogenesis has been achieved with the birth of the mouse ‘Kaguya’ (Kono et al., 2004). This has opened up the hope of making parthenogenesis a successful method of cloning in domestic animal.

The failure of live birth after parthenogenetic embryo transfer has been due to several factors like:

1. Lack of expression of paternally imprinted gene in parthenogenetic embryos.
2. The embryo development needs sequential expression of development related genes.
3. Fetal development require paternally expressed gene even after implantation.
4. Some developmental related gene are lacking in haploid embryos as there is presence of only single set of chromosome.
5. There may be lethal gene expression in parthenogenetic diploid embryos.
6. There may be over expression of developmental genes in tetraploid embryos as over expression or under expression both are detrimental to growth and development of embryo etc.

The preceding information underlines the failure of parthenogenesis in mammal to live birth. The major reasons are absence of paternal genes, which are required for normal embryonic development. This constraint may be overcome if the whole chromosome sets carried to parthenogenetic embryos. The haploid parthenogenetic embryos showed undetectable level of embryonic development related genes like Nanog, Sox-2, Klf4 and Foxd3 at early embryonic developmental stage in comparison to diploid parthenogenetic embryos. This may be one of the major reasons for poor developmental competence of parthenogenetic haploid embryos (Singh et al., 2013). We observed higher expression of maternally expressed genes in parthenogenetic derived embryonic cell colony compared to in vivo and IVF derived embryonic cell colony. There was no NDN gene expression in diploid parthenogenetic derived embryonic cell colony. Ranjan, (2013) found perturbed expression of developmental related gene in parthenogenetic embryos in caprine. In three parthenogenetic diploid embryo recipients ultrasonography on day 40 revealed that there was fluid filled uterine body with solid fetus like structure. These might be dead fetus and had started resorption. The progesterone profile also corroborated the assumption of pregnancy (Ranjan et al., 2013a).Whereas, Kharche et al., (2012b) observed the presence of fluid filled cavity containing clear hyperechoic amniotic ring and developing foetus in one recipient on day 30 post parthenogenetic embryo transfer. Pregnancy did not sustain further and the recipient aborted a foetus on day34 post transfer. Sexing of parthenogenetic foetus showed single band of amelogenin gene indicating female cell DNA. Microsatellite analysis revealed that the recipient has not contributed genetically to the parthenogenetic foetus confirming the identity of aborted fetus of parthenogenetic origin.
Applications: Parthenogenesis does not occur in mammal naturally, but artificially parthenogenetic embryo can be produced by various methods. Parthenogenetically produced embryos are important tools to study the origin, phylogeny and genes regulating the growth and development of early embryos. Stem cells production from parthenogenetic embryo is cost effective and these are important in therapeutic and research fields in future. Pluripotent, stable buffalo ESC lines derived from IVF, parthenogenesis, and HMC embryos may be genetically manipulated to provide a powerful tool for studies involving embryonic development, genomic imprinting, gene targeting, cloning, chimera formation, and transgenic animal production. (Muzaffar et al., 2012). Singh et al., (2012) isolated ESC-like cells from parthenogenetic blastocyst hold properties of ESCs and express markers of pluripotency.

The parthenogenetic activation of oocytes represents a valid tool to investigate the comparative roles of paternal and maternal genomes in controlling early embryo development. Furthermore, parthenogenetic activation is relevant to cloning research, because artificial activation of oocytes is an essential component of nuclear transfer protocols (Kim et al., 1996). An optimized activation protocol may enhance better or complete reprogramming of the reconstructed embryo, which might in turn increase the chance of success in cloning.

Due to increasing interests in genomic imprinting, potentiating ES cells and cloning, much attention is again being paid to parthenogenetic and tetraploid development. Although parthenogenetic embryos are defective in postimplantation development, in particular by exhibiting limited development of extra-embryonic membranes, ES cells can be derived from parthenogenetic blastocyst .Tetraploid embryos have limited developmental potency; usually die during postimplantation, and exhibit defects, in particular in the forebrain and eyes, the vertebral axis and heart. Nonetheless, tetraploid embryos can be used as complements for cloning offspring from ES cells (Wang et al., 1997). In the ES cell-tetraploid aggregates, tetraploid cells are selected to form extraembryonic membranes, contributing to functional placenta (Nagy et al., 1990). This feature has been used to complement placenta deficiency in development of cloned embryos.

Recently, parthenogenesis has attracted wide attention because of the potential for deriving pluripotent lines. Embryonic stem cells (ESCs), typically derived from the inner cell mass (ICM) of the mammalian blastocyst, are in fact of fundamental value for developmental research and both cell and tissue replacement therapy. However, the development of human ESC based clinical therapies has hitherto been limited because of the ethical dilemma involving the destruction of a human embryo. Therefore, the establishment of embryonic stem cells (ESCs) from parthenogenetic embryos has attracted attention as an alternative way to derive pluripotent stem cells for application to cell transplantation therapy or drug screening, since it does not involve the destruction of viable embryos (Revazova et al., 2007). Thus, Parthenogenesis is emerging as an ideal solution to overcome these ethical problems.

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Climate Change: Bovine Productive, Reproductive and Adaptive Performance and Mitigation Strategies

S.V. Singh, R.C. Upadhyay, O.K. Hooda, Beenam and A.K. Singh

Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal

Global warming is likely to impact productivity of livestock due to their sensitivity to temperature changes. Air temperature, humidity, wind velocity and solar radiation are the main climatic variables that affect livestock productivity and reproduction in tropical climate. In the present paper sensitivity of cattle and buffaloes to extreme weather events/ sudden temperature (Tmax, Tmin) and temperature humidity index (THI) change have been described. A sudden change (rise or fall) in maximum/minimum temperature during summer and winter season was observed to negatively affect the normal physiology, growth, milk production and reproduction of cattle and buffaloes. The decline in minimum temperature (>3°C) during winter and increase (>4°C) during summer than normal average temperature were observed to decrease milk production up to 30% on the next or subsequent days after extreme event(s). The return of animals to normal milk production/ growth /physiology depends on severity and time period of thermal stress/ extreme event occurrence. Lower THI showed relatively small effect on milk production and growth performance. Studied also showed that the lactation period of animals shortened during extreme summer when THI were more than 80 and reproductive functions were affected adversely. Heat stressed buffaloes, exotic and crossbred cattle did not exhibit estrus or exhibited estrus symptom for short period. Both milk production and reproductive functions of livestock species including cattle and buffaloes are likely to be further affected negatively due to expected higher global warming effects/ extreme events in future.

Mitigation strategies i.e. adaptation, shelter modification and feeding strategies should be developed to overcome the possible negative impacts of extreme events/ climate change on productive and reproductive performances of livestock species.

Introduction: Cattle, buffalo, sheep, goat and birds are homeotherms and are sensitive to thermal stress. Studies showed that the negative effects of hotter summers will outweigh the positive effects of warmer winters on production efficiency of animals. Higher the ambient temperature increases, the more the animal’s production decreases. IPCC predicted an annual mean surface temperature rise by 3 to 5°C (A2 scenario) and by 2.5 to 4°C (B2 scenario) up to the end of century, with more pronounced warming in the northern parts of India (IMD, Pune), which would cause a drastic decline in livestock productivity. Temperature and humidity interact to cause stress in animals. Higher the temperature and humidity, the greater will be the stress and discomfort to animals and the more will be the reduction in the animal’s ability to produce milk, gain weight and reproduce. The number of days it takes for cows to reach their target weight in dairy and meat animals and milk production and conception rate in cattle/ buffaloes decreases depending upon severity and duration of stress. As a result of rapid global warming, milk and meat production are projected to decline in a warmer world (Hatfield et al, 2008). The projected increases in air temperatures will negatively affect confined animal operations, increasing production costs as a result of reductions in performance associated with lower feed intake and increased requirements for energy to maintain healthy livestock. These costs do not account for the increased death of livestock associated with extreme weather events such as heat waves. Night time recovery in physiological functions is an essential for survival, when animals are stressed by extreme heat. A feature of recent heat waves is the lack of nighttime relief and which causes the deaths of livestock species. (Hatfield et al, 2008). Warming also affects/ help in survival of parasites and disease pathogens. The earlier arrival of spring and warmer winters allow greater proliferation and survival of parasites and disease pathogens (Hatfield et al, 2008). Changes in rainfall distributions are likely to further lead to spread vector borne diseases due to higher humidity. Heat stress reduces animals’ ability to cope with other stresses, such as diseases and
parasites due to lower immunity status. Sustaining livestock production would require modification of shelter system to reduce thermal stress on animals, using the understanding of the chronic and acute stresses that livestock will encounter to determine the optimal modification strategy (Singh and Upadhyay, 2008 and Hatfield et al, 2008). Changing livestock species as an adaptation strategy is a much more extreme, high-risk, and in most cases, high-cost option than changing crop varieties. Accurate predictions of climate trends and development of the infrastructure and market for the new livestock products are essential for making this an effective response.

Climate and Climate Variability in India: The climate of India is mainly dominated by the high temperature (April to September). The whole year can be divided into four seasons based on the similar meteorological conditions viz. (i) Winter season (January and February) (ii) Hot weather season (March to May) (iii) hot humid season (June - September) (iv) Post monsoon season (October to December). Year to year deviations in the weather and occurrence of climatic anomalies / extremes in respect of these four seasons are:-

(i) Cold wave, fog, snow storms and avalanches
(ii) Hailstorm, thunderstorm and dust storms
(iii) Heat wave
(iv) Tropical cyclones and tidal waves
(v) Floods, heavy rain and landslides, and
(vi) Droughts

The cold and heat waves are the major threats to the livestock productivity in different parts of India. The occurrence of these events during different years of last hundred years (1901-1999) is given the following tables 1 and 2. After 2000, heat waves further intensified in different parts of India. According to the Glossary of Meteorology (AMS, 1989) heat wave is “a period of abnormally uncomfortable hot and usually humid weather of at least one day duration, but conventionally lasting several days to several weeks”. An operational definition often used for a heat wave is three to five successive days with maximum temperatures above a threshold.

Cold wave/ wind chill are the apparent temperature felt on the exposed animal’s body owing to the combination of temperature and wind speed. As wind velocity increases, heat is carried away from the animal’s body at a faster rate, driving down both the skin temperature and eventually the internal body temperature below their normal temperature and to a state of hypothermia.

**Table 1.** Number of cold waves recorded in different states of India in different years

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Table 2. Number of heat waves recorded in different states of India in different years

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Source: De et al; 2005

Projections of Climate Change over India for the 21st Century

Based on modeling and other studies, the following changes due to increase in atmospheric GHG concentrations may arise from increased global anthropogenic emissions:

- As per IPCC, annual mean surface temperature rise by 3 to 5°C (A2 scenario) and 2.5 to 4°C (B2 scenario) by end of this century. The warming will be more pronounced in the northern parts of India as per the simulation studies carried by Indian Institute of Tropical Meteorology (IITM), Pune.
- Indian summer monsoon (ISM) is a manifestation of complex interactions between land, ocean and atmosphere. The simulation of ISM’s means pattern as well as variability on inter-annual and intra-seasonal scales has been a challenging ongoing problem. Some simulations by IITM, Pune, have indicated that summer monsoon intensity may increase beginning from 2040 and by 10% by 2100 under A2 scenario of IPCC.
- Changes in frequency and/ or magnitude of extreme temperature and precipitation events. Some
results show that fine-scale snow albedo influence the response of both hot and cold events and that peak increase in extreme hot events are amplified by surface moisture feedbacks.

**Climate change and availability of feed resources:**

As per the IPCC (2007) report, climate change will further negatively impact the Indian agriculture and would adversely affect livestock production in the India (Dinar et al. 1998). Due to poor availability of good quality of feed and fodders in India (3.4% area under pasture), animals are generally maintained on poor quality grasses available in the pastures or are stall-fed, mainly on crop residues. As per the Govt. of India (2002) estimate, India is already deficit in feed and fodder viz. dry fodder (22%), green fodder (62%) and concentrates (64%). These shortages would be further aggravated by the adverse effects of global warming/climate change on agricultural/ fodder crops.

Adverse consequences of climate change would be more visible on livestock species in areas where high ambient temperature could be associated with decline in rainfall, increased evapotranspiration or increase in the incidence of droughts. A drought in 1987, affected over 168 million cattle in India, due to decline in feed and fodder availability and serious water shortages. In one of the worst drought affected state of Gujarat, 18 million cattle out of 34 million were reported to have diec before it rained the next year. A 1999–2000 drought in the arid state of Rajasthan in the north-western part of the country, which is highly drought-prone affected 34.5 million cattle; in the subsequent year about 40 million cattle were affected by drought (CSO 2000). The drought damaged 7.8 million ha of cropped area in the state and fodder availability fell from 144 to 127 million tons. Any increase in the frequency and intensity of droughts in the arid and semi-arid regions in India would perhaps have the greatest impact on the pastoral families, as they have to migrate to arable areas to secure their livelihoods.

**Effect of long term and extreme events on milk production and reproduction in India:**

The impact of temperature rise/change was assessed on milk production of cattle and Murrah buffaloes and a decline in milk production was observed with a rise in THI and T_max. Analysis of the potential direct effects of climate change in 2020/2050 and global warming on summer season milk production of Murrah buffaloes indicated that a rise of 1.0 or 1.2°C during March-August for India (Region 23- HADCM3 A2/B2 scenario) will marginally effect milk production but temperature rise of more than 2°C over existing temperatures for time slices 2040-2069 and 2070-2099 will cause higher incidence of silent estrus, short estrus and decline in reproduction efficiency of buffaloes. Animals with limited water access will experience warming effect more than that of buffaloes dissipating heat by water wallowing (Upadhyay et al. 2009).

A sudden rise in T_max during summer and a fall in T_min cause a negative impact on milk yield of cattle. The increase in T_max (>4°C) than normal during summer and decline in T_min (>3°C) during winter was observed to impact the milk production negatively in crossbred cattle and buffaloes. The decline in yield varied from 10-30% in first lactation and 5-20% in second and/ or third lactation. The extent of decline in milk yield was less at mid lactation than either late or early stage. The negative impact of sudden temperature change i.e. cold wave or heat wave on milk yield of cattle/buffaloes were not only observed on next day of extreme event but also on the subsequent day (s) after extreme event, thereby indicating that T_max increase during summer and T_min decrease during winter cause short to long term cumulative effect on milk production of cattle and buffaloes. The return to normal milk yield took 2-5 days with a variable response. The decline in milk yield and return to normal yield after and extreme event was also dependent on subsequent day (s) T_max and T_min. The R^2 was non significant and very low for cool period observed during Feb-April / Sept-Nov and actual affect on milk production was minimum. This indicated that low THI (<75) had a relatively small effect on milk production performance. The lactation period of buffaloes were shortened by several days (3-7) during extreme summer when THI was more than 80. The expressions of estrus and reproductive functions were also negatively impacted. Excessively distressed buffaloes with higher rectal temperature (more than 40 °C) did not exhibit estrus or exhibited estrus symptoms for short duration.
that often remained undetected (Upadhyay et al. 2009).

**Global extreme events and their impacts on animal performance and survival:** Extreme weather event that adversely impacted livestock includes the severe heat waves of 1995 and 1999 in the Midwestern states which caused nearly 5,000 animals deaths in each year (Busby and Loy, 1996; Hahn and Mader, 1997; Hahn et al., 2001). Major death losses in the United States and elsewhere e.g. dairy cows in southern California, 1977 (Oliver et al., 1979); feedlot cattle in Nebraska, 1992 (Hahn and Nienaber, 1993), and 1999 (Mader et al., 2001). A 1995 (July 10-15) heat wave in the Midwestern United States resulted in more than 4000 feedlot cattle deaths in Iowa and Nebraska, as well as numerous human deaths in Chicago and elsewhere. During this heat wave event, there were extended periods during five days of the heat wave (July 10-14) when the THI values were 84 or above. One contributing factor to the cattle losses was the continuous exposure to THI values above critical threshold, so there was no opportunity for recovery in physiological functions at night (Scott et al., 1983). Accompanying higher solar radiation loads (clear to mostly clear skies) and low to moderate wind speeds were further contributing factors in the area of highest risk. For cattle in other locations with 20 or more daily THI-hrs in the "Emergency" category (THI 84) for only one or two days, the animal heat load was apparently dissipated with minimal or no mortality (Hahn, 1999). The economic toll from this heat wave event for cattle feeders in Iowa alone was estimated to be $28 million as a result of death and performance losses (Smiley, 1996). Retrospective analysis of hourly climatic records during the 1995 heat wave event was used to evaluate characteristics of heat waves (e.g., intensity, duration, recovery time) that cause feedlot cattle deaths; the results, in terms of daily THI-hrs at or above the Livestock Weather Safety Index (LWSI) thresholds for the Alert, Danger, and Emergency categories, provide a valuable approach to environmental management practices (Hahn and Mader, 1997). This THI-hrs analysis of the 1995 heat wave and others have reinforced the LWSI thresholds for categories of risk, and support an environmental profile for single heat wave events that create conditions likely to result in deaths of Bos-taurus cattle in feedlots: 15 or more THI-hrs per day for three or more successive days at or above a base level of 84 (Emergency category of the LWSI) with minimal or no nighttime recovery opportunity. Death losses can be expected if shade, precautionary wetting, or other relief measures are not provided during such conditions. Conditions in the "Danger" category of the LWSI also may cause mortality in highly vulnerable animals (e.g., new entrants to the feedlot; those at or near market weight; animals not yet acclimated to hot weather; sick animals, especially with respiratory problems). Successive heat waves with intervening cool periods can create excessive heat loads and potentially lethal conditions for cattle even when the conditions during secondary heat waves are comparatively moderate. This is likely a result of increased feed intake during the cool periods. It should be further noted that costs associated with death losses, while drastic, are often greatly surpassed economically by performance losses (growth, efficiency) of surviving cattle (Ballin, 1982).

In the Northern Plain states, with greater than normal snowfall and wind in the winter of 1996/1997, up to 50% of the newborn calves and over 100,000 head of cattle were lost in many areas (Mader, 2003). In the winter of 2000/2001 (Hoelscher, 2001), feedlot cattle efficiencies of gain and daily gain decreased approximately 5 and 10% from previous years as a result of late-autumn and early-winter moisture combined with prolonged cold stress conditions. In January 2007, Colorado faced the most severe snow storm in the past sixty years, causing decreased hay supplies and large death losses to livestock. The exceptional drought in Southern High Plains that began in the fall of 2010 and continued for a year caused incredible losses as calves were placed early in feedlots, culled at much higher rates than normal, or moved to regions where grass and hay are more readily available.

Cattle mortalities also increase during periods of extreme heat stress (Hahn, 1985). Heat stress can decreased dry matter ingested and increase dry matter digestibility (Lippke, 1975) and decrease the rate of weight gain (Mitlohner et al., 2001). But the extent of production loss is often difficult to estimate because heat stress effects are typically hidden among high natural and managerial sources of variation (Linvill and pardue, 1992). Animals exposed to cold weather require more energy to
maintain their body reserves and to maintain their body temperatures (Vinning, 1990). In the winter, the influence of wind can have a negative impact on cattle performance and its effects are magnified when combined with cold temperatures. One way cattle compensate for colder weather is to increase feed intake. However, cattle have a physical limit on how much they can consume. Once that point is reached, they will need higher quality feeds and supplements to compensate for the increased energy requirement.

Deng, et al. (2007) use the Temperature Humidity Index (THI) to analyze the impact of weather on dairy cow production in the southeast, where summer temperatures are high with high relative humidity. The THI index is used to account for the interaction between temperature and humidity. They reported that milk yields decline as the rectal temperature increased, and with the same high temperature, cows exposed to low humidity performed better than those exposed to high humidity. THI can be calculated using Johnson (1963) formula as follows:

$$\text{THI} = 0.72 (t_{db} + t_{wb}) + 40.6$$

*Where*: $t_{db}$ and $t_{wb}$ are dry bulb and wet bulb temperatures ($^\circ C$) respectively

Heat stress begins to occur in dairy cattle/ buffaloes when the THI is > 72. Some of the signs that the dairy cattle and buffaloes exhibit with the increases in THI, range from mild changes in metabolism and milk production to animals death depending upon the stress levels.

For assessing the cold stress, the Wind Chill Index (WCI) is used to indicate the cold stress levels on animals. Wind chill is the apparent temperature felt on exposed skin of animals due to wind speed. The following formula (Paul Allman Siple and Charles Passel) is used to calculate WCI, when temperatures fall below 45°F.

$$\text{WCI} = 0.0817 \times (3.71 \times \text{wind}^{0.5} + 5.81 - \text{wind} \times 0.25) \times (TD - 91.4) + 91.4$$

When temperatures are between 46°F and 59°F, the following formula is used.

$$\text{CSI} = [(TD - 45/14) \times TD + (59 - TD)/14] \times \text{WCI}$$

*Where* WCI = Wind Chill Index, wind = wind speed in miles / hour, CSI = Cold Stress Index and TD= Dry Bulb Temperature ($^\circ F$)

The combined effects of temperature and wind are often expressed as a wind chill index. The wind chill index, rather than ambient temperature, is used to estimate effective temperature when considering the severity of cold stress. For example, when the temperature is 20°F with no wind, the wind chill index is 20°F. At the same ambient temperature, 5, 15 and 25 mph winds would result in a wind chill index (or effective temperature) of 13°, 4° and -7°F, respectively. By any means reducing the exposure of animals to wind will dramatically reduce cold stress. In general, a cow’s energy requirements increase 1% for each degree the wind chill is below 32°F. For a wet cow, the increased energy requirement begins at 59°F and increases 2% for each degree drop.

**Table 3:** Probabilities of extremely warm, wet and dry seasons 2080–99 suggested by IPCC GCM model projections in Asia (*in per cent*)

<table>
<thead>
<tr>
<th>Sub region</th>
<th>Season</th>
<th>Extreme warm</th>
<th>Extreme wet</th>
<th>Extreme dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>East</td>
<td>DJF</td>
<td>96</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MAM</td>
<td>98</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>JJA</td>
<td>100</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SON</td>
<td>10</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>South</td>
<td>DJF</td>
<td>99</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MAM</td>
<td>100</td>
<td>32</td>
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<tr>
<td></td>
<td>JJA</td>
<td>96</td>
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<tr>
<td></td>
<td>SON</td>
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<td>39</td>
<td>3</td>
</tr>
</tbody>
</table>
Climate change and animal adaptability: Weather and extreme events have adverse effects on several aspects of animal production (Upadhyay et al; 2007). There is a range of thermal conditions within which animals are able to maintain a relatively stable body temperature by means of behavioral and physiological means (Bucklin et al; 1992). Heat stress results from the animal’s inability to dissipate sufficient heat to maintain homeothermy. High ambient temperature, relative humidity and radiant energy compromise the ability of animals to dissipate heat. As a result, there is an increase in body temperature, which in turn initiates compensatory and adaptive mechanisms to reestablish homeothermy and homeostasis. These readjustments generally referred to as adaptations, may be favorable or unfavorable to economic interests of humans, but are essential for survival of the animal (Stott, 1981).

Thus, an increase in air temperature, such as that expected in different scenarios of climate change, would affect directly animal performance by affecting animal heat balance. There are four modes of energy transfer i.e. radiation, conduction, convection and evaporation, which is governed by physical laws. Several physical parameters control heat transfer by each mode. Air temperature affects energy exchanges through convection and evaporation (Shashikant et al., 2010; Hahn, 1976). When temperature increases, evaporation becomes the most important way of heat loss, since it does not depend on a temperature gradient (Shibu et al. 2008). Under that circumstance the combination of temperature and humidity acquire more relevance, since increased humidity enhances temperature effects. The comfort limit depends on level of production. Animals producing at higher level are more sensitive to heat stress (Johnson, 1987; Singh and Upadhyay, 2008, 2009). Not only intensity of stress, but also the length of the daily recovery period is important in determining animal responses (Hahn et. al; 2001 and Upadhyay et. al; 2007).

Animal diseases: Global warming is likely to cause an increase in animal diseases that are spread by insects and vectors. Higher temperature and humidity will favor spread and growth of insects/ vectors. Incidences of both protozoan and viral diseases affecting livestock will spread in susceptible population. Incidence of protozoan diseases like Trypanosomiasis and Babesiosis are likely to increase in high producing crossbred cattle and may be higher in future. Some of the viral diseases may also reappear and affect both small and large ruminants’ population. Frequency and incidence of mastitis and foot diseases affecting crossbred cows and other high producing animals may increase due to increase in number of stressful days. Climatic conditions favorable for the growth of causative organisms during most part of the year due to temperature rise will facilitate spread of diseases in other seasons and also increase area for their spread.

Mitigation strategies to overcome the effects of climate change: Since climate change could result in an increase of heat stress, all methods to help animals cope with or at least alleviate the impacts of heat stress could be useful to mitigate the impacts of global climate change on animal responses and performance. Three basic managemental tools/ schemes for reducing the effect of thermal stress have been suggested (Kumar et al; 2009):

(a) Physical modification of the environment;
(b) Development of genetically less sensitive breeds and
(c) Improved nutritional and managemental practices.

Physical modification of the environment: The methods for micro environment modification include: shades, ventilation, combination of wetting and ventilation. Shades are the simplest method to reduce the impact of high solar radiation/ climate change. Shades can be either natural or artificial. Tree shades have proved to be more efficient (Hahn, 1985). If sufficient natural shade is unavailable, appropriate shelter should be constructed. Different aspects concerning design and orientation of
shades and different roofing materials have been suggested by different workers for different agroclimatic condition for various species of animals. Shades are effective in reducing heat stress/physiological responses in the dairy animals (Singh and Upadhyay, 2008, 2009). The protected animals show lower physiological responses (RR, PR, RT & ST) during afternoon and yield more milk and protein (Singh and Upadhyay, 2009). The artificial shade structure did not differ from tree shades in terms of the effects on animal well-being (Valtorta et al, 1997). Proper ventilation in a shelter is important for the relief from heat stress, if possible, natural ventilation should be maximized by constructing open-sided constructions (Bucklin et al; 1992). Forced ventilation provided by fans is a very effective method for lowering the temperature (Kumar et al; 2009). An effective way of cooling dairy cattle and buffaloes are spray evaporative cooling. Several cooling devices viz.: mist, foggers and sprinkling systems are available. However, the single use of a sprinkling and fan system for 30 minutes before milking has proved to be useful to relief dairy animals from heat stress in terms of efficiency to reduce the impact of heat waves under a grazing system (Valtorta et al; 2002).

**Feeding strategies:**

- Increase number of feedings/day particularly during morning, afternoon and night hours i.e. feeding during cooler hours to reduce SDA of feeds.
- Maintain energy intake with decreased dry matter intake.
- Increase dietary protein density to compensate lower intake.
- Increase dietary mineral concentration (Na, K etc.).
- Ratio / balance of cations (Na & K) and anions (Cl & S) are also important.
- Feeding Total Mixed Ration (TMR) should be preferred over component or separate ingredient feeding.
- Well balanced TMR- diet formulation at optimum fibre level- encourages DMI; minimize rumen fermentation fluctuation & pH declines.

**Supplementation of antioxidants:** For amelioration of adverse effect of thermal stress, several studies have been carried out at NDRI, Karnal and their results are given here. The feeding of vitamin E showed a positive impact by lowering the levels of thermal stress markers, viz. HSP72 mRNA expression in lymphocytes and antioxidant enzymes (SOD and CAT) levels by improving levels of a-tocopherol in blood of growing, heifers and lactating buffaloes Murrah buffaloes during summer and winter seasons. Higher milk yield in experimental group of lactating buffaloes compared to control group further indicated the beneficial effect of vitamin E supplementation during extremes of climatic conditions (Lallawmkimi et. al., 2013). Ganaie et al. (2013) showed the beneficial effect of vitamin C feeding to Primiparous Murrah buffaloes during summer season. The results indicated that the deviations in immune status and oxidative stress caused due to thermal stress restored towards normalcy by supplementation of vitamin C. Kumar et al. (2013) conducted the experiment on Sahiwal cows by supplementing chromium during summer and winter season. The results of the present study indicated the beneficial effect of chromium supplementation over the control group by improving the immunity and growth performance.

**Additional means of reducing Heat Stress effects:** Selective crossbreeding- The exotic breeds of cattle which are more heat tolerant due to more sweat gland density (Jersey) should be given more preference over less heat tolerant (Holstein Friesian).

**Summary:** Summer weather challenges agriculture and animals in many regions of the world, whether as a result of current natural variability or potential global change. A consequence of thermal stress associated with summer/ winter conditions reduces animal’s performance and in some cases, death from extreme events (e.g., heat waves/ cold waves). In terms of environmental management, the impacts can be reduced by recognizing the adaptive ability of the animals and by
proactive application of appropriate counter-measures (sunshades, evaporative cooling by direct wetting or in conjunction with mechanical ventilation, etc.). Quantification of the impacts of normal summer/ winter weather and potential global change allows livestock producers to gain a better understanding of the magnitude of production and death losses in both situations. Projected economic losses resulting from climate-induced reductions in production may justify mitigation through changes in management practices. Specifically with regard to potential climate change, the capabilities of livestock managers to cope with the effects are quite likely to keep up with the projected rates of change in global temperature and related climatic factors. However, coping will entail costs such as application of environmental modification techniques, use of more suitably adapted animals, or even shifting animal populations. Assessment of potential economic impacts associated with global change on key areas of animal agriculture needs to be made available for use in allocating strategic adjustments and resources to minimize adverse effects on socioeconomic stability.

Acknowledgements: The authors are grateful to Director, NDRI, Karnal for providing necessary facilities for conducting the different experiments. The financial assistance received under NPCCC and NICRA project for research work is highly acknowledged. The authors also acknowledge the contribution by the other staff members of the project team.

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ASAS, ADSA, DesMoines, IA.


♦ ♦ ♦
Barbari, Jamunapri, Marwari and Sirohi bucks maintained their deep body temperature within the normal range despite large variation in environmental temperature during different thermal environments (hot-dry, cool and moderate) if housed in shed to protect them from solar radiation during hot-dry period and from low temperature during cool period of the year. The mechanism used by different breeds to get rid of the excess heat load during hot period varied. Sirohi and Jamunapari bucks lose excess heat through more efficient mechanism of higher sweating rate. Marwari bucks used respiratory evaporative heat loss to a greater extent mainly because long shaggy hair coat interfered in cutaneous evaporative heat loss. Barbari bucks utilized both efficient sweating mechanism as well as higher respiratory evaporative heat loss. The higher demand of heat loss during hot period in Barbari bucks was possibly due to more heat load as a result of higher metabolic rate in this breed than all other three breeds. The cardiac activity in Barbari was also higher than all three breeds.

Consistently significant lower mean rectal temperature of Sirohi bucks in all the three thermal environments than that in Marwari, Jamunapari and Barbari bucks indicated inherent lower body temperature of this breed. The metabolic rate was also lower in this breed in comparison to other three breeds. The bucks of all the four breeds reduced their metabolic rate during hot thermal environment. Water intake and urine excretion in Sirohi bucks was also significantly lower than other three breeds. Physiologically Sirohi bucks appeared to be best suited to combat the thermal stress in semi-arid climate.

**Introduction:** The climatic factors most likely to influence the distribution and abundance of domestic animals are, firstly, those which control the distribution and growth of plants and secondly, those which directly influence the animals. The second group includes solar radiation, environmental temperature, rainfall, humidity and air movement. In arid and semi-arid zones of India, where the important indigenous breeds of goats like Beetal, Barbari, Jamunapari, Sirohi, Marwari, Jakhrana, Kutchi, Gohilwadi, Zalawadi, Mehsana and Surti are localized, air temperature prevails above 40°C in summer, which sometimes reaches up to 50 °C. The effect of high air temperature is furthermore intensified by solar radiation. In winter, it falls below 0 °C. The rainfall is scanty and skewed.

The climatic elements acting on homeotherms evoke physiological responses as they attempt to maintain energy and fluid balance. The magnitude of the responses depends on the thermal state of the environment. While these environmental variables act in different combinations and ways, but are integrated by an animal. Obviously, the thermoregulatory effort by an animal will be governed by its own thermal status. Temperature sensitive biochemical reaction within animal body maintain all energy transfer processes which involve anabolism and catabolism of body tissues.

The efficiency of animal production depends on the maintenance of energy costs as much as on feed intake. Climatic extremes are known to increase the net energy cost of maintenance above the thermoneutral values as decline in energy intake restricts the metabolizable energy available for production. All the productive process are associated with a high metabolic rate and hence, with a high rate of heat production making hot environment more stressful.

The goat breeds found in hot arid and semi-arid zone of the country are known to differ in their physiological adaptability to high temperature coupled with or without direct solar radiation. At Central Institute for Research on Goats, Makhdoom, systematic information on the breed specific thermoregulatory responses in some of the goat breeds of hot arid and semi-arid zone of India
(Jamunapari, Barbari, Sirohi, Marwari,) have been studied (Kumar, 1998).

Clearly, there is a need to understand the goat’s adaptive mechanisms in order to more efficiently employ this animal to boost the economy of the poor people.

**Physiological Responses of Goats in Hot Dry and Cool Environment**

**Respiratory Rate:** Bucks of Barbari breed had significantly lower mean respiratory rate than the bucks of Marwari breed at moderate environment, but in hot environment there was no significant difference in mean respiratory rate of Barbari and Marwari breeds. The mean respiratory rate of Marwari and Barbari breeds was significantly higher than that in Sirohi and Jamunapari breeds in hot environment. Critical difference test showed that the morning respiratory rate of Marwari bucks was significantly (P<0.05) higher than that in Sirohi, Jamunapari and Barbari breeds. It indicated that the Marwari bucks were not able to loose the excess heat from body during cool hours as efficiently as the bucks of other three breeds. The shaggy long hair coat in Marwari was probably responsible for this. The texture of the coat is thus very important.

**Heart Rate:** The mean heart rate in Sirohi and Marwari breeds was significantly (P<0.05) lower than that in Jamunapari and Barbari breeds and in Jamunapari than that in Barbari bucks. The morning heart rate in Sirohi bucks was significantly (P<0.05) lower than that in bucks of all other three breeds and in Marwari bucks than that in Jamunapari and Barbari and in Jamunapari in comparison to that in Barbari bucks. The heart rate in the afternoon in Sirohi and Marwari breeds was significantly (P<0.05) lower than that in Jamunapari and Barbari breeds and in Jamunapari than that in Barbari bucks. It is evident that the heart rate in all the three environments was highest in Barbari followed by Jamunapari. Sirohi and Marwari both breeds habitat of hot-arid zone had lower heart rate than the two breeds of semi-arid zone. Barbari being the smallest out of 4 breeds had highest heart rate. Values of deviation in heart rate from morning to afternoon were comparatively less in hot environment than that in moderate and cool environments. The heart rate increased from morning to afternoon in all the three thermal environments but the magnitude of displacement was lower in hot environment than in moderate and cool environments.

**Rectal Temperature:** No significant difference in mean rectal temperature of the bucks in cool, moderate and hot thermal environments indicated that the bucks of all the four breeds were able to maintain almost similar mean rectal temperature despite of large variation in environmental temperature. Consistently significant lower mean rectal temperature of Sirohi bucks in cool, moderate and hot thermal environments than that in Marwari, Jamunapari and Barbari bucks indicate inherent lower body temperature of Sirohi bucks than the other three breeds.

**Resting Heat Production:** The overall mean values of resting heat production were significantly lower (P<0.05) in hot environment than that in cool and moderate environments. There was no significant difference in resting heat production in cool and moderate environments. Resting heat production, kcal per kg of metabolic body weight per day, was significantly (P<0.05) higher in Barbari breed than that in Jamunapari, Marwari and Sirohi breeds. Differences between the breeds were not significant during cool period. The resting heat production was significantly (P<0.05) lower in Sirohi breed than that in Barbari breed in moderate environment. But in hot environment the resting heat production in Marwari bucks was significantly lower (P<0.05) than Barbari breed only. The resting metabolic rate of Barbari bucks was consistently higher in all the three environments and the overall values were significantly higher from those of other three breeds, indicate its inherent high metabolic rate. The overall metabolic rate of Marwari and Sirohi bucks were comparatively lower than that of Jamunapari bucks and significantly lower than that of Barbari bucks. A lower resting metabolic rate is advantageous to life in arid region where water and food are at a premium, since this would reduce water loss and food requirements (Yousef, 1985). Barbari is a prolific breed. That might be the reason for its higher metabolic rate.
Dry Matter Intake: The analysis of variance indicated that the dry matter intake per 100 kg of body weight and per unit metabolic body weight basis differed significantly (P<0.01) between the thermal environments and between the breeds. Interaction between thermal environments and the breeds was not significant (P>0.05). Critical difference test further revealed that the dry matter intake per 100 kg body weight and per unit metabolic body weight in moderate environment was significantly higher than that in cool and hot environments. Dry matter intake was significantly (P<0.05) higher in Barbari bucks in comparison to that in the bucks of all other three breeds. In the present study, dry matter intake, on percent body weight and metabolic body weight basis, in hot environment was about 10% lower than that in moderate environment.

Water Intake: The analysis of variance of total water intake (free + through feed) per 100 kg body weight per day indicated significant (P<0.01) differences due to thermal environments and breeds as well as due to interaction between thermal environment X breed (P<0.05). Total water intake was significantly (P<0.05) higher in hot environment than that in cool and moderate environments. It was significantly lower in cool environment than in moderate environment. Total water intake in Sirohi bucks was significantly (P<0.05) lower than that in Marwari, Jamunapari and Barbari breeds. Water intake increased with increase in ambient temperature. Increase in total water intake in hot environment in comparison to that in cool environment was 161.54% in Marwari, 165.20% in Sirohi, 196.58% in Jamunapari and 215.51% in Barbari breed. Increase in total water intake in hot environment from moderate environment was 79.25% in Marwari, 114.22% in Sirohi, 98.63% in Jamunapari and 75.56% in Barbari breed. Significantly lower water intake of Sirohi bucks even at the high ambient temperature in the present investigation indicates lower water requirements of this breed for thermo-regulatory purposes and better adaptability in desert conditions. Water ingestion in the morning was almost negligible in cool period. But in hot period bucks ingested sufficient amount of water in the morning hours also. Water intake was 368.43%, 225.27%, 542.26% and 584.99% higher in the afternoon as compared to morning in cool environment in Marwari, Sirohi, Jamunapari and Barbari breeds, respectively. In moderate environment, water intake in the afternoon was 76.20%, 161.00%, 58.11% and 65.70% higher than that in the morning. But in the hot environment, water intake in the evening was 3.91% less than the water intake in the morning in Marwari bucks. Sirohi, Jamunapari and Barbari bucks drank 87.63, 43.76 and 12.58 percent respectively more water in the evening than in the morning in hot environment. Water intake per kg of dry matter intake was maximum in hot environment. The values were almost double in comparison to that in cool and moderate environments. In the present investigation water intake (l) per kg of dry matter intake in Marwari, Sirohi, Jamunapari and Barbari breeds in cool environment were 2.0, 1.85, 2.12 and 1.60 respectively; 2.61, 2.06, 2.33 and 2.20 respectively in moderate environment and 4.72, 4.62, 5.17 and 4.33 respectively in hot environment.

Sweating Rate: The analysis of variance for sweating rate (g/m2/hr) indicated that the mean sweating rate varied significantly (P<0.01) due to thermal environments and interaction between environment X breed. In hot environment, it was significantly higher (P<0.05) in Barbari bucks than that in all other three breeds and in Jamunapari bucks than that in Marwari and Sirohi. It was significantly higher in Sirohi bucks than that in Marwari bucks. When the heat load on an animal, whether it be metabolic or environmental exceeds its own body temperature, evaporation becomes the only mode of heat dissipation available in order to maintain a stable body temperature. Evaporative heat loss from the skin surface is dependent on rate of sweating, humidity of the surrounding air and wind speed. Significantly higher sweating rate in Barbari breed followed by Jamunapari, Sirohi and Marwari breeds in hot environment indicated maximum heat load (metabolic-environmental) on Barbari bucks. Long hairy coat of Marwari bucks might have interrupted the heat loss through cutaneous evaporation.
Urine Excretion: The analysis of variance of urine excretion (l/100 kg B.Wt./day) indicated that the urine excretion was significantly (P<0.01) different between the breeds and due to interaction of thermal environment X breed. The urine excretion was also significantly (P<0.05) different between the thermal environments. Critical difference test revealed that the urine excretion was significantly (P<0.05) lower in hot environment in comparison to that in cool and moderate environments. The urine excretion in Sirohi bucks was significantly (P<0.05) lower than that in Marwari, Jamunapari and Barbari bucks. At high environmental temperature, when water loss through evaporation is markedly intensified, the kidney play a big role in regulating the water content in the body. The kidneys of animals adapted to hot-dry conditions contain more long loop of Henle than those of animals in cool or wet environment. An interaction between cerebral thermal sensors, sensors regulating thirst and release of Antidiuretic hormone by the cells of supraoptic nuclei of posterior pituitary gland has been suggested in increasing the permeability of water from kidney tubules (Taylor, 1970; Fitzsimons, 1979; Baker, 1982). The mechanism of action of antidiuretic hormone (ADH) involves cyclic Adenosine Mono Phosphate in increasing permeability. In the present study, urine excretion was 60.74% of the total water intake in cool environment. In moderate environment it was 42.46% and in hot environment it was drastically reduced to 18.45% of the total water intake. In cool environment, the bucks of different breeds excreted urine in the same proportion of their water intake. The pattern was more or less similar in moderate environment. But in hot environment, Barbari bucks drank significantly higher water, but the urine excretion was significantly lower than the other breeds, indicating higher water economizing capacity of this breed by reducing water loss through urine and utilizing more water for evaporative purposes to ameliorate the effect of heat load. Breed wise ratio of urine excretion to water intake in cool environment was 65.64, 54.00, 62.18 and 60.80 percent in Marwari, Sirohi, Jamunapari and Barbari breeds respectively. In moderate environment, ratio was 38.32, 47.28, 50.23 and 36.84 percent and in hot environment, it was 24.21, 19.54, 18.01, 7.96 percent in Marwari, Sirohi, Jamunapari and Barbari breeds respectively.

Faeces voided and moisture loss through faeces: The analysis of variance for daily faecal output, kg/100 kg body weight revealed that the faeces output varied significantly due to thermal environments (P<0.01) and breeds (P<0.05), but not due to interaction between thermal environment X breed. Critical difference test further showed that the daily faeces output in hot environment was significantly lower than that in cool and moderate environments and in cool environment than that in moderate environment. Reduction in faeces output in hot environment from that of moderate environment was 30.28 percent. The weight of faeces in Sirohi breed was significantly (P<0.05) lower than that in Marwari and Barbari but not than that in Jamunapari breed.

The analysis of variance for daily moisture loss through faeces per 100 kg body weight indicated that it was significantly (P<0.01) different between the thermal environments but not between the breeds. Critical difference test further showed that the daily moisture loss through faeces was significantly lower in hot environment than that in cool and moderate environments and in cool environment than that in moderate environment. Reduction in water loss through faeces in hot environment from that of moderate environment was 35.38 percent. The moisture content of faeces were 58.33, 61.32 and 54.37 percent in hot, moderate and cool environments respectively. Percent faecal water content in the present investigation were comparable to sheep (63%), camel (53%) (Maloiy, 1972) and Bedouin goats (50%) (Shkolnik et al., 1972) but lower than cattle (80%) (Thornton and Yates, 1969). The amount of water loss through faeces, when examined as percentage of total ingested water, was maximum in cool environment followed by moderate and hot environment. In cool period, it was 19.02, 21.34, 21.35 and 21.49 percent in Marwari, Sirohi, Jamunapari and Barbari bucks respectively. In moderate environment it was 15.98, 20.75, 18.44 and 15.53 percent in Marwari, Sirohi, Jamunapari and Barbari breeds respectively. In hot environment it was 8.34, 4.41, 5.86 and 4.81 percent in Marwari, Sirohi, Jamunapari and Barbari bucks respectively. Ratio of total water excreted through urine and faeces to
that of total water intake accounted 54.3, 68.03, 68.67 and 52.37 percent in Marwari, Sirohi, Jamunapari and Barbari bucks respectively in moderate environment. The ratio decreased in hot environment and was 32.55, 23.95, 23.87 and 12.77 percent in Marwari, Sirohi, Jamunapari and Barbari bucks respectively. Expected water diverted towards evaporative cooling accounted 76.05% in Marwari, 67.45% in Sirohi, 76.13% in Jamunapari and 87.23% in Barbari breeds in hot environment.

References


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Cellular thermo-tolerance and gene expression in livestock

K S Roy and C S Prasad

Stress Physiology Lab, NIANP, Adugodi, Bangalore

Introduction: The cumulative effect of all the environmental factors, like ambient temperature, relative humidity, radiant energy, air velocity and metabolic heat associated with maintenance and productive processes are the major contributor to heat stress that lower the efficiency of reproduction and production in animal. Heat stress in livestock is the outcome of animal’s inability to dissipate sufficient amount of heat to maintain homeothermy. The gene expression in animals under heat stress changes due to – (a) activation of heat shock transcription factor1 (HSF1) (b) increased expression of heat shock protein (HSP) and decreased expression and synthesis of other proteins (c) increased glucose and amino acid oxidation and reduced fatty acid metabolism (d) endocrine system activation of the stress response (e) immune system activation via extracellular secretion of HSP. If the stress continues, these gene expression changes lead to an altered physiological state referred as acclimation- a process largely controlled by altered gene expression in response to endocrine signals. In the acclimated state, metabolism is adjusted to minimize detrimental effects of increased thermal heat load. As a whole when the livestock face difficulty for coping up with stress, their productivity gets affected in terms of weight gain and meat quality, milk production, reproduction, and finally compromised immune systems that makes animal vulnerable to diseases, maladaptive behaviors that execute negative impact on animal and lower survival rates in younger stocks.

Cellular resistance to heat stress: The hyperthermia resulting from heat stress can compromise cellular function and result in physiological changes unfavorable to animal production. This is particularly true for reproduction, like elevations in testicular temperature impede spermatogenesis and culture of oocytes and embryos at temperatures characteristic of those experienced by heat-stressed cows can compromise subsequent embryonic development (Roth and Hansen, 2004). The cattle that evolved in hot climates have acquired genes that protect cells from the deleterious actions of elevated temperature. Genetic resistances to cellular effects of elevated temperature are seen in both B. indicus as well as in B. Taurus. The genetic differences in cellular resistance to elevated temperature in cattle is the first example in endotherms of genetic adaptations in cellular resistance to elevated temperature (Roy and Collier, 2012). It is possible that the same gene or genes conferring cellular thermotolerance are present in indigenous, Senepol, and Romosinuano, especially because of the contribution of B. indicus genotypes to these cattle breeds (Magee et al., 2002). Identification of the genes conferring cellular thermotolerance offers the possibility of transferring these genes to heat-sensitive breeds to improve reproduction and other physiological systems compromised by hyperthermia. Presently little is known regarding the molecular basis for the improved cellular resistance to elevated temperature in thermo-tolerant cattle. The capacity for transcription in response to elevated temperature seems to be important for expression of genetic differences because there were no differences between indigenous and Holstein embryos in resistance to elevated temperature at the two-cell stage, a time when the embryonic genome is largely inactive (Memili and First, 2000).

Indigenous genotypes to increase thermo-tolerance in cattle: The indigenous cattle exposed to heat stress experience less severe alterations in feed intake, growth rate, milk yield, and reproduction than do cattle from B. taurus breeds that are not adapted to warm climates. In addition to being thermo-tolerant, indigenous cattle are tick resistant and efficient at digesting poor quality forages. Not much use has been made of indigenous genotypes to develop cattle for beef and dairy production in the tropics and semi-tropics. However, other genetic characteristics of indigenous cattle that limit their usefulness as beef and dairy animals including poor meat tenderness, low milk yields and lactation persistency, a long prepubertal period, short duration of estrus, and poor temperament.
Utilization of indigenous crossbreds in hot climates becomes more beneficial relative to purebred European breeds as the overall level of feed resources and other inputs decline. An alternative scheme to crossbreeding for utilizing the indigenous genotype for livestock production in hot climates is to incorporate those indigenous genes that confer thermo-tolerance into European breeds while avoiding undesirable genes. Olson et al. (2003) have identified a phenotype characterized by development of a very short, sleek hair coat that is inherited as if controlled by a single dominant gene. Cattle inheriting the slick hair gene are able to better regulate body temperature. Identification of specific gene loci conferring thermo-tolerance in indigenous cattle could be followed by crossbreeding and selection for the favorable allele using phenotypic traits or molecular markers. Efforts have been made to identify specific loci in indigenous crossbreds that affect carcass and growth traits (Casas et al., 2003) but no effort has been described for identifying genetic markers for thermostolerance. Care must be taken in doing so to avoid inadvertent selection of genes for reduced production (since genes causing low feed intake and low milk yield would increase thermo-tolerance). This problem could be avoided by identifying candidate genes known to be involved in thermostolerance. The utilization of thermo-tolerant breeds has been exploited by beef industry in many of the countries with hot climate. However, the short duration of estrus in indigenous cattle is one of the major factors that limit the widespread use of AI in these breeds. This problem can be overcome with the use of fixed-time artificial insemination protocols, developed specifically for indigenous cattle. Additionally, in vitro and in vivo embryo transfer has been increasing in tropical countries like Brazil, particularly in the Nelore breed (B. indicus, over 90 million animals are in Brazil). European breeds usually predominate in the dairy industries of tropical and subtropical regions because of the higher milk yields as compared to indigenous cattle. Utilization of indigenous crossbred in hot climates may become more beneficial relative to purebred European breeds looking into the feed deficits and decline in other inputs which could lead to better economic gain.

**Heat Shock Transcription Factor:** A transcription factor family known as the HSF has been implicated as important first responders during the onset of elevated cell temperature (Trinklein et al., 2004; Page et al., 2006). These transcription factors coordinate the cellular response to thermal stress and affect expression of a wide variety of genes including HSPs (Akerfelt et al., 2007). The physiological importance of HSF is exemplified by the evolutionary conservation between yeast (Saccharomyces cerevisiae), fruit flies (Drosophila melanogaster), vertebrates, and plants (Pirkkala et al., 2001). Although mammals express HSF1, 2, and 4, HSF1 is primarily responsible for inducing HSP gene expression during hyperthermia (Pirkkala et al., 2001). The current model of HSF1 transcriptional activity indicates that nonstressed cells contain folded HSF1 monomers bound to HSP within the cytoplasm. Upon heat stimulus, the HSP dissociate from HSF1 monomers, which then unfold and bind to two other HSF1 monomers to form trimers before their nuclear translocation. Once in the nucleus, homotrimeric HSF1 binds promoters containing heat shock elements (HSE) to activate heat stress target gene transcription. Although HSF1 has traditionally only been associated with regulation of HSP, recent evidence now links it to regulation of carbohydrate metabolism, transport, cytoskeleton, and ubiquitination during HS (Page et al., 2006). The HSF1 gene has been mapped to chromosome 14 in cattle (Winter et al., 2007); however, investigations of HSF1 regulation and function are limited in bovine species despite the importance of HSF1 to the initiation of the heat stress (HS) response.

**Heat shock protein70 (HSP70) Gene:** The HSP70 is a 2440-base pair gene containing a 212-base pair leader sequence and a 242-base pair downstream or 3′-untranslated region. There are two regulatory elements in the 5′-region that interact with heat shock transcription factors (HSFs). These HSFs bind to the promoter element during stress and are sufficient to induce HSP70 transcription. An important consideration regarding HSP70 regulation involves the apparent conflict between transcription of message and HSP70 translation. There is evidence suggesting that transcriptional activation of the HSP70 gene is independent of protein synthesis. For instance, in cell culture experiments, HSP70 mRNA can increase in response to a challenge, although there is little
HSP70 protein produced (Bruce et al., 1993; Hensold, 1990). There are also data demonstrating that both transcriptional and posttranscriptional regulatory steps are required for HSP production (Morimoto et al., 1994; Peterson and Lindquist, 1989). Studies of oxidative stress suggest that HSP promoter activity and protein accumulation may be uncoupled.

**Heat shock proteins and cellular thermostolerance:** The prime physiological functions associated with the stress-induced accumulation of the inducible HSP70 was acquired thermostolerance, which is defined as the ability of a cell or organism to become resistant to heat stress after a prior sub-lethal heat exposure (Mizzen and Welch, 1988; Moseley, 1997). The phenomenon of acquired thermostolerance is transient in nature and depends primarily on the severity of the initial heat stress. In general, the greater the initial heat dose, the greater the magnitude and duration of thermostolerance. The expression of thermostolerance following heating will occur within several hours and last 3–5 days in duration. Additional supporting evidence includes observations that have linked the kinetics of thermostolerance induction and decay with parallel changes in HSP70 induction and degradation (Li, 1985). Advances in molecular biology techniques have provided researchers with tools to address the issue of link between HSP induction and thermostolerance more directly. Cellular manipulations that either block HSP70 accumulation or overexpress certain HSPs have been shown to either increase or decrease heat sensitivity (Johnston and Kucey, 1988; Roy and Collier, 2012). The precise mechanisms for the improvement in cellular thermostolerance in association with an increase in HSP levels have not been delineated; it is acceptable to postulate that proteins in the HSP70 family are involved in preventing protein denaturation. The heat stress results in translational arrest within a cell, and this arrest is proportional to both the intensity and duration of the applied heat stress. One interpretation of these results is that a primary function of HSPs during cellular stress is to maintain translation and protein integrity. Cells that were made thermostolerant also produced less HSP during a second challenge compared with previously unheated cells, suggesting there is a regulation of HSP synthesis that is dependent on the levels of these proteins existing within the cell.

**Role of HSP70 in stress tolerance:** The mechanisms by which HSPs confer stress tolerance are not well understood. Attention has primarily been focused on the role of HSP70 as a chaperone and its potential ability to contribute to cellular repair processes in response to interventions such as heat, oxidative stress, activation of proteases, release of lysosomal and proteolytic enzymes, and alterations of the cytoskeleton. Several important cytoprotective functions have been credited to HSPs and, in particular, the HSP70 family. These include folding of proteins in various intracellular compartments, the maintenance of structural proteins, the refolding of misfolded proteins, translocation of proteins across membranes and into various cellular compartments, prevention of protein aggregation, and degradation of unstable proteins (Bader et al., 1992; Palleros et al., 1991). Interestingly, it has also been noted that HSPs can play a role in apoptosis. HSP27, HSP70, and HSP90 proteins are predominantly antiapoptotic, whereas HSP60 is proapoptotic. Moreover, it appears that these HSPs function at multiple points in the apoptotic signaling pathway to elicit this response (Garrido, 2001).

**Conclusion:** The reaction to heat stress is a highly conserved cascade of protein activation and altered gene expression in response to a variety of stressors. The gene expression component of this network is under heat shock transcription factor regulation. The central role that heat shock proteins have in cytoprotection during heat stress is demonstrated by the fact that HSP overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heat stroke. The heat stress response is fully integrated with the physiological stress response and considered as a component of a system-wide gene network coordinated across a variety of cells and tissues to minimize effects of the thermal stress on cellular functions. Considerable work is needed to define these networks and delineate opportunities for improving efficiency of domestic animals in hot climate. Understanding the genetic regulation of cutaneous evaporative heat loss, determining the role of HSF in coordinating cellular metabolism, thermostolerance and genetic regulation of nutrient partitioning during thermal stress needs special attention for further research.
Suggested Readings:


Peterson R. B. and Lindquist S., 1989. Regulation of HSP70 synthesis by messenger RNA


Microbial Manipulation for Sustainable Livestock Production

J.P. Puri, Former Professor & Head
Department of Veterinary Physiology, LLR University of Veterinary and Animal Sciences, Hisar, Haryana

Livestock is an important component of Indian economy, in terms of income, employment, nutritional security and foreign exchange earning. The livestock owner also earn livelihood by selling products obtained from livestock and also live stock. Our country is endorsed with a very large number of livestock with diversity in terms of species. The world’s best dairy buffaloes, draught cattle, carpet wool sheep and high profile goat breeds are found in this region. White revolution has placed India as the leading producer on the milk map of the world. Now a days small farmers are becoming more and more dependent on animal husbandry instead of agriculture. There are several factors which affect the productivity of live stock. The research workers in animal science field, in our country as well in the world have the same aim i.e. to improve productivity. Nutrients inputs are subjected first to fermentative digestion by micro-organisms in the rumen and then to glandular digestion. As a result of several decades of continuous research on ruminal fermentation, metabolism and microbiology it could be said that there is a big scope of microbial manipulation to improve the transformation of poor quality feeds into milk and meat. The development of recombinant DNA technology and molecular biology have lead rumen microbiologists to apply these techniques to the rumen micro flora. General objectives of rumen fermentation manipulation can be summarized as follows:

1. To improve fiber digestion.
2. To reduce protein degradation or to produce amino acids.
3. To modify the ratio of fermentation products.
4. To inhibit the growth and metabolic activities of undesirable organisms.
5. To obtain bacterial production of substances those are of benefit to the host metabolism.

To increase the fiber degradation, cellulose is the primary target for manipulation, because it is the major polysaccharide, its digestion in the rumen ranges from 30 to 65 % which is much less than for other polysaccharides. In India sub-continent the animals are getting straw based diet or some grasses which have very poor nutritive value and digestibility. To overcome this, research has been concentrated to improve the nutritive value by using various techniques to improve the feed utilization.

**Enrichment of poor quality roughages by physical and chemical treatments:** Physical treatments include chaffing, soaking & steam treatment. Chemical treatments include various methods using several chemicals to improve nutritive value & digestibility of poor quality roughages. The most promising chemical treatment is urea treatment with moisture & ensilement, as urea is widely available, cheap and easy to handle. Ammoniation of rice straw by 4% urea, 40 % moisture and one month ensilement increased CP contents about three times & digestibility by 15 to 20 units, rumen fluid volume and flow rate of ruminal contents have also been found to increases significantly by feeding urea treated rice straw (Puri, 1989). Feeding of urea treated rice and wheat straws have been found beneficial in growing calves and buffalo calves (Puri & Gupta, 2001). Various other workers (Saadullah et al., 1993 Singh & Gupta, 1987; Ghebrehiwet et al., 1988 Prasad et al., 1994a) also found urea treatment very efficient in improving the nutritive value of straws.

**By-pass protein technology:** Another technology which could be used for improving the feed utilization is by-pass nutrients technology. The high quality protein particularly are not utilized properly because they are hydrolyzed more rapidly in the rumen, the ammonia evolved is not used fully by the micro flora, which is converted to urea and excreted out of the body. Thus the nutrients,
which otherwise can be digested and utilized more efficiently in the abomasum and small intestine, leads to their under utilization. The good quality protein must escape rumen degradation and pass to abomasum and small intestine to supply sufficient amounts of amino acids. To achieve this, proteins can be fed in protected form. Feeding of bypass protein is more beneficial for high yielding animals. Such situation exists in all the developing countries, including India where use of bypass protein feed technology can be fully exploited for augmenting growth and milk production. A significant increase in growth was found in crossbred calves fed formaldehyde treated Cakes (Kumar and Walli 1994b; Tiwari and Yadav 1994; Chatterjee and Walli, 1998a). Feeding of bypass protein has yielded positive results for milk production (Chaturvedi and Walli, 2000 and Chaturvedi and Walli, 2001) and in buffaloes (Chaturvedi and Walli, 1998b). Similarly, soybean seed treated with formaldehyde, which causes protection of both protein and fat, when fed to buffaloes resulted in beneficial effect, especially in the later part of lactation (Srivastava et al., 1994).

Combination of urea treated straw & protected proteins has been found beneficial. As feeding of protected protein may lead to decrease in ammonia-N in the rumen which is very essential for the synthesis for the amino acids. The deficiency of ammonia-N can be taken care of by feeding urea treated roughages. Hence the combination of two techniques will be more useful. Madan and Puri (1997) fed formaldehyde treated mustard cake along with urea treated wheat straw to buffaloes and found that total-N, ammonia-N TCA precipiable–N and TVFAs in the rumen increased significantly, indicating that this combination is beneficial for effective feed utilization. In another experiment Madan et al., (1997) found that rumen fluid volume, rumen fluid dilution rate (%h) and rumen fluid out flow rate increased when protected protein and urea treated straw diet was fed together. Feeding of these diets in combination increased DMI & growth rate in buffaloes (Puri et al., 2004).

Anaerobic fungi: It has been established that rumen anaerobic fungi possess very well developed polysaccharide degrading enzyme system. However, by increasing fungal biomass or stimulating fungal growth in rumen, not only the fiber digestion is enhanced, the flow of amino acids to the small intestine could also be increased (Kamra, 1999). Gulati et al., (1989) suggested cultivation of anaerobic fungi on a commercial scale and then use it as probiotic for enhancing fermentation. An increase of 15.37% in growth rate of crossbred calves was reported recently (Dey et al., 2002). When fungal culture of Orpinomyces species (C-14) was administered at weekly intervals as probiotics.

Ionophores: Since the approval of monensin as a feed additive for ruminant diets in the mid-1970s, research on the effect of ionophores on ruminal fermentation has multiplied rapidly. As a result, several ionophores have been discovered and approved as feed additives for beef cattle, and have greatly improved the efficiency of beef production. Because of the cost effectiveness of ionophores, their rate of adoption in beef cattle increased rapidly to the point that nowadays virtually all feedlots in the US include an ionophore in their supplement. Ionophores, like monensin, lasalocid have already established their use, having a positive effect on rumen fermentation. Monensin fed to buffaloes @ 150mg/head/day in two doses showed that rumen fermentation was shifted towards more production of propionic acid which is beneficial for the animal. A decrease in protozoal population was observed and the blood glucose level was found to increase showing better feed utilization. (Chander Bhan and Puri, 2000). Ionophores play a key role in the prevention of digestive disorders. Feeding ionophores cause reduction in ruminal counts of gram-positive lactate producing bacteria which is believed to be the principal cause of acidosis prevention (Owens et al., 1998; Coe et al., 1999) Studying the effect of ionophores requires experimental induction of acidosis which is extremely challenging (Nagaraja & Titgemeyer, 2007). Impact of ionophores on acidosis is achieved by modulation of feed intake (Coe et al., 1999; Salinas-Chavira et al., 2009)
**Probiotics:** The inclusion of probiotics in beef cattle diets is perhaps the second most adopted practice after ionophores. The mode of action of probiotics feeding is believed to adapt the rumen to the presence of large quantities of lactic acid, which in turn will stimulate the growth of lactate utilizers (Beauchemin et al., 2003; Krehbiel et al., 2003). Once lactate utilizing bacteria counts increase, the ability to metabolize lactate derived from rapid carbohydrate fermentation also increases, preventing the risk of acidosis. Elam et al., (2003) indicated that growth performance by feedlot cattle was not greatly affected when feeding L. acidophilus (strains NP45 & NP51) plus Propionibacterium freudenreichii (strain NP24), but Vasconelos et al., (2008) reported a 2% increase in G:F when feedlot cattle received the same probiotic and strains. Recently the use of lactate utilizing bacterium Megasphaera elsdenii as a probiotic has yielded interesting results in acidosis prevention and performance enhancement. Drenching M. elsdenii intraruminally has been effective in increasing ruminal pH and decreasing lactate concentration (Henning et al., 2010a,b). Interestingly, a link between ruminal abundance of M. elsdenii and milk fat depression has been identified (Palmonari et al., 2010), which can be extremely important in the future development of probiotics in dairy diets.

**Genetic manipulation of rumen microbes:** Biotechnological tools for genetic manipulation have been extended to rumen microbes to create bugs with desired traits viz. increased cellulolytic activity, reduced proteolytic and methanogenic activity and controlling the growth of organism producing lactic acid, as reviewed by several workers (Smith and Hespell, 1983, Mackie and White, 1990 and Gregg 1995). Before transferring the recombined genetic material into rumen bacteria, genes have to be cloned in E. coli or some native rumen bacteria. The genes which have been cloned in E. coli are endoglucanase, xylanase, B-glucosidase, amyrase and glutamine synthetase from the donor source of Bateroides fibrisolvens, Ruminococcus flavefaciens, Fibrobacter succinogenes, Neocallimastix frontalis and Streptococcus bovis (Wallae, 1994).

Some of the recent studies on genetic manipulation of rumen micro organisms include, transfer of acetylxylan esterase from Neocallimastix patriciaeur to B. fibriolvens (Dalrymple et al., 1997), endoglucanase from R. flavefaciens 17 to S.bovis JB 1 (Whitehead and Flint 1995) and xylanase from E. ruminantium to B. fibrisolvens OB 156 (Kobayashi et al 1995). Neelkantan et al (1996) were able to isolate potential cellulolytic ruminococcal isolates with endogucanase activity from buffalo rumen. The endoglucanase from R. albus A6 was purified. Molecular cloning of the endoglucanase gene in E. coli was successfully achieved. The cell clones were further characterized. SAV 1 & P SAV 2 plasmids were characterized for shuttle vector construction.

But the final success in the genetic manipulation of rumen organism has still eluded the scientists, as the genetically engineered bugs with desired traits have failed to give gene expression in the mixes population of rumen environment. Efforts are on, in many parts of the world, including India (Chandrashekharaiash et al., 2000 a, b) for the genetic manipulation of rumen bacteria and to use this biotechnological tool for increasing food production from ruminants. This approach is likely to take a long time before success is achieved especially with regards to the stability of genetically modified organisms under in vivo conditions of rumen. But till that time, there is an ample scope to do more research on several biotechnologies, to make these technologies commercially more effective to increase the growth and milk production from ruminants, including buffaloes.

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Final Report of NARP II/ World Bank Project on “Improving Productivity of Dairy Animals”
NDRI, Karnal, India.


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Free radicals and phyto antioxidant in metabolic diseases- current status and future prospects

V. Leela
Professor, Department of Veterinary Physiology, Madras Veterinary College, Chennai

Introduction: In recent years there is an upsurge in the areas related to newer developments in prevention of diseases especially the role of free radicals and antioxidant. There has been growing interest in research into the role of phyto antioxidants in food and health (Gulcin, 2012).

Free radicals – friends or foes? Concepts of free radicals and cellular damage exists from way back from world war II when the Hiroshima and Nagasaki bomb blast ended with the massive deaths and concomitant ill effects in the then existed population. In 1954, Gerhman and Gilbert proposed that the lethal effects of ionizing radiation might be ascribed to the formation of reactive oxygen species. Since then free radical biochemistry has gained notoriety (Gilbert et al., 1981). Free radical is the term used in broader sense which commonly means reactive oxygen species (ROS), reactive nitrogen species (RNS) and other atoms with unpaired electron or in exited states that lead to free radical generation or those species that result from free radical reactions. Free radicals are generally short lived (half life of milli or micro or nano seconds) which are playing a central role in implicating the metabolic diseases as well as ageing (Harman 1956; Halliwell and Gutteridge, 1997)

ROS and RNS: Both of these are produced in a well regulated manner to maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules and also act as second messenger to modulate cellular functions. Most of the cells can produce super oxide anion, hydrogen peroxides and nitric oxide on demand. Some earlier studies showed that exogenous H2O2 could mimic the action of insulin growth factor. Similarly nitric oxide act as redox sensitive transcription factor mediate its physiological role in vasodilatation and neurotransmission. Other sources of free radicals include redox cycling of xenobiotics, exposure to physico-chemical agents like ionizing radiations (including x-rays, γ rays, UV light). Damage induced by ionizing radiations in biological systems is mediated by product of radiolysis of H2O2 by generating hydrogen radical, hydroxyl ion, hydrated electrons, hydrogen peroxide, peroxyl radical, superoxide anions etc. (Von Sonntag 1987). Cigarette smoke contains a large amount of reactive oxygen species. Recent immunological studies claimed that generation of free radicals by antibodies irrespective of the specificity. ROS have been implicated in many diseases and in aging process. These free radicals cause oxidative tissue damage and antioxidant systems minimize or prevent deleterious effects of the ROS (Valko et al., 2007). In many patho physiological conditions, the generation of free radicals are evident and there by worsening the pathological conditions.

Antioxidants: By simple definition, there are substances that neutralize free radicals or their actions (Sies 1996). Nature has endowed each cell with adequate battalions against the harmful effects of the free radicals. Every cell has superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thios and di-sulphide bonding which actually act as buffering systems of the cells. Vitamin E (α-tocopherol) is an essential nutrient, act as chain breaking antioxidant that prevents propagation of free radicals reactions in all mammalian cell membrane. Similar function is attributed by Vitamin C (ascorbic acid) also. Other non enzymatic antioxidants include carotenoids, flavenoids and related polyphenols and glutathione.

Levels of antioxidant action: Antioxidants, which are capable of neutralizing free radicals or their actions, act at different stages. They act at the level of prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS (SOD, catalase). Interception of free radicals is mainly by radical scavenging (Vit C, Vit E, glutathione, thiol compounds, carotenoids and flavenoids). At the repair and reconstruction level, repair enzymes are involved (Sies, 1996; Cadenzas
Concepts of oxidative stress: The pro-oxidants are kept in check in normal healthy physiological system by various levels of antioxidant defense. Upon exposure to adverse physico-chemical, environmental or pathological agents (pollutants/ cigarette smoke/UV rays/toxic chemicals/over nutrition/ advanced glycation end products in diabetes) disturbances is brought about in delicately maintained balance promoting ill effects of pro-oxidants resulting in oxidative stress. It is currently proposed that most of the patho physiological conditions are driven to worst due to oxidative stress.

Molecular damages induced by free radicals: All the biological molecules present in the mammalian body are at risk of being attacked by free radicals. Such damaged molecules can impair cell functions and may even end in cell death eventually resulting in diseases. Biological molecules such as lipids in the lipid membrane of cells can be succumb to lipid peroxidation (LP) which is initiated by ROS (Gulcin et al., 2010). Unsaturation promotes the rate of LP. LP is highly detrimental to the functioning of cell. Peroxidation reaction can be terminated by number of reactions. By use of Vit E, peroxidation can be stopped and recycling of the biomolecules can be achieved by Vit C or GSH. Carbohydrate are attacked by ROS and produces carbon- centered radical which then leads to chain breaks in macro molecules such as hyaluronic acid. DNA and proteins are highly vulnerable to free radical attack. The C₄-C₅ double bond of pyrimidine is particularly sensitive to ROS attack generating spectrum of oxidative pyrimidine/purine damage products. Protein hydroperoxides can generate additional radicals particularly upon interaction with transition metal ions. The oxidized proteins rendered due to reaction of free radicals make the proteins functionally inactive and sometimes gets accumulated further worsen the functionality of cellular system. Lipofuscin, an aggregate of peroxidized lipids and proteins which accumulates in lysosomes of aged cells is an indicator of ROS damage.

Significance of antioxidants in relation to disease: Antioxidants may prevent or improve different diseased conditions (Knight, 2000). Zinc is an essential trace element and an important co-factor for many mammalian enzymes, which also includes the isoenzyme form of SOD seen in cytosol. Selenium is next important element and a co-factor for glutathione peroxidase. Vitamin E and other forms of tocopherol are efficient lipid soluble antioxidants that function as a chain breaker in lipid peroxidation in cell membranes. Vitamin E is considered to be a standard antioxidant to which other compounds with antioxidant activities are compared, especially in terms of its biological activity and clinical relevance. Vitamin C, carotenoids, flavanoids act as excellent radical quenchers.

Antioxidants and prevention against diseases: Based on the earlier studies, it is evident that supplementing single antioxidant is ineffective in prevention against any diseases. Requirement for antioxidant in Indian conditions differ from western countries due to the nutritional differences. There are also numbers of dietary supplements rich in antioxidants tested for their efficiency. For the past 10 years, many such studies have been made in our laboratory in birds and rats. It was found that variety of plant compounds possess antioxidant property, which are capable of protecting against oxidant damage.

Antioxidants and therapeutics: Among ancient systems of therapy in India, Ayurvedic medicines are usually customized to an individual constitution. This system employs unique holistic approach and set a tradition in bio prospecting of new therapies from medical plants. It is not surprising we still relay on traditional medicine based therapies which largely involve plants and their combination as primary healthcare.

Indian culinary plants: There is a large number of culinary plants exhibit excellent antioxidant activities, which includes Allium cepa, Allium sativum, Azadirachta indica, Cinnamom verum, Curcuma longa, Emblica officinalis, Momordica charantia, Nigella sativa, Ocimum sanctum, Trigonella foenumgraecum, Withania somnifera and Zingiber officinalis. In respect of the above, I
would like to give some data on our studies with *Ocimum sanctum*, *Ocimum basilicum*, which showed excellent antioxidant effect when studied with selenium on broiler chickens. The LPO index and plasma antioxidative enzyme status clearly indicated free radical quenching capabilities of these plants when included in 0.5% of broiler ration. Dietary supplementation of *Allium sativum* and *Allium cepa* significantly increase the activity of antioxidative enzyme (SOD, GSH-Px, and catalase) in broiler chickens. We suggest that *Allium sativum* and *Allium cepa* are useful nutritional antioxidants and their supplementation nullifies the tissue damage or oxidative stress caused by the rapid growth rate in broilers. In another study, we evaluated aqueous extract of Cinnamon bark, coriander and cumin seeds for their antioxidative, antihyperglycemic and antihyperlipidemic activities in STZ induced diabetic rats. Studies on plasma glucose, serum cholesterol, triglycerides and plasma urea concentration revealed significant reduction in these parameters confirming their claimed properties. Plasma insulin and total protein concentration were significantly improved in cumin treatments. Histochemical studies supported glycogen deposits indicating antidiabetogenic and cryoprotective action.

**Conclusion:** The idea of growing crops for health rather than for food/fiber is slowly changing plant biotechnology and medicine. Rediscovery of the connection between plants and health is responsible for launching a new generation of botanical therapeutics that include plant-derived pharmaceuticals, multicomponent botanical drugs, dietary supplements (polyphenols and flavanoids), functional foods and plant-produced recombinant proteins.

**Concept of functional food:** These are that provide more than simple nutrition and supply additional physiological benefit. As the dietary habits are specific to populations and vary widely, it is necessary to study the disease-preventive potential of functional micronutrients in the regional diets. This concept in mineral nutrition has evolved an evolution by TANUVAS, providing TANUVAS mineral mixture which is region specific and proved to enhance health and production in bovines. Similarly there can be functional antioxidant food which can be popularized by providing plants with increased levels of essential vitamins and nutrients.

**References**

Nutrigenomics: Animal Science perspectives

J.P. Ravindra and C.G. David
National Institute of Animal Nutrition and Physiology, Bangalore

Introduction: Modern nutritional research is aiming at health promotion and disease prevention and at performance improvement. The concept of developing nutritionally enhanced or functional food requires the understanding of the mechanisms of prevention and protection, the identification of the biologically active molecules and the demonstrated efficacy of these molecules. As a consequence of these ambitious objectives, the disciplines “nutrigenetics” and “nutrigenomics” have evolved.

Nutrigenomics has emerged as a novel and multidisciplinary research field in nutritional science that aims to elucidate how diet can influence animal health and addresses the inverse relationship, i.e. how diet influences gene transcription, protein expression and metabolism. Nutritional genomics is conceptually based on the following considerations: (i) dietary components may alter gene expression; (ii) diet can be a risk factor for disease; (iii) some diet-regulated genes may affect incidence, onset and progression of chronic disease; (iv) the degree of the dietary impact on the health/disease balance may depend the individual’s genetic makeup; (v) dietary intervention adapted to individual nutritional requirements, nutritional status and genotype may prevent or mitigate chronic disease.

The Omics sciences: The major challenges for Omics (genomic/transcriptomics, proteomics and metabonomics) in nutrition and health still lie ahead of us, some of which apply to Omic disciplines in general, while others are specific for omic discovery in the food context:

(i) The integration of gene and protein expression profiles with metabolic fingerprints is still in its infancy as we need to understand how to select relevant sub-sets of information to be merged, and how to resolve the issue of the different time-scales, at which transcripts, proteins and metabolites appear and act.

(ii) Health and wellness are poorly understood compared to many diseases.

(iii) Omics in nutrition must be particularly sensitive: it has to reveal rather many weak than a few abundant signals to detect early deviations from normality.

(iv) In the food context, health cannot be uncoupled from pleasure, that is, food preference and nutritional status are inter-connected.

Objectives of Nutrigenomics: The objective is to integrate genomics (gene analysis), transcriptomics (gene expression analysis), proteomics (global protein analysis) and metabonomics (metabolite profiling) to define a “healthy” phenotype. This eventually should result in personalised nutrition for maintenance of individual health and prevention of disease.

Perspectives on human health: Throughout the 20th century, nutritional science has focused on identifying vitamins, minerals and other nutrients, defining their use in terms of human health. As the nutrition-related health problems of the developed world shifted to issues such as over-nutrition, obesity, type-two diabetes, cancer, and cardiovascular diseases, the focus of modern medicine and of nutritional science changed accordingly. In order to address the increasing incidence of these diet-related-diseases, the role of diet and nutrition has been and continues to be extensively studied. To prevent the development of disease, nutrition research is investigating how nutrition can optimize and maintain cellular, tissue, organ and whole body homeostasis. Despite accomplishing >600 disease-multiple-gene association studies, only very few, conclusive sets of genetic markers have emerged so far for chronic diseases. On top of this multiplicity of genes implicated in disease prevention and
health maintenance, the complex interplay between these genes and the environment, which is in this context nutrition and diet, needs to be taken into consideration. The impact of diet on health conditions can manifest at virtually any stage of life: even early influences in the form of pre-, peri- and postnatal nutrition may imprint metabolism later in life.

The differentiation of adipocytes is a transcriptional regulatory cascade, involving the member of the CCAAT-enhancer binding protein (C/EBP) family, SREBPs and the adipocyte determination and differentiation dependent factor 1, which enhance the expression of PPARγ. The latter induces exit from the cell cycle and stimulates the production of adipocyte specific genes. Normal adipocytes protect lean tissue from the lipotoxic effects of excess or oxidised lipids. The high-fat diet-induced adipocyte hypertrophy and insulin resistance is mediated by PPARγ. In fact, the development of insulin resistance during obesity (the type II diabetes) can also be considered as a protective mechanism reducing glucose-derived lipogenesis in cells.

Gut health: Diet is a potent mechanism for altering the environment of cells in most organs, particularly in the gastrointestinal tract. The role of the gut microbiome in systemic responses to dietary nutrients, drugs and toxins is just as important as tissue metabolism. Manipulation of the diet may be a way of changing microflora to treat intestinal disorders indirectly in addition to direct effects of changing the character of nutrient supply on gene function that controls intermediary metabolism.

Personalized diets: As nutrigenomics seeks to understand the effect of different genetic predispositions in the development of diseases, once a marker has been found and measured in an individual, the extent to which s/he is susceptible to the development of that disease can be quantified and personalized dietary recommendation can be offered.

Functional foods: Nutrigenomics also aims to demonstrate the effect of bioactive food compounds on health, which should lead to the development of functional foods that will develop and maintain health according to the needs of the individual. Nutrigenomics is a rapidly emerging science still in its beginning stages.

Application of transcriptomics tools: The integration of microarray analysis into basic and applied nutrition and food research provides new insights into the effects of nutrition and food ingredients like fats, carbohydrates, proteins, carotenoids, vitamins, minerals, flavonoids and xenobiotics at the molecular level. The results of such high-throughput screening approaches can change our fundamental understanding of cellular processes on the molecular level. The relationship between specific nutrients or diet and gene expression may help to identify these effects and facilitate the prevention of common diet related diseases as discussed below:

- About 33% of the genes liver of rats fed a soya protein diet differed from those of casein-fed animals. Compared with casein, soya protein changed the expression of 120 genes involved in lipid metabolism, antioxidant activity and energy metabolism (Tachibana et al. 2005).
- Chemopreventive effect of fish oil is due to direct action of n-3 PUFA and not to reduction in the content of n-6 PUFA. It was also noted that dietary fat composition alters the molecular portrait of gene expression profiles in the colonic epithelium at both the initiation and promotional stages of tumour development (Kitajka et al., 2004).
- Iron deprivation results in a large spectrum of differentially expressed genes in the duodenal and more distal segment’s epithelium (Collins, 2006).
- Dietary haeme (derived from red meat) and calcium are modulators of colon cancer risk. The results obtained confirmed that dietary haeme increased the cytotoxicity of faecal water in the colon and elevated epithelial proliferation, a risk factor in colon carcinogenesis, moreover calcium-reduced cytotoxicity and inhibits haeme-induced effects (Van der Meer-van Kraaij et al., 2005).
- Vegetables particularly carrot affects the expression of genes involved in carcinogenic and anti-carcinogenic processes in the lungs of mice susceptible to lung cancer (Breda et al., 2005).
Clinical applications:

- Phenylalanine-restricted tyrosine supplemented diets and galactose-free diets are prescribed for the nutritional treatment of type 1 phenylketonuria and galactosemia (galactose 1-phosphate uridylyltransferase deficiency), respectively, based on routine genetic tests.
- Polymorphisms in apolipoprotein E modify the clinical effectiveness of Cognex and the potential dietary benefits of vitamin E in Alzheimer disease stating that not all genes that are important to clinical outcome variables are directly involved in the pathogenesis of the disease or nutritional benefit.

Perspectives on animal health: Just as in humans, animal health is directly related to diet and nutrition. Subtle changes in animal diets can "turn on" or "turn off" specific genes responsible for cellular health. This gene regulation in turn impacts the overall health of the animal.

Dietary fat has profound effects on gene expression. Specific fatty acid regulated transcription factors have been identified. In mammals, these factors include peroxisome proliferator-activated receptors (PPARα, -β/δ and -γ), oestrogen receptors (ERs) and sterol regulatory element binding proteins (SREBPs). They are regulated either by direct binding of (oxidised) fatty acids, fatty acyl-coenzyme A or oxidised fatty acid (eicosanoid) regulation of cell surface receptors and that of intracellular calcium levels as well as activation of signalling cascades. At the cellular level, the physiological response to fatty acids will depend on the quantity, chemistry and duration of the fat ingested. These mechanisms are also involved in the control of carbohydrate and lipid metabolisms, cell differentiation and growth and cytokine, adhesion molecule and eicosanoid production.

Changes in body composition – increased dietary fat

Increased dietary fat or higher NEFA from fat mobilisation caused by starvation may enhance hepatic oxidation and decrease esterification of fatty acids by reducing fatty acid synthase expression, thereby preventing triglyceride accumulation in the liver. Ingestion of high-fat diets downregulates insulin-responsive glucose transporter (GLUT4) protein expression in adipose tissue, thereby increasing leptin gene expression. In horses, the expression of muscle GLUT-4 depends not only on the dietary carbohydrate source (sugar or starch), but also on physical activity.

Feed restriction

Short-term (2-day) did not change either the number or the size of fat cells in white adipose tissues owing to a declined mRNA expression of PPARγ2, glucocorticoid receptors (GR) and of their modulator, the 11β-hydroxy-steroid dehydrogenase type 1 (11β-HSD1).

Long-term (14-day), however, resulted in an increased number of smaller adipocytes, demonstrating the recovered expression of PPARγ2, GR and 11β-HSD1 mRNA (Arai et al., 2004). This type of adaptation may partly explain the fast regaining of extra kilos after weight reduction programmes.

Dietary carbohydrates, both independently and through insulin effect, deeply influence the transcription of the fatty acid synthase gene in a number of species.

Intake of different oils: Ingestion of high amounts of oleic acid or n-3 fatty acids downregulates the expression of leptin, fatty acid synthase, lipoprotein lipase and phosphoenolpyruvate carboxykinase (PEPCK) in retroperitoneal adipose tissue of pigs. There is no effect in subcutaneous adipose tissue.

Reduced fat mass in rats fed a high oleic acid-rich safflower oil diet is associated with changes in the expression of hepatic PPARα and adipose SREBP-1c regulated genes, called also as adipocyte determination and differentiation factor (Hsu and Huang, 2006).
Effect of protein-rich diets: Protein-rich diets cause a shortage of mRNA necessary for expression of the fatty acid synthase gene in the adipocytes, resulting in the moderation of total body fat. Such an effect cannot be observed in the liver tissue (Clarke, 1993). Hepatic fat synthesis, in turn, can be inhibited by providing unsaturated fatty acids in the diet.

Essential fatty acids: Dietary essential fatty acids are the precursors of eicosanoids. Among the eicosanoids derived from arachidonic acid, prostaglandin E2 is known to possess immunosuppressive actions. The immuno-modulatory roles of dietary fatty acids are mediated, at least partly, through the alteration of prostaglandin biosynthesis.

Conjugated linoleic acids (CLAs): CLAs are potent activators of PPARs and, in turn, this function is thought to be related to the anticarcinogenic effect of certain CLAs. Moreover, CLAs induce apoptosis in adipocytes. The anti-inflammatory effect of some CLAs can be explained by the inhibition of proinflammatory cytokine mRNA expression, including interleukin-6 (IL-6), tumour necrosis factor α (TNFα) and, on the contrary, by the induction of gene expression of PPARγ both *in vivo* (in weaned piglets challenged with lipopolysaccharide) and in blood mononuclear cell culture (Changhua et al., 2005).

Milk is one of the richest natural sources of CLAs. The ruminant milk fat derives from two sources: approximately half of it comes from the uptake of blood fatty acids, whilst the remaining part is formed by the *de novo* fatty acid synthesis in the mammary gland. Composition and function of the rumen microflora have great influence on the pattern of fatty acids available to the mammary gland. Under certain circumstances (for example, with ingestion of diets low in effective fibre), the rumen environment and bacterial population differ from the average. Altered microflora will produce CLAs other than rumenic acid; the concentration of *trans-10, cis-12* CLA may increase. The latter is an efficient inhibitor of milk fat synthesis in the mammary gland and is part of the biohydrogenation theory of milk fat depression. (Bauman et al., 2006). This means that a natural bacterial product in low amounts is capable of influencing mammary gene expression and thereby controlling milk fat production.

Practical application of CLAs: Cis-9, trans-11 CLA or rumenic acid, present in milk fat and beef meat, has been found to have anticarcinogenic effect in rats, where it was found to be capable of reducing the incidence of mammary cancer (Aimitis, 2004). By repressing the activation of COX-2 transcription, mixtures of CLA isomers were able to counterbalance carcinogenic inflammation in cell culture. In the case of colon cancer cells exclusively the *trans-10, cis-12* CLA was capable of inhibiting cell cycle progression via induction of a cyclin-dependent kinase inhibitor, the p21 (Cho et al., 2006). Some of these compounds (the type of effective molecule depends on the disease, but mostly the *cis-9, trans-11* CLA have also proved to have antiatherogenic, antiobesity and antidiabetic characteristics both against type I and type II diabetes.

Metals and gene function: Bivalent metals strongly influence gene expression. *Zinc or cadmium* application enhance the transcription rate of the metallothionein (MT) gene in intestinal tissue. *Cadmium* acts also in prolonging the half-life of MT mRNA in hepatocytes. This effect on the half-life prolongation of MT mRNA is metal and tissue specific: the influence of cadmium is stronger than that of zinc, and the intensity of effect in spermatocytes and spermatids is higher than in hepatocytes and fibroblasts (De et al., 1991).

*Zinc* also acts as

- Part of the .zinc-fingers., fixing activator proteins to the active segments of the DNA.
- Appropriate zinc supply is essential to the balanced regulation for gene expression of pro-inflammatory enzymes like cyclooxygenase-2 (COX-2).
- The dietary supplementation with zinc oxide increases insulin-like growth hormone I (IGF-I) and IGF-I receptor gene expression in the small intestine of weanling piglets (Li et al., 2006).
Iron influences transferrin and ferritin concentrations by exerting an effect on mRNA stability and the translation rate (Bremner and Beattie, 1990).

Selenium deficiencies can influence the patterns of protein synthesis in mice by regulating the expression of specific genes at the transcriptional level. Genes that were up-regulated by selenium deficiencies in mice included those associated with stress responses, cell cycling and growth, and cell adherence (Rao et al., 2001). Changes in gene expression in intestinal tissues with the expression of over 2500 genes being influenced by selenium supplementation, and at least 100 of these can be directly or indirectly associated with reproductive functions.

Role in Reproduction: Direct effects of dietary selenium on gene expression in key reproductive tissue have yet to be examined data from these studies can be used to identify candidate genes or biomarkers that are clearly regulated by various dietary forms of selenium in some tissues. There are at least three examples that are worth examining.

1. The formation of the active form of the thyroid hormone is accomplished through the action of the selenium-dependent enzyme, type 1 5-deiodinase, which deiodinates the relatively inactive thyroxin (T\(_4\)) to triiodothyronine (T\(_3\)), the most active form of thyroid hormone. The delay in the conversion of T\(_4\) to T\(_3\) has been associated with increased embryonic mortality in poultry (Christensen, 1985) and can be expected to have the same effects in other species where strict metabolic regulation of energy metabolism is needed for proper maintenance and development of embryos.

2. Oxidative stress on reproductive tissue and during embryo development is believed to be a major determinant affecting reproductive efficiency and has been suggested to be a leading cause of male infertility. The selenoproteins, glutathione peroxidase 1 and glutathione peroxidase 3, were both up-regulated by the use of selenium yeast and sodium selenite in the diets of mice. These are key proteins functioning as antioxidants in many tissues and would be expected to have a major impact on the reproductive tissue, sperm quality, and embryo development.

3. Thioredoxin is an electron carrier protein involved in many aspects of cell cycling and in maintaining antioxidant systems. The regulation of the thioredoxin system is believed to be key in differentiation and morphogenesis in embryonic tissue and can influence early embryo viability and maturation. Thioredoxin mediates estradiol effects on antioxidant systems, which influences embryo survivability and implantation, as well as fertility in pigs. While at least one component of the thioredoxin system, thioredoxin reductase, is a selenium-containing enzyme which would be regulated by selenium availability, thioredoxin itself is not a selenoprotein.

Vitamins may influence gene expression:

- **Vitamin A** exerts its regulatory function in the form of retinol and retinoic acid. The most important target tissues are in the adrenal glands, testes, cerebellum, kidneys, prostate, cerebral cortex, skin and the viscera. After retinoic acid binds to its receptor, it will stimulate the transcription and translation of vitamin A-responsive genes, including some involved in cell differentiation (growth hormone, glycerolphosphate dehydrogenase and leptin production among others).
- The actions of **active vitamin D** are mediated by nuclear hormone receptors. The vitamin D receptor directly binds to DNA at vitamin D responsive elements as a homodimer or heterodimer to activate gene transcription. Ligand binding to the vitamin D receptor forms a complex of coactivators that modulates gene expression in different cell types
- A role for **biotin** in gene expression has been recognised by the significant depression of ornithine transcarboxylase gene expression in biotin deficiency. The consequent loss of enzyme activity is the basis for hyperammonaemia (Yuichi et al., 1996).
The influence of diet on the **phenotypic manifestation** in some cases is drastically determinant viz. the prenatal (maternal) nutrient supply may influence not only the development of adipose tissue, but also the colour of the offspring mouse pups (Waterland and Jirtle, 2004). Incidence of this so-called **metabolic imprinting** significantly depends upon the type of placenta, too.

**Application of transcriptomics in animal and feed sciences:** Evidence from the recently published transcriptomics based nutritional studies performed in livestock species suggests that, with appropriate study design, it is feasible to apply transcriptomic methods successfully in animal feed and nutritional research. In the context of nutrition and micronutrient research in livestock species, transcriptomic methods have been popularly applied; however, it has been widely discussed albeit primarily in other studies using cell lines and animal models. Under such type of approach, a multitude of genes regulated at the mRNA level by dietary components has been identified and this, in turn, has provided new insights into the biological processes affected by nutritional parameters. In livestock species, the major application of nutrigenomics tools is to how effectively being utilized for dairy and meat industries.

An Effective utilization of microarray technology was beneficial to study mammary gland tissues (milk production and udder health), muscle growth and development and myogenesis process (beef production) and the role of gut microflora on nutritional diet intake in ruminants (health and food safety).

**Milk production and udder health:** Study of Ron et al. (2007) has effectively been hybridized Affymetrix microarray (MG-U74v2) in identification of 249 differentially expressed probe sets common to the three experiments along the four developmental stages of puberty, pregnancy, lactation and involution. In context to candidate genes for milk production traits, a total of 82 expressed genes were identified in mammary gland tissue with at least 3-fold expression over the median representing all tissues tested in GeneAtlas.

**Meat Industry:** The bovine cDNA microarray for beef industry was mainly investigated for muscle fibre number and fibre composition of muscle is largely determined during prenatal development.

- Study of Lehnert et al. (2007) provided a detailed description of molecular events accompanying skeletal muscle differentiation in the cattle, as well as gene expression profiling for muscle growth and development and developmentally regulated in bovine foetal muscle. Their study also highlights the developmental expression pattern of \textit{FSTL1} and \textit{IGFBP5}, which have previously been implicated in myogenesis regulation, as well as describing the changing representation of a recently-described ncRNA (\textit{NEAT1} orthologue) in developing cattle muscle.
- In pig, nutrigenomics tools were effectively utilized in analysis of regulation of myogenesis and its biochemical pathways (Te Pas et al., 2007). Combination of biochemical pathway and microarray results revealed the biological insight of porcine myogenesis process is controlled by two distinct waves, i.e. Notch signaling pathway and the WNT signalling pathway.

**Animal health and food safety:** In context to the above prospective in birds and ruminant, microarray technology was successfully utilized (Paustian et al., 2008) by analysing comparatively genome of \textit{Mycobacterium avium} subspecies obtained from multiple host species. Study showed several polymorphic regions within the genomes of \textit{M. avium} subspecies obtained from a variety of host animals. They further concluded that genome diversity in \textit{M. avium} subspecies appears to be mediated by large sequence polymorphisms that are commonly associated with mobile genetic elements.
**Application of proteomics in animal and feed sciences:** Proteomics is the study of all the proteins in a particular cell, tissue or compartment. The major tools of proteomics are two dimensional (2D) gel electrophoresis and mass spectrometry (MS). Proteomics is concerned with over 100,000 proteins in mammals. In experiments on animals, the scope of the investigations is usually restricted to assessment of the influence of dietary components on the proteome of selected organs, for example, the liver. The proteome represents the protein equivalent of the genome, which is determined by the sequence, the type and number of its nucleotides. In contrast to this static nature of the genome, the proteome represents a tremendously dynamic object, which is influenced by a variety of parameters. However, arraying of proteins is more difficult than the arraying of DNA, because they have to maintain their correctly folded conformations. In contrast to these technical problems, genome-wide screens for protein function are of biological importance for many applications such as: analysing protein expression profiles, monitoring protein-protein interactions, identifying protein posttranslational modifications, screening the substrates of protein kinases, examining the protein targets of small molecules, and proteomic analysis as a function of bioprocess cultivation conditions.

- Two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight MS were used to detect the changes in the proteome of liver of rats fed diets with or without folic acid. Special attention was directed on liver, because it is the main tissue of folate storage and metabolism. The results obtained indicate that folate deficiency modifies the abundance of several liver proteins consistently with adaptive tissue response to oxidative and degenerative processes Chanson et al. (2005).

- Proteomic analysis was quite effective and useful to evaluate the effect of dietary methionine on breast-meat accretion and protein expression in skeletal muscle of broiler chickens (Corzo et al., 2006). Via a tandem mass spectrometer, a total of 190 individual proteins were identified from Pectoralis major muscle tissue; three of them were recognized which differed distinctly between the treatment proteome and could be considered as potential biomarkers regulated by a methionine deficiency in broiler chickens.

More recently, protein microarray technology has been developed for the parallel identification, quantification, and functional analysis of different proteins. In principle, these applications will allow the substitution of single-plex systems.

Among livestock species, the application of protein array has not been cited in context to its application towards animal nutrition and feed science research. However, it has effectively been utilized in identification of novel bacterial antigens/pathogens and in early diagnosis of M. paratuberculosis infections in cattle (Bannantine et al., 2008).

**Application of metabolomics in animal and feed sciences:** Metabolomics represents the final step in understanding the function of genes and their proteins. The aim of metabolomics is to determine the sum of all metabolites (other substances than DNA, RNA or protein) in a biological system: organism, organ, tissue or cell. Techniques employed to investigate the metabolome include nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). The scope of metabolomic analysis is mainly restricted to the assessment of the influence of dietary components on the metabolome of selected organs or tissue in animal nutrition studies.

- Metabolomic analysis was implemented to detect the changes in the biochemical profiles of plasma and urine from pigs fed with high-fibre rye bread. Using an explorative approach, the studies disclosed the biochemical effects of a wholegrain diet on plasma betaine content and excretion of betaine and creatinine Bertram et al. (2006).

- Proton nuclear magnetic resonance microscopy (H-1-NMR) was used to determine the metabolite profiles in the liver of rats used as an animal model to characterize the toxicity of triazol fungicides and identifying biomarkers of exposure and/or effect (Ekman et al., 2006).
Hierarchical metabolomics is useful in evaluating potentially undesirable changes in the overall metabolite composition of transgenic plants. Comparison of total metabolites in tubers of GM and conventional potatoes indicated that GM potatoes with increased content of inulin-type fructans were substantially equivalent to traditional cultivars (Catchpole et al., 2005).

**Promoting research:** Nutrigenomics holds great promise for discoveries in veterinary and human medicine, including profiles and characteristics of dietary and body protein metabolism, development of food allergy, absorption and metabolism of nutrients, their functions in uterine development, growth, reproduction and health, finding biomarkers of nutritional status and diseases and even assisting in target-designed drug development.

In the veterinary field, nutrigenomical databanks make possible the selection for metabolic disease resistance. Hopefully, in the near future, nutrigenomics may provide us with clearly determined, cell-physiologically appropriate nutrient allowances for production animals. Data will enable us to screen susceptible breeds or individuals and to give guidance for optimised or individualised diets to prevent the onset of polygenic, nutrition-related disorders in genetically predisposed individuals for companion and pet animals and reliable, effective functional feeds for both categories.

Future of Nutrigenomics in Animal Sciences needs to focus in addition to further prospects in the following areas to understand

- The role of nutritional management in performance of animals (production/disease).
- The aging process in animals.
- The role of nutrigenomics and immune system, diseases and reproduction

**Conclusion:** Nutrigenomic approaches will enhance researcher’s abilities to maintain animal health, optimize animal performance and improve milk and meat quality. Nutrigenomics is a rapidly emerging science still in its beginning stages. It is uncertain whether the tools to study protein expression and metabolite production have been developed to the point as to enable efficient and reliable measurements. Also once such research has been achieved, it will need to be integrated together in order to produce results and dietary recommendations.

**Key References and Suggested reading**


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122
Nutraceutical concept for gut health in poultry

A. V. Elangovan
National Institute of Animal Nutrition and Physiology, Bangalore

India ranks 3rd and 4th position in table egg (6-7% annual growth) and broiler meat (10-12% annual growth) production, respectively. Over the years, there has been a tremendous genetic improvement of both broilers and layer stock. The poultry industry is faced with a number of challenges, not only the availability of feed ingredients but also to produce high quality products in a cost effective manner. In India, maize and soybean meal formed the major source of energy and protein sources in the diet of broiler and layer chicken until recently. With the escalating feed ingredient costs, definitely the role of feed additives and maintenance of gut health with alternate feed ingredient use is a challenge for the broiler chicken. The first few days after hatch are the most critical period for the development and survival of commercial chickens. The newly hatched bird must undergo a physiological and metabolic change as its primary source of nutrients from the yolk is replaced with an exogenous diet. During this adjustment period, the hatchling draws on its limited body reserves to fuel the rapid physical and functional development of the gastrointestinal tract in order to attain the capacity to digest feed and assimilate nutrients necessary for growth and development. The sooner the young bird achieves this functional capacity, the sooner it can utilize dietary nutrients, grow efficiently to its genetic potential and resist disease. It requires all the nutrients as per requirement as well as additives to maintain gut health.

Nutraceuticals are dietary supplements that have specific physiological and microbiological functions in the gastrointestinal tract. Maintenance of a good symbiotic relationship between the host animal and its intestinal micro-flora is the critical component in the development of good nutritional strategies. Commercial farming conditions place various stressors on the intestine which can lead to digestive disturbances, which are often manifested as a scour type problem. Gut health has been the major focus of research in recent years. The maintenance or enhancement of sound gut health is complex than simple modulation through probiotics or prebiotics. The gut harbours more than 640 different species of bacteria, digests and absorbs the vast majority of nutrients, and accounts for 20% of body energy expenditure, also has the largest immune organ in the body (Kræhenbühl and Neutra, 1992). Gut health is highly complex and encompasses the macro- and micro-structural integrity of the gut, the balance of the microflora and the status of the immune system. These may affect the nutrient partitioning and utilization for organ development, tissue growth and immune system maturation (Kelly and Conway, 2001; Kelly and King, 2001).

Under practical conditions, many chicks gain access to feed only 24-48 hours after hatching because of variable hatching time, hatchling servicing, and delivery to the farm. This delay in feed consumption causes the mobilization of body reserves to the support metabolism and thermal regulation. Therefore, the time from hatching of chicks to the onset of feeding is a critical period in the future performance potential of commercial chicks. Close to and shortly after hatch, segments of the GIT and digestive organs increase in size and weight more rapidly in relation to body weight than do other organs and tissues (Lilja, 1983; Noy and Sklan, 2001). An infected gut is not efficient in digesting and transporting nutrients, a well-developed gut is essential for the ability of poultry to resist disease (Ao and Choct, 2006).

Before the extensive use of probiotics, for many decades since the early 1950’s, antibiotic growth promoters had been used in poultry feeds in order to maximize efficiency of production and quality of product and to control diseases in chickens. The action of feed grade antibiotics on bacteria included inhibition of their growth, modification to their carbohydrate and protein metabolism, damage to cell wall formation and disruption to their nucleic acid synthesis. Feeding antibiotics reverses microbe-induced growth depression by maintaining a healthy gut and in turn increasing the
utilization of nutrients (Anderson et al., 1999). Pedroso et al. (2006) found that changes in the composition, instead of reduction in the number of bacterial genotypes in the intestinal bacterial community induced by antibiotics, is responsible for improved growth performance in chickens.

There are numerous factors influencing gut microflora like hygiene conditions, pathogen load of the ingredients, humidity of the shed, feed additives viz., enzymes, probiotics, prebiotics or acidifiers. However, dietary factors such as composition, processing, digestibility and feeding method also influences gut microflora (Choc, 2009). Choc et al. (1996) observed that addition of soluble NSP to a broiler chicken diet drastically increased volatile fatty acid production in the ileum due to microbial fermentation, which was easily reversed when the NSP were depolymerised with an enzyme especially B-glucanase and xylanase.

**Enzymes:** Since the commercial introduction of exogenous enzymes for the past three decades, their use has increased greatly, not only in traditional markets where wheat and barley are the major grains, but also in countries where maize and sorghum form a major part of the poultry diet. Enzyme supplementation in diets based on especially wheat and barley with soluble NSP and low apparent metabolizable energy values has resulted in most beneficial and uniform production performance in poultry (Choc, 2009). However, in India, the use of wheat and barley for both broiler or layer diet has been negligible and the beneficial effect of enzyme supplementations has not been very apparent in maize soybean meal based diets (Mandal et al., 2005; Elangovan et al., 2004).

Soluble non-starch polysaccharides in poultry diets have an antinutritional activity, which is manifested by wet droppings and poor utilization of nutrients. Microbial enzymes especially B-glucanases and xylanases targeting these polymers have been extensively used with highly positive results in both enhancement of performance and reduction of excreta volume and moisture. The bird’s endogenous enzymes might be limited in the types and amounts of enzymes necessary to utilize a high carbohydrate and vegetable protein diet at an early age, thus nutrient digestibility may not be up to its maximum. Exogenous enzymes have been used commercially for a number of years to improve nutrient digestibility in broiler diets and to supplement the bird’s developing endogenous enzymes, probably to meet the fast growth, efficient feed utilization and gut health.

Future research should be targeted for the complete breakdown of celluloses and hemicelluloses to ensure that individual monosaccharides are utilized. The mode of application of enzyme is also an important means whether added in feed; insufficient time is available in the GI tract of especially broilers where the feed does not stay for sufficient time for the enzymes to act upon. Other alternatives are whether enzymes can be added to a feed and reconstituted, how far it will be practical and economical. As there is much emphasis on early feeding with higher amino acid levels, enzyme should have a major role in augmenting the nutritive value of feeds.

**Probiotics:** Broiler chickens in the current production trend are more prone to stressors, infections and diseases, and thereby efforts are being made through proper nutrition and management that will lead to microbiological control, allowing for more consistent production responses. With the reduction / ban in the use of feed antibiotics, probiotics are currently being used as an main feed additive in poultry (Panda et al., 2008; Yang and Choct, 2009). It has been primarily used to establish normal intestinal flora and to prevent or minimize the disturbances caused by enteric pathogens. There has been varied reports on the probiotic supplementation in broiler chicken on growth performance whereas more consistency on the immunity as well as gut microbial profile (Ramarao et al., 2004; Khaksefidi and Rahimi, 2005; Elangovan et al., 2011).

Most of the earlier reports indicated a non significant effect on the growth performance and carcass characteristics of broiler chicks fed different probiotics (Pelican et al., 2004; Smulikowska et al., 2005). The study of Awaad et al. (2003) indicated *P. acidilactici* and *Saccharomyces boulardii* as probiotics was beneficial to control *C. perfringens* and *Salmonella typhimurium* colonization in the caecum of broilers. Ramarao et al., 2004 observed that on feeding probiotic, the total bacterial,
coliform, and *Escherichia coli* counts in crop and caecal contents were low in broilers. With *Lactobacillus* supplementation (Tollba *et al.*, 2004), there was significant decrease in pH of the intestines (duodenum, jejunum, ileum and caecum) and total count of *Escherichia coli* and *Salmonella pullorum*. In the study of Elangovan *et al*. (2011), supplementation of GalliPro (*Bacillus subtilis* 8 x 105 CFU/g feed) in maize-soybean meal based diet did not influence the growth performance of broilers reared in either battery cages or floor, but was helpful in augmenting humoral immunoresponsiveness and reducing entero-pathogens of the crop and intestine.

**Prebiotics:** The search for suitable growth promoters in diets of broiler chicken has been one of the focuses in poultry nutrition, which has received emphasis in the last few years in the process of production of safe foods of animal origin. Oligosaccharides and polysaccharides, such as inulins (fructans), fructo-oligosaccharides (FOS) and arabinogalactans and fucans are the potential prebiotics in use (Vidanarachchi *et al*., 2010; Samanta *et al*., 2012a). Studies have shown that various oligosaccharides and polysaccharides may act as bioactive compounds or prebiotics in poultry feed, exerting growth-promoting effects. Recently, xylooligosaccharides (XOS) has been successfully produced from natural grass, pigeon pea stalks, corn cobs, corn husks, sugarcane bagasse (Samanta *et al*., 2012a, 2012b, Natasha *et al*., 2012). Mannan-oligosaccharide, obtained from the mannan on the surface of *Saccharomyces cerevisiae* is a high-affinity ligand binding competitively to bacteria and preventing their binding to bowel cells resulting in the failure of the bacteria to colonize and cause infection in the host. Waldroup *et al*. (2003) reported that addition of Bio-MOS in broiler diet had no significant effect on any parameter of growth and carcass characteristics. In another study, MOS containing diet was found to produce comparable body weight and feed conversion ratio than those of antibiotic supplemented diet in broilers but significantly lowered mortality compared to antibiotic diet (Hooge, 2004).

In the study of Samarasinghe *et al*. (2003), MOS at 2 g/kg was found to improve weight gain and showed a remarkable inhibition of duodenal coliform bacteria, yeast and mould in the caecum, and all viable microbes in the ileum. Zhou and Zhang (2003) observed that Bio-MOS @ 0.5 g/kg in diet of broilers significantly decreased *E. coli* in the ileum. Caecal bacterial load was significantly reduced by 3 logs with the addition of MOS to the diet (Stanley and Sefton, 2000). In the study of Elangovan *et al*. (2005), addition of mannan-oligosaccharide in conventional maize-soy diet did not influence the growth performance of broilers reared in battery cages, but was helpful in reducing the total viable counts and enteropathogens of intestine and total viable counts of meat samples.

**Organic acids:** The pH of the gut varies dramatically from the mouth, proventriculus and gizzard to the caecum of birds. The release of hydrochloric acid keeps the proventriculus and gizzard highly acidic. A low stomach pH (2.0 to 4.0) is required to initiate protein digestion by the enzyme pepsin and to prohibit bacterial growth. Small intestine pH ranges from 4.0 to 6.0. In the large intestine, the pH can vary between 6.0 and 7.0. The higher pH favors growth of the bacterial population. Any undigested feed residue will be fermented into lactic acid and volatile fatty acids (acetic, propionic and butyric). These VFAs provide a ready source of energy to the intestinal enterocytes, modify the motility of the intestinal tract, and help maintain the mucosal barrier and resistance to pathogenic challenges.

The balance between the beneficial (*Lactobacilli, Bifidobacter, Streptococci and Enterococci*) and pathogenic organisms (*E. coli, Salmonella*) is influenced by the gut pH, type and level of feed substrate and health status of the animal. The likelihood of reduced efficiency of enzyme action and increased proliferation of pathogenic bacteria in the gut increases at pH levels above 6.0. Maintenance of optimum pH values throughout the digestive tract will improve action of digestive enzymes on feed substances to deliver available nutrients to the animal and prevent excess undigested material being available for bacterial growth (Eidelsburger, 1988; Roth and Kirchgessner, 1998). The organic acids includes lactic, fumaric, citric, sorbic, malic, propionic and formic. The acid can act by dissociating in the gut and produce hydrogen ions, thereby modifying the pH of the intestine. If it does not readily
dissociate then the acid can pass through the cell wall of the bacteria and dissociate within the bacterial cytoplasm, increasing the cellular hydrogen ion concentration. The remaining part of the acid molecule disrupts the cellular DNA formation and protein synthesis. Lactic acid is particularly effective in this respect against *Staphylococcus aureus*, *Clostridium*, *E. coli*, *Salmonella* and *Enterobacteriaceae*. All acids have different dissociation constants (pKa values), the lower values indicate stronger acidification power.

**Conclusions:** The feeding of the broiler during the first week needs special care as it forms nearly 16% of its life, further the development of the gastro-intestinal tract and other associated organs are at its peak. The importance of early feeding helps in overall development of the chick organs and helps in further improvement in terms of mortality, stimulation of immune system and also building stronger immune system with less mortality post-hatch and better performance in broiler chicken. Strategic should be aimed at use of appropriate nutraceutical products to prevent intestinal malfunctions and help realize full genetic potential. Knowledge of anatomy, physiology, biochemistry, molecular biology and microbiology are all needed to design appropriate nutritional solutions for the future.

**References**


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Early studies by Burr and Burr in the year 1930, on rats fed with diet deficient in fat observed altered reproductive performance such as ovulation failure and delay in onset of estrus. Supplementation fat to those rats re-established the normal reproductive performance. Thus, the scientists believed that fatty acids have definite role in reproduction. Later, scientists concentrated on the fat supplementation in different animals species both in ruminants and non-ruminants of both sexes. The factors studied were establishment of puberty, maternal recognition of puberty, ovarian activity, secretion of reproductive hormones in females and semen quality parameters in males.

It is proved that some specific polyunsaturated fatty acids (PUFA) may pass intact the reticulo-rumen and be absorbed from the small intestine, allowing in this way the improvement of reproductive efficiency directly on the target tissue of the reproductive system of the female or by an indirect effect mediated by the endocrine system.

Several studies have shown that PUFAs of the omega-3 family such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are usually derived from fish play an important role in animal performance. Furthermore, lipid supplements partially resistant to biohydrogenation in the rumen have been developed such as calcium salts of long chain fatty acids (Ca-LCFA) which can be absorbed by limiting biohydrogenation. PUFAs act as mediators in a series of processes in several reproductive tissues, including fluidity of cell membrane, intracellular signalling and susceptibility to oxidative damage. Changes in chain length, degree of unsaturation and position of the double bonds in the acyl chain of fatty acids may have a major impact on reproductive function and play a role in livestock reproduction.

**Omega-3 and prostaglandins:** Prostaglandins (PG) are difficult to measure directly due to their relatively short half-life. Therefore, the inactivated prostaglandin metabolite (13, 14-dihydro-15-keto PGFM, is generally quantified.

The effects of omega-3 on contents of endogenous PG or the PGFM metabolite in ruminants in vivo have been studied following either direct lipid infusions in sheep or omega-3 or omega-6 supplementation in cattle. Intravenous infusions of omega-3 may be associated with reduced plasma PGFM concentrations in sheep. While plasma concentrations of PGFM and PGE\(_2\) were higher when sheep were infused with two lipid emulsions compared with saline, PGFM and PGE\(_2\) were lower on day 13–15 of the oestrous cycle when the lipid contained a lower omega-6:omega-3 ratio. Reduced concentrations of PGFM may be due to the effect of omega-3 on enzymes associated with PG synthesis. For example, myometrial concentrations of PGHS-2 mRNA (the rate-limiting enzyme in the synthesis of 2-series PG, were lower in ewes intravenously infused with a lipid high in long-chain omega-3 compared with long-chain omega-6.

While plasma fatty acids were not measured, the lower plasma PGFM was associated with a higher proportion of EPA and DHA and a lower proportion of LA in milk and caruncles.

Overall, results from the majority of infusion and feeding studies indicate that plasma PGFM concentrations are lower when animals are fed diets with a lower omega-6 : omega-3 ratio. However, there are a number of limitations to the existing research. Firstly, many of the supplements used, including fish oil/meal or rumen-protected lipids, are expensive and not indicative of typical commercial diets. Secondly, there do not appear to be any direct feeding studies in sheep and, finally, the influence of omega-3 on PGFM concentrations in animals of varying parity still needs to be clarified.
Effect of omega-3 on markers of female reproductive success: Omega-3 and omega-6 PUFA affects many factors associated with the synthesis and metabolism of important reproductive hormones such as the steroid hormones, progesterone (P4) and estradiol (E2). Diets rich in omega-3 fatty acids lowered plasma cholesterol level which may reduce steroid hormone synthesis, as cholesterol is a precursor for both P4 and E2. However, high omega-3 reduced PGF2α level and thus prevents regression of the corpus luteum (CL). The prolonged life of CL sustains the P4 release. Higher plasma concentrations of EPA have been associated with enhanced peroxisome proliferator activated receptors (PPARs) which reduces the P4 clearance. Conversely, diets rich in omega-6 fatty acids have been to increase steroidogenic acute regulatory protein and PGE2 which may stimulate P4 production.

The exact mechanism is not known for the higher P4 and E2 production when omega-6 rich fat fed to ruminants than omega-3 rich supplements. While omega-3 and omega-6 may alter the supply of cholesterol as a substrate for steroid synthesis, the most significant effects of omega-3 and omega-6 are likely to be mediated through the synthesis of series-2 and series-3 PG and the subsequent effects of PG on P4 and E2. The interaction of omega-3 with steroid hormone production and metabolism may be further complicated by negative feedback, as E2 may reduce the activity of delta-6-desaturase, thereby reducing the availability of long-chain omega-3 substrates for synthesis of series-3 eicosanoids.

Follicle development: When dairy cows were fed diets high in omega-3 FAs, the mean diameter of the ovulatory follicle and CL was higher, while follicle size was reduced when cows were fed diets high in omega-6 FAs. However, diets high in omega-6 FAs may also improve follicle development, as the number of medium sized follicles was increased when beef cows were supplemented with omega-6 (from soybean) compared with long-chain omega-3 FAs from fish oil or saturated fat. In contrast, results from a number of studies on dairy cattle have indicated that the follicle number, follicle diameter or CL volume is not affected by omega-3 FAs compared with omega-6 FAs.

Oocyte maturation and quality: Fatty acids are an important energy source for oocytes and early embryos. The level of FAs could affect oocyte maturation directly by altering the fatty acid composition of oocyte lipids, or indirectly by influencing the concentrations of PG and other metabolites in the follicular fluid surrounding the oocyte. The environment in which the oocyte grows will have a direct impact in the early life of embryo. Oocyte maturation both in vitro and in vivo was found to be improved following the inclusion of long-chain (fish oil) omega-3 FAs, in maternal diets. In addition, the number, quality and chilling resistance of oocytes from two month old Merino lambs were improved following supplementation with long-chain omega-3 FAs and these results were related to higher omega-3 FAs concentrations in the phospholipid fraction of cumulus cells, plasma and erythrocytes. On the contrary, supplementation with omega-6 FAs may be detrimental to oocyte maturation as high concentrations of omega-6 FAs in follicular fluid surrounding the immature oocyte and within the oocyte itself can inhibit the resumption of meiosis at the germinal vesicle stage, preventing the oocyte from further maturation.

Onset of estrus and ovulation rate: The effect of omega-3 and omega-6 FAs on PG and steroid hormones also has the potential to affect the onset of estrus and ovulation. In particular, increased PGF2α or PGE2 associated with higher omega-6 could stimulate early luteolysis of the CL and earlier onset of estrus, while inhibition of PG by n-3 has the potential to have the opposite effect. The time to estrus was also one day shorter when ewes were intravenously infused with olive oil compared with soybean oil. The olive oil, which contained a higher omega-6 : omega-3 ratio than soybean oil, stimulated higher plasma concentrations of both PGFM and PGE2 providing evidence supporting the possible links between fatty acids, PG and estrus initiation.
While ovulation rate is increased when the energy content of the diet is increased through the addition of total lipid or the combination of omega-3 and omega-6 FAs, few studies have examined the effects of specific alterations in omega-3 or omega-6 FAs on ovulation rate in ruminants. Methodological problems also prevent an accurate assessment of these effects. For example, the non-significant alteration in ovulation rate in heifers fed omega-3 or omega-6 diets may have been masked by the super ovulation induced by FSH treatment. In addition, low numbers of animals in some studies prevents a meaningful interpretation of ovulation rate data. Therefore, the effect of high omega-3 diets on ovulation rate and estrus remain unclear.

**Embryo survival:** Successful maternal recognition of pregnancy and subsequent embryo survival depends on the release of trophoblast interferon from the embryo which blocks the expression of endometrial oxytocin receptors and endometrial secretion of PGF\(_{2\alpha}\), preventing regression of the P4-secreting CL. As described previously, diets high in omega-3 may reduce PGF\(_{2\alpha}\) synthesis which may prevent regression of the CL, allowing continued secretion of P4 that may improve embryo survival. Although a number of researchers have inferred a positive effect of omega-3 on embryo survival through reduced PGF\(_{2\alpha}\) secretion by BEND cells *in vitro*, few studies have directly measured the effect of omega-3 FAs on embryo survival *in vivo*.

Results from a small number of studies indicate that omega-3 FAs may be associated with reduced embryo mortality. In a study conducted on dairy cows, embryo mortality was numerically lower following supplementation with the omega-3 ALA from linseed. Although not statistically significant, the effect of omega-3 may have been masked by the very low mortality in all treatment groups. While embryo recovery rate was not altered when cows were fed a ruminally protected long chain omega-3 FA from fish oil, the number of degenerate embryos was reduced following dietary inclusion of omega-3 FAs. Due to limited number of studies, the direct effects of omega-3 FAs on pregnancy rates and reproductive capacity of commercial herds or flocks remains largely unknown.

**Pregnancy rate:** Lipid supplementation has improved pregnancy rates in cows, however, the outcome of omega-3 and omega-6 FAs on pregnancy rates in ruminants were variable. There are no published studies that have examined the direct effects of high omega-3 diets on pregnancy rates in sheep.

Pregnancy rates were higher and pregnancy losses were lower when dairy cows were fed diets high in omega-3 compared with omega-6 or saturated fat. However, feeding omega-6 rich diets have been associated with lower pregnancy rates, but still, not all results were consistent.

**Gestation length and parturition:** Feeding of omega-3 or omega-6 FAs influenced gestation length and time of parturition through altering the type and quantity of PG synthesised, as PG are essential for parturition. Incidence of premature labour were less in cows fed omega-3 due to reduced circulating inflammatory cytokines.

While there is strong evidence linking consumption of diets high in long-chain omega-3 in late gestation with longer gestation length in humans, fewer studies have been conducted with ruminants. In sheep, however, gestation length was extended by two days when ewes were supplemented with fish oil in the third trimester compared with saturated fats and gestation length was longer when ewes fed an algal DHA.

Similarly, in an attempt to study the effect of omega-3 FAs on preterm delivery induced by betamethasone prolonged the time to deliver compared with omega-6 FAs. This was due to reduced plasma level of PG and myometrial PGHS-2 mRNA in ewes infused with long-chain omega-3 FAs. On the contrary, in cows there was no difference in gestation length or placental expulsion rate in dairy cows supplemented long-chain omega-3 or omega-6 FAs, plasma PGFM level was maintained without any change.
**Effect of omega-3 on male reproduction:** Prostaglandins may also play an important role in male reproduction, as there are several effects of PG on sperm motility and quality. In heat stressed bulls, alteration in the semen quality has been a consistent feature and this could be reversed by feeding omega-3 FAs in the bull diet. This could be explained by the higher incorporation of the DHA in the sperm which prevents oxidative damage to the sperms.

Diets enriched with omega-3 FAs and vitamin E improved post-thawed sperm quality in goats. Recently, in a study using rams found higher concentration of the sperms per ml when omega-3 FAs were included in diet. However, quality of the liquid semen was not improved.

It can be concluded that fat supplements with higher level of omega-3 fatty acids could enhance the reproductive performance of the ruminants. This unexplored area needs further investigations in animals maintained in hot climate like our country.

**References**

On Request

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Heat stress: As it affects nutrient digestibility and methane emission in ruminants

A. K. Madan and Brijesh Yadav

Department of Veterinary Physiology, College of Veterinary Science &A.H, DUVASU, Mathura, UP

The livestock sector is evolving in response to rapidly increasing demand for livestock products. Ruminant population is the main driver for growth of livestock sector besides pig and poultry. Rise in environmental temperature due to climate change alters basic physiology of rumen which negatively affects production. Dry matter intake begins to decline in an adaptive response to heat stress. Increased environmental temperature reduces gut motility, rumination, ruminal contractions and depresses appetite in ruminants. Heat stress reduces the total production of volatile fatty acid (VFA) with individual variation and also results in changes in ruminal pH. Passage rate and retention time of digesta is also influenced by rise in ambient temperature and thus affects digestibility. Change in microbiota due to heat stress may change fermentation pattern in rumen resulting in variation in digestibility, VFA production and also methane emission.

Effect of heat stress on rumination and rumen motility

Alteration of dynamic characteristics of digestion is recognized as a possible mechanism through which heat stress can affect nutrition of animals (Sejian et al., 2012). Increased environmental temperature reduces rumination time (Soriani et al., 2013) and depresses appetite (Dikmen et al., 2012) by having direct negative effect on appetite centre of hypothalamus (Baile and Forbes 1974). Available data suggest that rumination is depressed during dehydration and heat stress (Soriani et al., 2013 and Aganga et al., 1990). Also, blood flow to rumen epithelium is depressed during heat stress and reticular motility and rumination is decreased whereas, volume of digesta in rumen of beef cows, Bedouin goats (Silanikove, 1985), riverine buffalo and Egyptian buffalo (Marai and Haeeb 2010) is increased. Higher concentration of lactic acid and lower ruminal pH was observed in heat-stressed cattle, which may imply that a higher lactic acid concentration and lower ruminal pH might be involved in inhibiting rumen motility during heat stress (Mishra et al., 1970). There is no clarity on involvement of gastrointestinal hormones and peptidergic neurons in mediating effect of temperature on gastrointestinal motility. Some of gastrointestinal hormones that influence motility also affect feed intake in ruminants (Grovum 1981). Large number of biologically active compounds produced in gut that may influence motility and passage makes this area very complex (Bloom 1978).

Effect of heat stress on VFA production and ruminal pH

Heat stress reduced total production of volatile fatty acid (Kelly 1967 and Tajima et al., 2007). Ratio of acetate to propionate decreased during heat stress and more specifically, molar concentration of acetate decreased whereas propionate and butyrate concentration increased non-significantly (Nonaka et al., 2008). Effects of ruminal temperature on a dual-flow, continuous-culture system on in-vitro fermentation characteristics was investigated and found that high ruminal temperature decreased total VFA concentration as compared with normal ruminal temperature, however; ruminal temperature did not affect molar proportion of VFAs (Salles et al., 2010 and King et al., 2010). Decrease in molar concentration of volatile fatty acid during heat stress was mainly attributed to decrease in roughage intake (Kelly 1967) and variation in fermentation pattern due to changes in microbial population (Uyeno et al., 2010). High ruminal temperature increased culture pH from 5.73 to 5.82 on averages whereas an increase in ruminal pH form 5.82 to 6.03 was reported during heat stress in lactating dairy cattle.

Effect of heat stress on nutrient digestibility

Some authors reported an increase in diet digestibility in cattle exposed to hot environments (Christopherson 1985; National Research Council. 1981; Weniger 1992 and Nonaka et al., 2008). In
contrast, negative or no relationships between high ambient temperatures and diet digestibility have been reported in dairy cattle (Mathers et al., 1989; McDowell et al., 1969) and small ruminants (Silanikove, 1985). Positive effect of high environmental temperature on digestibility of feed is attributed to either reduction in passage rate of digesta (Christopherson 1985 and Christopherson and Kennedy 1983), changes in feed composition or reduction in DMI (Christopherson 1985). DM digestibility in Ayershire cattle was significantly higher at 33°C than at 20°C with moderate quality diet, but was similar at 33°C and at 20°C with a high-quality diet (Mathers et al., 1989). Digestibility patterns of different feed components during hot dry and hot humid thermal exposures were studied (Korde et al., 2007) and found that digestibility of CP, OM, NDF, ADF and NFCD increased during both type of exposures as compared to cool comfort whereas digestibility of NDF, ADF and NFCD was lower during hot-humid exposure compared to hot-dry. High ruminal temperature did not affect DM and NDF digestibility whereas it tended to decrease OM digestibility as compared to normal ruminal temperature (66.6 vs. 67.4%) (King et al., 2011). Digestibility pattern at different thermal exposures were investigated and it has been reported that digestibility at 25 and 30°C did not change whereas digestibility increased at 35°C and then decreased at 40°C thermal exposure (Yadav et al., 2012a). The decrease in nutrient digestibility at 40°C could be attributed to change in rumen environment (pH, rumen temperature, rumen motility, rumen flora and fauna) due to higher intensity of thermal stress (Yadav et al., 2012a). Higher concentrations of lactic acid and lower ruminal pH were observed in heat-stressed cattle, which may imply that a high lactic acid concentration and lower ruminal pH might be involved in inhibiting rumen motility during heat stress (Mishra et al., 1970). Variation in rate at which feed passes through digestive tract is a major factor in positive relationship between environmental temperature and digestibility (Christopherson and Kennedy 1983). Increase in diet digestibility in heat-stressed ruminants was explained by increased mean retention time in whole gastrointestinal tract (Bernabucci 2011). Slower passage rate and longer mean retention time of digesta have been described in dairy cows (Nonaka et al., 2012) and heifers (Nonaka et al., 2007) maintained under hot environment. A significant increase in DM digestibility was reported at 33°C as compared to 20°C in Holstein heifers (Nonaka et al., 2007). An improvement of diet digestibility during a short time exposure of Holstein heifers to hot conditions, which reduced when exposure was prolonged (Bernabucci et al., 1995). In same study, changes in diet digestibility observed under hot environment were not related to DMI and passage rate of digesta. Although a positive relationship between digestibility, especially of fiber components, and ambient temperatures has been reported (Lu 1989), high temperatures had little effect on diet digestibility in dairy cows (McDowell et al., 1976) and goats (Lu 1989). Digestibility coefficients of dry matter, organic matter, neutral detergent fiber and acid detergent fiber in sheep were not affected by short exposure (10 days) to a THI of 82, but were lower after prolonged exposure to heat (McDowell et al., 1969). Dilution of rumen content due to higher water intake, reduction in rumen bacteria activity, decline in rumen motility and reduction of saliva production may be responsible for digestibility changes when animals are chronically exposed to extreme temperature humidity index (Bernabucci et al., 2009). It was also opined that ewes, chronically exposed to heat exhibited lower diet digestibility and lower pH with cellulolytic and amylolytic bacteria concentrations, slower digesta passage rate and lower osmolarity of rumen content, indicating a possible impairment of bacterial activity and high dilution of rumen fluid. Negative effect of such a depression of rumen bacterial activity on diet digestibility might have overcome positive effects caused by decline in DMI and digesta outflow rate, resulting in net reduction of diet digestibility in chronically heat-stressed ewes. The microbiota composition was significantly different at elevated environmental temperatures and humidity (Tajima et al., 2007). In another study, four Holstein heifers exposed at three temperatures (20°C, 28 °C and 33 °C) in climatic chamber for two weeks resulted in increase in relative populations of Clostridium cocoides–Eubacterium rectal group and genus Streptococcus while genus Fibrobacter decreased in response to increasing temperature (Uyeno et al., 2010). The change in microbiota due to thermal exposure may change fermentation pattern in rumen resulting in variation in digestibility of different feed components and also composition of fermentation products. Other than alteration of bacterial activity,
different responses in digestibility in ewes exposed to thermal exposure for different times might be related to changes of ruminal and intestinal absorption of nutrients (Christopherson 1985).

**Effect of heat stress on methane production in rumen**

Methanogens belong to a separate domain archaea in kingdom of *Euryarchaeota* and are found in a wide range of other anaerobic environments (Liu and Whitman 2008). Through a series of biochemical reduction of carbon dioxide (CO$_2$) with hydrogen (H$_2$), methanogens use acetate and methyl group-containing compounds to produce methane (CH$_4$) (methanogenesis). Usually, CH$_4$ is produced by two types of methanogens, the slow-growing methanogens (regeneration time about 130 h) that produces CH$_4$ from acetate (*Methanosarcina*) and fast growing methanogens (generation time 4–12 h) that reduce CO$_2$ with H$_2$. In rumen, methanogenesis occurs mostly by fast-growing methanogens as ruminal retention time is too short to permit establishment of slow growing species. Methane emissions in ruminants also account for a 2% to 12% of gross energy loss of feeds depending upon type of diets (Johnson and Johnson 1995). Methane is one of by-products formed from degradation of carbohydrates during enteric fermentation in feed and anaerobic digestion in manure. Rumen is most important part of methane production in ruminants like cattle, while methane is mainly produced in large intestines for monogastric animals like pigs. Enteric fermentation accounts for about 80% of methane in dairy cow (Monteny et al., 2001). Methane production from enteric fermentation is a function of rate of organic matter fermentation, type of volatile fatty acid produced and efficiency of microbial biosynthesis (Shibata and Terada 2010). Major factors that affect methane production in ruminants are pH, volatile fatty acids, diet, feeding strategy, animal species and environmental stresses. Optimum pH for methane production is 7.0–7.2, but gas production can occur in pH range of 6.6–7.6. However, beyond this range, activity of fiber degraders reduces (Dijkstra et al., 2012).

The CH$_4$ emission rate exhibited significant diurnal variations with two peaks which were probably related to feeding routine. Daily CH$_4$ emissions increased significantly with activity of cows ($r = 0.61$) while daily CH$_4$ emissions were negatively correlated to indoor air temperature ($r = -0.84$). This suggests that increased daily indoor air temperatures due to seasonal changes may bring about decreased animal activity which may decrease release of CH$_4$ from dairy cows (Ngwabie et al., 2011). The methane production was found to be correlated with DMI (Ramin and Huhtanen 2012) whereas forage portion of diet has also been used to predict methane production (Hippenstiel et al., 2012). Effect of different thermal exposure on methane emission were studied (Yadav et al., 2012b) and found that methane emission per kg DMI was reduced during 35°C and as compared to exposure at 25 and 30°C, then increased at 40°C. Lowest methane emission at 35°C might be due to higher digestibility whereas methane production at 25 and 30°C was higher as digestibility was lower than at 35°C temperature exposure. This could be due to availability of more organic matter for microbes to convert into methane and hence methane production increased at 25 and 30°C. Highest methane emission per kg DMI at 40°C might be attributed to lower organic matter digestibility and shift in methane producing microbes and other microbial fermentation, due to change in rumen environment because of higher environmental temperature (Yadav et al., 2012b). An increase in methane emission was observed during higher ruminal temperature (King et al., 2011). Methane production from enteric fermentation was a function of rate of organic matter fermentation, type of volatile fatty acid produced and efficiency of microbial biosynthesis (Shibata et al., 2010).

**Conclusion and future action plan**

Effect of heat stress has been well established on digestibility and production of VFAs but there had been only few studies related to effect of heat stress on alteration of molar concentration of volatile fatty acids, methane production and population of flora and fauna in rumen. There are only few studies related to change in ruminal microbial population in response to heat stress. Methane is one of the main products of ruminal fermentation which might be also affected by heat stress. In scenario of climate change, methane emission from livestock animals is of paramount importance.
therefore, it necessitates a comprehensive study relating to microbial population being affected by heat stress especially methane producing bacteria. In order to mitigate and formulate successful strategies to combat negative effects of increased environmental temperature on rumen function it is highly pertinent to carry out studies to ascertain physiological and microbiological basis of alteration in rumen function during heat stress.

References


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INTRODUCTION

Stress in living beings may be considered as a physical, chemical, or emotional factor that causes bodily or mental unrest and that may be an etiological factor for a disease. Physical and chemical factors causing stress include trauma, infections, toxins, illnesses, and injuries. Emotional causes of stress and tension are variable. Stress is a fact of everyday life. When something happens to us, we evaluate the situation mentally. If it is threatening to us, we decide how to deal with this situation, and what skills we can use. If the situation outweighs the skills, then the situation is “stressful” and we react with the classic “stress response.” If we decide that our coping skills outweigh the situation, then we don’t see it as “stressful.”

Stress is a normal part of life. In small quantities, stress is good; it can motivate and help us to become more productive. However, too much stress, or a strong response to stress can be harmful. How we perceive a stress provoking event and how we react to it, determines its impact on our health. We may be motivated and invigorated by the events in our lives, or we may see some as “stressful” and respond in a manner that may have a negative effect on our physical, mental, and social well-being. Stress can come from any situation or thought that makes you feel frustrated, angry, or anxious. Everyone sees situations differently and has different coping skills. For this reason, no two people will respond exactly the same way to a given situation.

SOURCES OF STRESS

One can experience stress from four basic sources:

1. **The Environment** – the environment can impose intense influence on the body and compel us to adjust. Examples of environmental stressors include weather, noise, crowding, pollution, traffic, unsafe and substandard housing, and crime.

2. **Social Stressors** – we can experience multiple stressors arising from the demands of the different social roles we occupy, such as parent, spouse, caregiver, and employee. Some examples of social stressors include deadlines, financial problems, job interviews, presentations, disagreements, demands for your time and attention, loss of a loved one, divorce, and co-parenting.

3. **Physiological** – situations and circumstances affecting our body can be experienced as physiological stressors. Examples of physiological stressors include rapid growth of adolescence, menopause, illness, aging, pregnancy, giving birth, accidents, lack of exercise, poor nutrition, and sleep disturbances.

4. **Thoughts** – Our brain interprets and perceives situations as stressful, difficult, painful, or pleasant. Some situations in life are stress provoking, but it is our thoughts that determine whether they are a problem for us.

TYPES OF STRESSORS

Stress is not always a bad thing. Stress is simply the body’s response to changes that create taxing demands. Many professionals suggest that there is a difference between what we perceive as positive stress, and distress, which refers to negative stress. In daily life, we often use the term “stress” to describe negative situations. This leads many people to believe that all stress is bad for you, which is not true. Mainly there are two types:
**Positive stress** – is advantageous and has the following characteristics:

- Motivates, focuses energy
- Is short-term
- Is perceived as within our coping abilities
- Feels exciting
- Improves performance

An example of positive personal stressors includes:

- Receiving a promotion at work
- Starting a new job
- Marriage or commitment ceremony
- Buying a home
- Having a child
- Moving
- Taking or planning a vacation
- Holiday seasons
- Retiring
- Taking educational classes or learning a new hobby

**Negative stress** - is disadvantageous and has the following characteristics:

- Causes anxiety or concern
- Can be short or long-term
- Is perceived as outside of our coping abilities
- Feels unpleasant
- Decreases performance
- Can lead to mental and physical problems

An example of negative personal stressors includes:

- The death of a partner
- Filing for divorce
- Losing contact with loved ones
- The death of a family member
- Hospitalization (oneself or a family member)
- Injury or illness (oneself or a family member)
- Being abused or neglected
- Separation from a spouse or committed relationship partner
- Conflict in interpersonal relationships
- Bankruptcy/money problems
- Unemployment
- Sleep problems
- Children’s problems at school
- Legal problems
- Inadequate or substandard housing
- Excessive job demands
- Job insecurity
- Conflicts with team mates and supervisors
- Lack of training necessary to do a job
- Making presentations in front of colleagues or clients
- Unproductive and time-consuming meetings
- Commuting and travel schedules
MUSCULAR STRESS

Almost everybody has experience with muscular aches and pains. A slip on the ice pulls a muscle in your back. You exercise too strenuously after a sedentary week and are bothered by stiffness the next two days. The repetitive motions from long periods of typing or painting the ceiling leave you with a sore shoulder. Overexertion can cause muscle pain, so can stress. Increased muscle tension is an important part of the "fight or flight" stress reaction to demand and pressure. Noradrenaline from the sympathetic nervous system alerts the muscles to tense up in preparation for action. Tense muscles get set to act quickly in response to threat or danger. You move faster and have greater strength during an emergency because of this extra boost.

Muscular complaints are the most common physical sign of stress. Most of these only last for a short time and are low intensity for most people, but in others they can be long-lasting and therefore have major implications for basic functioning. They are the most frequent reason for sick leave and even disability.

Muscular stress may manifest itself in any of these ways:

- Back pain
- Headaches from muscle contraction
- Tight muscles
- Jaw pain
- Shakiness or difficulty sitting still

Suffering from any of these can interfere with work, family life, and simple activities of daily life. The human body has more than 690 separate muscles and accounts for more than 40 percent of body weight. People often take them for granted, expecting good performance day in and day out. Any one of these muscles can become overly fatigued, injured, or develop spasms. Muscle fibers are designed to tense and then relax. A muscle can go through this tense/relax cycle indefinitely. As you walk, one set of muscles tenses while the opposing set of muscles relaxes. However, a muscle under sustained tension without an alternating relaxation phase eventually develops spasm and pain. Sustained tensions from emotional stress, poor posture, or certain repetitive movements do not allow this relaxed phase to occur.

Noradrenaline alerts the muscles to tense up in preparation for action; this is called the “fight or flight” response. Tense muscles get set to act quickly in response to threat or danger. Exercising can produce a temporary stress on some body functions, but its health benefits are indisputable. It is only when stress is overwhelming, or poorly managed, that its negative effects appear. Understanding the relationship between stress and your muscles can help you tend to the special needs of this important organ system. The good news is that these straightforward interventions can make big difference in healing and protecting your muscles.

EVALUATION OF MUSCULAR STRESS

Resting Heart Rate

Check your resting heart rate (pulse) after relaxing for a period of time. You will need a watch or clock with a second hand (or digital seconds). First, find your radial pulse on the thumb side of your wrist or your carotid pulse on your neck just under the jaw. For sixty seconds count the number of beats that you feel.

Breathing Pattern

Sit in the chair so that your back is primarily straight up and down against the back of the chair. Place one hand on your abdomen with your palm covering your navel. Place your other hand on the upper part of your chest with the palm of that hand just above the heart. For a minute or two notice your breath as it goes in and comes back out. Become aware of your hands as you breathe in and out.
Which one seems to move more? Is it your abdominal hand or your chest hand? Or do they both move equally?

Try this second technique to see if you get the same results. First, breathe out and empty your lungs. Count to three as you inhale deeply. Now, hold it. Did your shoulders go up? Did you feel like the air filled the upper part of your lungs? If so, you probably lean toward chest breathing. If you are a diaphragmatic breather, you would feel your abdominal area expand, your belt tighten, and fullness in the lower part of your lungs and chest.

Respiration Rate

For about a minute count how many natural, effortless breaths you take in a minute. Be sure to breathe as normally and naturally as possible. Each inhalation and exhalation cycle is considered one breath. The number of breathes in one minute is your respiration rate.

Stress-o-meter

Another self-assessment is the Stress-o-meter. Think back over the last month of your life. Include all of your waking moments, as you think back. Give yourself a rating according to the following scale. A score of “1” would indicate that you feel your life has been relatively stress-free during that period. You have felt blissful, calm, peaceful and serene at all times. You have been able to adapt and “flow” with situations as they arise. A “10” score would mean that you felt very high anxiety most of the time. You may have had periods bordering on neurosis, suicidal, or very depressed feelings. A score of 10 would mean that this was a month packed with high levels of stress. Your perception of stress primarily determines how your body responds. The Stress-o-meter increases your awareness of the level of stress you perceive in your life. When we exercise we can follow a perceived exertion scale that will give us some idea of how hard we are exercising. We can determine our intensity level. Similarly, we can use the Stress-o-meter to assess our general levels of perceived stress over the past month.

Many factors are involved in determining a general level of stress. A couple physiological measures that relate to increased stress are increased heart rate and increased respiration rate. The average pulse rate for an adult is approximately 70-80 beats per minute. The average respiration rate is around 12-16 breaths per minute. A faster heart beat or breathing rate might be an indicator of higher than desired stress levels.

Perceived Stress Scale

A more precise measure of personal stress can be determined by using a variety of instruments that have been designed to help measure individual stress levels. The first of these is called the Perceived Stress Scale (PSS). The PSS, while originally developed in 1983, remains a popular choice for helping us understand how different situations affect our feelings and our perceived stress. The questions in this scale ask about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don't try to count up the number of times you felt a particular way; rather indicate the alternative that seems like a reasonable estimate.

For each question choose from the following alternatives:

0 - never
1 - almost never
2 - sometimes
3 - fairly often
4 - very often
1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and stressed?
4. In the last month, how often have you felt confident about your ability to handle your personal problems?
5. In the last month, how often have you felt that things were going your way?
6. In the last month, how often have you found that you could not cope with all the things that you had to do?
7. In the last month, how often have you been able to control irritations in your life?
8. In the last month, how often have you felt that you were on top of things?
9. In the last month, how often have you been angered because of things that happened that been outside of your control?
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

**Figuring your PSS score:**

You can determine your PSS score by following these directions:

First, reverse your scores for questions 4, 5, 7, & 8. On these 4 questions, change the scores like this: 0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0.

Now add up your scores for each item to get a total. **My total score is ______.**

Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress.

Scores ranging from 0-13 would be considered low stress.
Scores ranging from 14-26 would be considered moderate stress.
Scores ranging from 27-40 would be considered high perceived stress.

The Perceived Stress Scale is interesting and important because your perception of what is happening in your life is most important.

**Symptoms of Muscular Stress:**

- Headaches
- Tense muscles, sore neck and back
- Fatigue
- Anxiety, worry, phobias
- Difficulty falling asleep
- Irritability
- Insomnia
- Bouts of anger/hostility
- Boredom, depression
- Eating too much or too little
- Diarrhea, cramps, gas, constipation
- Restlessness, itching, tics

**Kinds of Muscular Stress**

A) **Myalgia**, or muscle pain, is a symptom of many diseases and disorders. The most common causes are the overuse or over-stretching of a muscle or group of muscles. Myalgia without a traumatic history is often due to viral infections. Longer-term myalgias may be indicative of a metabolic myopathy, some nutritional deficiencies or chronic fatigue syndrome. The most common
causes of myalgia are overuse, injury or strain. However, myalgia can also be caused by diseases, disorders, medications, or as a response to a vaccination. It is also a sign of acute rejection after heart transplant surgery. Sudden cessation of high-dose corticosteroids, opioids, barbiturates, benzodiazepines, or alcohol can induce myalgia.

**Considerations**

Muscle pain is most frequently related to tension, overuse, or muscle injury from exercise or physically-demanding work. In these situations, the pain tends to involve specific muscles and starts during or just after the activity. It is usually obvious which activity is causing the pain. Muscle pain also can be a sign of conditions affecting your whole body, like some infections (including the flu) and disorders that affect connective tissues throughout the body (such as lupus). One common cause of muscle aches and pain is fibromyalgia, a condition that includes tenderness in your muscles and surrounding soft tissue, sleep difficulties, fatigue, and headaches.

**Home Remedy**

For muscle pain from overuse or injury, rest that body part and take acetaminophen or ibuprofen. Apply ice for the first 24 - 72 hours of an injury to reduce pain and inflammation. After that, heat often feels more soothing. Muscle aches from overuse and fibromyalgia often respond well to massage. Gentle stretching exercises after a long rest period are also helpful. Regular exercise can help restore proper muscle tone. Walking, cycling, and swimming are good aerobic activities to try. A physical therapist can teach you stretching, toning, and aerobic exercises to feel better and stay pain-free. Begin slowly and increase workouts gradually. Avoid high-impact aerobic activities and weight lifting when injured or while in pain. Be sure to get plenty of sleep and try to reduce stress. Yoga and meditation are excellent ways to help you sleep and relax. If home measures aren't working, your clinician may prescribe medication or physical therapy, or refer you to a specialized pain clinic. If your muscle aches are due to a specific disease, follow the instructions of your doctor to treat the primary illness.

**Prevention**

- Warm up before exercising and cool down afterward.
- Stretch before and after exercising.
- Drink lots of fluids before, during, and after exercise.
- If you work in the same position most of the day (like sitting at a computer), stretch at least every hour.

B) Hyperkinesia, also known as hyperkinesis, comes from the Greek hyper, meaning "increased," and kinein, meaning "to move." Hyperkinesia is a state of excessive restlessness which is featured in a large variety of disorders that affect the ability to control motor movement, such as Huntington’s disease. It is the opposite of hypokinesia, which refers to decreased bodily movement, as commonly manifested in Parkinson's disease. Many hyperkinetic movements are the result of improper regulation of the basal ganglia-thalamocortical circuitry. Overactivity of a direct pathway combined with decreased activity of an indirect pathway results in activation of thalamic neurons and excitation of cortical neurons, resulting in increased motor output. Often, hyperkinesia is paired with hypotonia, a decrease in muscle tone. Many hyperkinetic disorders are psychological in nature and are typically prominent in childhood. Depending on the specific type of hyperkinetic movement, there are different treatment options available to minimize the symptoms, including different medical and surgical therapies.

Studies have been done with electromyography to trace skeletal muscle activity in some hyperkinetic disorders. The electromyogram (EMG) of dystonia sometimes shows rapid rhythmic bursts, but these patterns can almost always be produced intentionally. In the myoclonus EMG, there are typically brief, and sometimes rhythmic, bursts or pauses in the recording pattern. When the bursts
last for 50 milliseconds or less they are indicative of cortical myoclonus, but when they last up to 200 milliseconds, they are indicative of spinal or brainstem myoclonus. Such bursts can occur in multiple muscles simultaneously quite quickly, but high time resolution must be used in the EMG trace to clearly record them. The bursts recorded for tremor tend to be longer in duration than those of myoclonus, although some types can last for durations within the range for those of myoclonus. Future studies would have to examine the EMGs for tics, athetosis, stereotypies and chorea as there are minimal recordings done for those movements. However, it may be predicted that the EMG for chorea would include bursts varying in duration, timing, and amplitude, while that for tics and stereotypies would take on patterns of voluntary movements.

**Amelioration of Muscular Stress**

**Deep Breathing**

Deep breathing is a simple but very effective method of relaxation. It is a core component of everything from the "take ten deep breaths" approach to calming someone down, right through to yoga relaxation and meditation. It works well in conjunction with other relaxation techniques such as Progressive Muscular Relaxation, relaxation imagery and meditation to reduce stress. To use the technique, take a number of deep breaths and relax your body further with each breath.

**Progressive Muscular Relaxation (PMR)**

Progressive Muscular Relaxation is useful for relaxing your body when your muscles are tense. The idea behind PMR is that you tense up a group of muscles so that they are as tightly contracted as possible. Hold them in a state of extreme tension for a few seconds. Then, relax the muscles to their previous state. Finally, consciously relax the muscles even further so that you are as relaxed as possible. By tensing your muscles first, you will probably find that you are able to relax your muscles more than would be the case if you tried to relax your muscles directly. Experiment with PMR by forming a fist, and clenching your hand as tight as you can for a few seconds. Then relax your hand to its previous tension, and then consciously relax it again so that it is as loose as possible. You should feel deep relaxation in your hand muscles.

This is something that you can do for yourself by following these steps:

- Sit quietly and comfortably.
- Close your eyes.
- Start by relaxing the muscles of your feet and work up your body relaxing muscles.
- Focus your attention on your breathing.
- Breathe deeply and then let your breath out. Count your breaths, and say the number of the breath as you let it out (this gives you something to do with your mind, helping you to avoid distraction). Do this for ten or twenty minutes.

**Ways to relax your mind**

- **Write.** It may help to write about things that are bothering you. Write for 10 to 15 minutes a day about stressful events and how they made you feel. This helps you find out what is causing your stress and how much stress you feel. After you know, you can find better ways to cope.

- **Let your feelings out.** Talk, laugh, cry, and express anger when you need to. Talking with friends, family, a counselor, or a member of the clergy about your feelings is a healthy way to relieve stress.

- **Do something you enjoy.** This can be:
  - A hobby, such as gardening.
  - A creative activity, such as writing, crafts, or art.
Playing with and caring for pets.
Volunteer work.

You may feel that you're too busy to do these things. But making time to do something you enjoy can help you relax. It might also help you get more done in other areas of your life.

- **Focus on the present.** Meditation and guided imagery are two ways to focus and relax your mind.
  - **Meditate.** When you meditate, you focus your attention on things that are happening right now. Paying attention to your breathing is one way to focus. For more information, see:
  - **Guided imagery.** With guided imagery, you imagine yourself in any setting that helps you feel calm and relaxed. You can use audiotapes, books, or a teacher to guide you.

**Ways to relax your body**

- **Exercise:** Regular exercise is one of the best ways to manage stress. Walking is a great way to get started. Even everyday activities such as housecleaning or yard work can reduce stress. Stretching can also relieve muscle tension.
- **Try techniques to relax:** Breathing exercises, muscle relaxation, and yoga can help relieve stress.
- **Yoga, tai chi, and qi gong:** These techniques combine exercise and meditation. You may need some training at first to learn them. Books and videos are also helpful. You can do all of these techniques at home.

**How to manage your stress**

_Understand how you experience stress._ Everyone experiences stress differently. How do you know when you are stressed? How are your thoughts or behaviors different from times when you do not feel stressed?

_Identify your sources of stress._ What events or situations trigger stressful feelings? Are they related to your children, family, health, financial decisions, work, relationships or something else?

_Learn your own stress signals._ People experience stress in different ways. You may have a hard time concentrating or making decisions, feel angry, irritable or out of control, or experience headaches, muscle tension or a lack of energy. Gauge your stress signals.

_Recognize how you deal with stress._ Determine if you are using unhealthy behaviors (such as smoking, drinking alcohol and over/under eating) to cope. Is this a routine behavior, or is it specific to certain events or situations? Do you make unhealthy choices as a result of feeling rushed and overwhelmed?

_Find healthy ways to manage stress._ Consider healthy, stress-reducing activities such as meditation, exercising or talking things out with friends or family. Keep in mind that unhealthy behaviors develop over time and can be difficult to change. Don't take on too much at once. Focus on changing only one behavior at a time.

_Take care of yourself._ Eat right, get enough sleep, drink plenty of water and engage in regular physical activity. Ensure you have a healthy mind and body through activities like yoga, taking a short walk, going to the gym or playing sports that will enhance both your physical and mental health. Take regular vacations or other breaks from work. No matter how hectic life gets, make time for yourself -- even if it's just simple things like reading a good book or listening to your favorite music.

_Reach out for support._ Accepting help from supportive friends and family can improve your ability to manage stress. If you continue to feel overwhelmed by stress, you may want to talk to a psychologist, who can help you better manage stress and change unhealthy behaviors.
Conclusion

In general, research for treatment of muscular stress has most recently been focusing on ameliorating symptoms rather than attempting to correct the pathogenesis of the disease. Therefore, now and in the future it may be beneficial to inform the learning of the disease's pathology through carefully controlled, long-term, observation-based studies. As therapies are supported by proven effectiveness that can be repeated in multiple studies, they are useful, but the clinician may also consider that the best treatments for patients can only be evaluated on a case-by-case basis. It is the interplay of these two facets of neurology and medicine that may bring about significant progress in this field.

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Regulation of corpus luteum function by locally produced growth factors in buffalo

Mahesh Gupta, S S Dangi, Gyanendra Singh, V P Maurya, Mihir Sarkar
Division of Physiology & Climatology IVRI, Izatnagar

Buffalo (Bubalus bubalis) is an important livestock species, concentrated mostly in the tropical and subtropical regions of the world. Asian Buffaloes representing 97.2% of world buffalo population lead the worldwide population of 189.8 million (FAOSTAT, 2011). In India the population of buffalo is one half as compared to cattle population, but contributes 55 % of the total milk production (62.8mtn in 2010) (FAOSTAT, 2011). Large population of buffalo (30–40%) remains unproductive due to reproductive problems like suboestrus, anoestrous, infertility that has hormonal etiology (Prakash, 2002; Madan and Prakash, 2007) incurring an estimated loss of 19–20 million tonnes of milk each year. Different conditions such as hot and humid season, insufficiency of nutrition, suckling of buffaloes, summer calvers, and early postpartum period are causes of frequent subestrus and increasing infertility in buffalo. Infertility in domestic animals is, in part, attributed to luteal dysfunction resulting in inadequate progesterone production (Foley 1996). Luteal insufficiency is considered one of the primary causes of embryonic mortality in buffalo (Campanile and Neglia 2007). Therefore, understanding of the endocrine, autocrine/paracrine factors that regulate luteal development and function is fundamental for the formulation of strategies aimed at improving fertility in buffalo.

Reproductive cycle in mammals is a process of chronological proliferation, differentiation and transformation of ovarian follicular cells followed by formation and regression of the corpus luteum (CL) in a cyclic manner (Schams et al. 2009). CL plays a central role in regulating the estrous cycle and in maintaining pregnancy. This function is performed largely by progesterone, which is the main steroid synthesized by the CL, a transient endocrine gland (Gregoraszczuk, 1994). The formation, maintenance, regression, and steroidogenesis of the CL are among the most significant and closely regulated events in mammalian reproduction. At the end of its life span, the CL undergoes a process of regression, leading to its disappearance from the ovary and allowing the initiation of a new cycle. In this process role of pituitary gonadotropins and growth hormone is well established (Schams et al. 2002). But recent reports also give evidences of essential modulatory role by locally produced factors such as steroid hormones, peptides and growth factors in the development of follicle, CL and regulation of reproductive cycle (Hyashi et al. 2003; Berisha and Schams 2005). These locally produced factors constitute a complex intra-ovarian autocrine/paracrine regulatory system to regulate the reproductive functions. Some the local growth factors that play important role in CL functions studied so far in buffalo are vascular endothelial growth factor A, C, D (VEGFA, C, D). Insulin like growth factor (IGF), leptin and ghrelin. Understanding these factors and their role in CL function will help formulation of strategies for improving fertility in buffalo.

VEGFA:

VEGF are growth factors that induce proliferation as well as migration and survival of endothelial cells. The VEGF have four different isoforms of 120, 164, 188 and 206 amino acids. The biological activities of VEGF are mediated through the two phosphotyrosine kinase receptors on endothelial cells, namely the VEGFR1 or‗fms-like tyrosine kinase‘ (Flt-1) and the VEGFR2 or ‘kinase insert domain-containing receptor‘ (KDR) (Neufeld et al. 1999). VEGF play an important role in CL development and function.

Corpus luteum has a heterogeneous tissue composition, comprising mainly of endothelial cells (EC), large luteal cells (LL) and small luteal cells (SL) besides fibroblasts, smooth muscle cells and immune cells (O’Shea et al. 1989). A precise interaction of the various cell types is essential for
growth of new blood vessels and establishment of a functional blood supply in the complex tissue. Growth of adequate vasculature is a fundamental requirement for development of CL during oestrous cycle and maintenance of pregnancy. Decreased luteal vascularity has been associated with inadequate luteal function in addition to its critical role in luteal regression (Reynolds et al. 1994). Therefore, the precise control of angiogenesis in the ovary is critical for normal luteal function. Among several molecules implicated as mediators of angiogenesis in the CL, VEGF appears to play a pivotal role in the regulation of vascular growth in the corpus luteum in several species including buffalo. In buffalo, time-dependent changes in VEGF mRNA and protein expression were observed with a very high correlation of VEGF system expression with vascular density and progesterone concentrations (Papa et al. 2007). mRNA expression and protein localization of VEGF isoforms VEGF120, VEGF 164 and VEGF 188 and their receptor VEGFR1 and VEGFR2 in buffalo corpus luteum (CL) during different stages of the oestrous cycle was demonstrated in our lab. The mRNA as well as protein expression of VEGF system was highest during the early and mid-luteal phase, which later steadily decreased after day 10 to reach the lowest level in regressed CL. (Chouhan et al., 2013a). The higher VEGF expression during early and mid luteal phase, indicating possible role of the VEGF system in the regulation of luteal angiogenesis and proliferation of luteal as well as endothelial cells through their non-angiogenic function. Further it was also demonstrated that VEGF treatment increase progesterone secretion and expression of progesterone synthesis intermediates (StAR, CYP11A1 and 3β-HSD mRNA) in luteal cell culture (Chouhan et al., 2013b). Positive effect of VEGF on cell survival and inhibitory effect on apoptosis was also found. All these observation clearly indicate that VEGF have important role in angiogenesis, cell proliferation and steroid synthesis in corpus luteum.

Lymphangiogenic growth factor (VEGF C and VEGF D):

Existence of lymphatic system in corpus luteum has been demonstrated in several species including domestic species pig and cattle. Recently Ali et al. (2013) demonstrated presence of lymphatic system in buffalo corpus luteum. Lymphatic system in CL has several important roles. Progesterone production is the key function of CL for initiation and maintenance of pregnancy. Early studies suggested that the luteal lymphatics transport steroid hormones from the ovary to the blood circulation; notably, the concentration of progesterone in ovarian lymph varied during estrous cycle (Hein et al., 1988). A primary function of lymphatics in the body is the maintenance of fluid homeostasis. The CL is a highly vascularized organ with a sustained, heightened blood flow to the CL-bearing ovary, and luteal blood capillaries are exceptionally permeable to plasma protein. Therefore, return of interstitial fluid and proteins to the vascular circulation may be a critical function of the lymphatics in the CL. VEGF C and VEGF D are growth factors that promotes lymphangiogenesis. They perform their action act through a common receptor VEGF R3. Lymphatic vessel endothelial hyaluronan receptor (LYVE-1) is one of the most specific and widely used lymphatic endothelial markers. Very recently mRNA and protein expression and localization of VEGF C, VEGF D, VEDF R3 and LYVE1 in different stages of corpus luteum during estrous cycle in buffalo was reported (Ali et al., 2013). There was stage wise difference in expression of above mentioned factors but their expression indicates existence of lymphatic system in buffalo CL for the first time. It was also observed that combination of VEGF C and VEGF D increases the expression of lymphatic endothelial marker LYVE1 in cultured luteal cells. These finding shows stimulatory effect of VEGF C and VEGF D on lymphangiogenesis in buffalo CL.

IGF System:

The insulin-like growth factor (IGF) system is a complex system consisting of two peptide hormones (IGF1 and IGF2), two types of cell surface receptors (IGFR1 and IGFR2) and six circulating binding proteins (IGFBP1-6) (Pavelic et al., 2007). All the components of IGF family act together to regulate the number of crucial biological outcomes such as cellular growth, proliferation, differentiation, survival against apoptosis, and migration (Khandwala et al., 2000; Pollak et al., 2004).
The expression and function of IGF family in CL was well established by different workers in different species of animals. But the existence of IGF family and their functions in buffalo corpus luteum was lacking. Recently Uniyal et al. (2012) reported mRNA and protein expression and localization of IGF1, IGF2, IGF R1, IGF R2 and IGFBP 1-6 in buffalo CL during different stages of estrous cycle. The results indicated that IGF system clearly expressed in buffalo CL with distinct variation in their transcript level during different stage of CL development. It was also observed that IGF1 increase VEGF and progesterone production in luteal cell culture in buffalo. All the observations clearly indicate IGF family members via their autocrine/paracrine effect play a significant role in promoting angiogenesis through production of VEGF in luteal cells, steroid synthesis through production of key steroidogenic factors and may act as proliferative, anti-apoptotic, mitogenic factors in bubaline CL, therefore, implying a key role of IGF system in regulation of reproductive cycle in water buffalo (Uniyal et al., 2012).

**Leptin:**

Leptin, a 16.4 kDa peptide hormone, product of the obese gene, is secreted primarily in adipocytes and is known to play a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure (Zhang et al., 1994). Apart from their role in the regulation of body weight and energy expenditure, evidence suggests that leptin also plays an important role in reproduction. Its role in reproduction includes important actions on the hypothalamus to bring about release of LH-releasing hormone, thereby triggering gonadotropin release and leading to development of the reproductive tract and induction of puberty (Caro et al., 1996). Recent evidences indicate direct role of leptin in ovarian functions. Expression of leptin and its receptor in bovine ovary was reported by Sarkar et al. (2010). mRNA and protein expression and localization of leptin and its receptor in bubaline CL during estrous cycle was demonstrated recently in our lab (Kumar et al., 2012). It was observed that expression of leptin and receptor along with mRNA expression of key steroid intermediates (StAR, P450scc and 3β-HSD) was highest during mid and late luteal phase and lower during early and regressing CL. Leptin concentration by ELISA was also higher during mid and late luteal stage (Kumar et al., 2012). These observations indirectly indicates role of leptin in luteal function and steroidogenesis in bubaline CL.

**Ghrelin:**

Ghrelin is a novel growth hormone-releasing acylated peptide, isolated from stomach. It is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a), a 7-transmembrane G protein-coupled receptor (Kojima et al., 1999). Principal functions of ghrelin are to increase food intake (hunger hormone) and to increase growth hormone secretion. A striking feature of ghrelin is its widespread pattern of expression (Van der Lely et al., 2004). Recently ghrelin has been reported to be associated with reproduction. Principal effects of ghrelin are on the neuroendocrine component of reproduction (Fernandez-Fernandez et al., 2004), evidence has emerged to indicate direct involvement of ghrelin in ovarian function. At systemic level, inhibitory effect ghrelin on hypothalamic pituitary axis has been reported in human, rat and pigs (Zhang et al., 2008 Muccioli et al., 2011). Expression of ghrelin and its cognate receptor has been demonstrated in the ovary of domestic species like sheep, goat (Chandra et al., 2012), pig (Rak-Mardyla et al., 2012) and cattle (Deaver et al., 2012). Recently it was found that ghrelin and its receptor are expressed in bubaline CL and their expression varies during estrous cycle. It was also observed that ghrelin have inhibitory effect of on progesterone production and mRNA expression of progesterone synthesis intermediates in cultured luteal cells of buffalo (Gupta et al., 2013). All these finding suggest possible regulatory role of ghrelin on buffalo CL.

Above discussion indicates that there is complex regulatory endocrine, autocrine, paracrine regulatory system which control CL formation, development, regression and functions. Thus these autocrine paracrine growth factors have important role in reproduction.
References


Melatonin and female reproduction - Emerging concepts

J. Kumar and D.K. Swain

Department of Veterinary Physiology, College of Veterinary Science &A.H, DUVASU, Mathura, UP

Introduction

The discovery of melatonin by Lerner and colleagues in 1958 heralded a new field of research in reproductive physiology. Ever since Huebner reported that a tumor of human pineal gland altered pubertal development (almost 70 years before identification of melatonin), it was surmised that some factor of pineal origin may be capable of influencing reproductive function (Russel et al., 2009). This led many scientists in first half of 20th century to experimentally examine association of pineal with reproductive status in a variety of species but with limited success in terms of demonstrating a functional relationship (Tamura et al., 2008). The findings were not sufficiently compelling to convince most, if any, reproductive biologists that pineal gland and reproductive system were functionally linked. Essentially concurrent with discovery of melatonin, cytological studies of pineal gland indicated that its metabolic activity was increased during darkness. These observations were soon supported by reports claiming that activity of one of enzymes that is required for melatonin synthesis (i.e., Hydroxyindole O-methyltransferase, now called Acetyl serotonin O-methyltransferase) was supposedly higher in pineal at night than during day (Tamura et al., 2009).

Synthesis and source of melatonin

Melatonin is an indoleamine originally discovered to be a secretory product of mammalian pineal gland and subsequently found to be produced in many different cells/organs and in all species of plant and animal kingdoms. Melatonin easily crosses cell membranes and all morphophysiological barriers, e.g., the blood-brain barrier, blood testes barrier and transovarian axis (Rodriguez et al., 2004).

The following diagram illustrates the mechanism of synthesis of melatonin from Tryptophan amino acid (Tamura et al., 2009). Tryptophan, which is taken up from blood, via four step pathway outline is converted to N-acetyl-5-methoxytryptamine (melatonin). Melatonin is best known for its production in cells of pineal gland from which it is quickly released in body fluids, i.e., blood and cerebrospinal fluid. Circulating melatonin has both receptor-mediated and receptor-independent actions. Many other cells also produce melatonin; in this case, indoleamine does not gain access to blood in any appreciable amounts but rather works near its site of synthesis as an autacoid or as a paracoid (Russel et al., 2009).
Role in oxidative stress

Melatonin acts as a potential antioxidant by showing scavenging action against reactive oxygen species and reactive nitrogen species (Tamura et al., 2008). The figure illustrates the actions of melatonin in reducing free radical-mediated molecular damage. Melatonin stimulates (blue lines) several antioxidative enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRd) and glutamylglycine ligase (GCL). It also inhibits (red line) the pro-oxidative enzyme nitric oxide synthase (NOS) (Liu and Borjigin, 2005). In addition to modulating activity of these enzymes, melatonin directly scavenges highly toxic hydroxyl radical (·OH), peroxynitrite anion (ONOO−) and possibly some other radical and non-radical products. Superoxide anion radical (O2•−), hydrogen peroxide (H2O2) and ·OH are referred to as reactive oxygen species (ROS); nitric oxide (NO) and ONOO− are referred to as reactive nitrogen species (RNS). O2 = molecular oxygen; e− = electron; Fe2+ = ferrous iron (Russel et al., 2009).
norepinephrine, which activates primarily β-adrenergic receptors to stimulate a cascade of molecular events that culminate in melatonin production and release (Galano et al., 2001). Seasonally changing photoperiods alter duration of elevated nocturnal melatonin production, a signal that provides mammals with time-of-year information. This message determines breeding season of both long-day and short-day breeders. In both cases, young are characteristically delivered in spring or early summer (Tamura et al., 2008). Light and dark regulation of biological clock (suprachiasmatic nucleus), pineal melatonin production and seasonal reproduction in photoperiodic mammals is represented in following figure. (ASMT- Acetyl Serotonin Omethyl Transferase; HIO MT- Hydroxyindole O-methyltransferase; NAT- Akylamine –N- Acetyl transferase) (Russel et al., 2009).

**Melatonin and female reproduction**

In ovarian follicle, melatonin impacts function of numerous cells, especially granulosa cells and ovum (oocyte). Action of melatonin in these cells are mediated via membrane receptors (MT, in particular MT1 and MT2) and also possibly via binding sites in nucleus and in cytosol. In addition
to its receptor-mediated actions, melatonin also functions as a direct free radical scavenger to reduce oxidative stress at the level of ovary; this beneficial action is carried out without an interaction with a receptor (Russel et al., 2013). Additional antioxidant functions of melatonin are achieved when the indole stimulates enzymes which metabolize free radicals to less toxic products. The antioxidative enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in thecal cells, granulosa cells and in the follicular fluid. Via these actions, melatonin reduces free radical damage, which would be especially bad for the ovum, and maintains these elements in an optimally functional state (Russel et al., 2009). The origin of melatonin in follicular fluid is blood and from its local synthesis in granulosa cells. C, cholesterol; LH R, LH receptor; FSH R, FSH receptor; NAT, N-acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase (currently known as acetylserotonin methyltransferase, ASMT); MIH, maturation-inducing hormone; MPF, maturation-promoting factor; GVBD, germinal vesicle breakdown; ROS, reactive oxygen species; IGF, insulin-like growth factor; TGF-β, transforming growth factor β. The possible roles of melatonin are outlined in the following figure (Tamura et al., 2008).

**Melatonin in regulation of follicular functions**

Melatonin and several of its metabolites are scavengers of ROS and RNS. Moreover, melatonin promotes expression and activities of several antioxidative enzymes (SOD, GPX, and GSR), while inhibiting activity of pro-oxidative enzyme nitric oxide synthase (NOS). Melatonin has been reported to reduce a damaged DNA product (8-OHdG) and a product resulting from oxidation of lipids (hexanoyl-lysine adducts) in FF (Russel et al., 2009). The level of oxidatively damaged molecules in the follicular fluid directly correlates with the quality of the ovum. Melatonin has been shown to stimulate maturation-inducing hormone (MIH), 20 b-dihydroxy-4-pregn-3-one), which
promotes maturation of the oocyte. Potential functions of melatonin during pregnancy are outlined in the lower figure. The information outlined below is mostly obtained from study of human and murine models. Large mammal studies are still to be done and understood (Russel et al., 2013). Melatonin readily crosses the placenta to enter fetal circulation. Day and night variation in melatonin levels provides fetal SCN with light and dark information. Melatonin has been found to protect the fetus from oxidative stress due to ROS and RNS. This indole may also enhance progesterone (P) synthesis by corpus luteum (and later by placenta) to assist in maintenance of pregnancy, while inhibiting premature release of oxytocin. In placenta, melatonin has similar protective effects against nitro-oxidative stress. Several investigators have proposed use of melatonin to reduce severity of preeclampsia and associated alterations. Although details of actions of melatonin in ovary and uterus are limited, experimental data published to date indicate likelihood of its improving microenvironment of both organs (Russel et al., 2009).

**Conclusion**

Melatonin has emerged as a powerful regulator of reproduction in both male and female. It is also known that it is secreted in ovarian mass that is from ovarian follicles and from placenta. The potential of melatonin in maintaining an optimally functioning peripheral reproductive system has been established. Melatonin’s functions, both in terms of its receptor-mediated and receptor-independent (e.g., antioxidant) actions, are, however, ubiquitous. Thus, melatonin may be critical for not only conserving reproductive health, but health in general. Diminishment of melatonin’s antioxidative protection during aging could certainly compromise favorable cell physiology at multiple reproductive organ levels. Also, since melatonin passes placenta and has proven antioxidant actions in fetus, it is possible that treatment of females with melatonin during pregnancy, especially when pregnancy occurs late in normal reproductive period, may reduce certain fetal problems associated with late-life pregnancies. Melatonin, even when given at extremely high doses to pregnant rats, has not been shown to have measureable untoward effects in either mother or fetus. These findings have opened new avenues to treat follicular abnormalities as well as birth related issues. In future melatonin can be used as therapeutic molecule in treatment of
female ailments associated with reproductive system.

References
Education, employment and nutrition hold the key to sustainable human development. The fast changing global scenario on food security and safety, shrinking natural resources (land, energy, water and biodiversity), trade linked issues and the climate change effects pose a major challenge to provide quality, safe, adequate and balanced nutrition to a large human population and keeping the livestock farming economically rewarding. Ensuring food and nutritional security and eliminating hunger, including hidden hunger, is the National priority. To meet this challenge, sustained scientific and knowledge based advancements rather than input based efforts are required in meat sector. India has achieved self-sufficiency in food grain production, thus has been able to eliminate calorie hunger. But it has not been able to eliminate the protein hunger. Now the focus has been shifted from "starch to protein self-sufficiency". Meat and meat products being one of the important components of balanced diet, this sector warrants action on many fronts to increase their availability at affordable prizes (Agnihotri et al., 2011).

Growing population and rising income along with fast changing food preferences are rapidly increasing demand for meat products in the country while globalization is boosting trade in livestock inputs and their products. Demand driven growth in livestock products sector will enable millions of poor to escape poverty trap provided adequate required inputs and support services are provided at an affordable cost. During the XI Plan, meat sector registered a growth rate of 4.1% being highest (8.0%) for buffalo meat (Table 4).

Livestock Population vis-a-vis Other Resources

India, with about 11% of the world livestock population, occupies a significant place numerically in respect of livestock wealth. With 2.4% of the land area of the world, and only 4.2% of the world’s fresh water, it maintains 1.21 billion human population which is more than 18% of world’s human population and about 529.70 million livestock and 648 million poultry (Table 1).

The cattle, buffalo, sheep, goat and pigs population which was 185.20, 97.90, 61.50, 124.4, 13.50 millions in 2003 reached to 199.10, 105.30, 71.60, 140.5, 11.1 millions, respectively in 2007 (18th Live stock census, DADF, M/o Agriculture). The average annual growth rate in population of these species during the corresponding period was 1.83, 1.84, 3.87, 3.1( -) 4.74 percent. Buffalo has surpassed the cattle population growth rate for various reasons viz., use for milk, meat and draft purposes as well as PFA standards for %fat and SNF favoring buffalo milk rather than cow milk production.

Though the country possesses about 57% of buffaloes, 14 % of cattle, 16 % of goat, 6 % of sheep and 1.5 % of world pig population but on production front the progress in yield/animal does not match with even world averages. Country has the largest livestock population, with most milk production, 6th in Meat Production and 3rd in egg and fish production, yet has high incidence of malnutrition, food insecurity, and rural poverty. India's food security situation continues to rank as "alarming"(IFPRI, 2011).

Production of Major Livestock Products

The White Revolution in the country simultaneous to Green Revolution has trebled milk production, now exceeding 127 million tons (2010-11). The egg production has increased significantly from 30.44 billion in 2000 to about 65.50 billion in 2010-11 (Table 2). The egg production has just doubled in ten years but our share in world total was only 2.13%. On meat production front, with production of 6.18 million tons meat annually, country ranks 6th in the world contributing about
2.00% to the world total meat production of about 295 million tons (FAO, 2012). India is the 6th largest producer of poultry meat (2.22 million tons) in the world, yet percentage share in world total was only 2.61%. Despite having largest livestock population, the volume of global trade in leather stands at US$ 137.96 billion (2011-12), with India contributing only US $ 4.86 billion (3.52 %) (Council for Leather Exports, India, 2010).

**Economic Contributions**

Value of output from livestock sector on the basis of current prices (2010-11) was Rs. 4, 61,434 crore which is about 28.40% of value of output of Rs.16,23,968 crore from total Agriculture and allied sector. Sector contributes approximately 4% to National GDP and 25% to Agricultural. The Economic contribution of meat was Rs.72, 444.22 crore. The economic contribution of milk (Rs.2, 62,215 crore) is higher than paddy, wheat and sugarcane. It not only provides high quality animal products but also utilizes non-edible agricultural by-products to convert them into quality proteins. It also provides skin as raw material base for the leather sector as well as fat, bones, bristles, blood, wool, fibers, hairs for the cottage industry.

Meat industry in India has great economic potential but received limited attention for its growth and development. Yet the value of output from meat group is Rs. Rs.72, 444.22 crore. It is envisaged to achieve 10% growth rate in meat sector during 12th Five Year Plan period. The earnings through meat and meat products export during 2010-11 were Rs. 9033.53 crore (Table 5).

**Productivity**

In general, productivity of our livestock is very low, in comparison to the world averages. Except buffalo, the average carcass yield from sheep, goat (Table 9) and pigs is lower being 12, 10, 35 kg as against the world average of 16, 12 and 79 kg, respectively (Table 6).

**Slaughter Rate**

As per FAO (2011) estimates, 10.60 million cattle, 10.89 million buffaloes, 24.45 million sheep, 59.66 million goats, and 9.40 million pigs were slaughtered for meat production.

**Availability is Lower than Requirements**

The per capita consumption of meat in the country was about 13.70 g/day i.e. 6.0 kg annually as against the 11 kg recommended. World average is 106.85 g. In USA, it is 337g, the highest followed by 331 g in Spain, 323 g in Australia. In China, it is about 148g/caput/day. Animal protein has its special significance in daily human diet because of its high biological value and being balanced and rich in essential amino acids, B-vitamins and certain essential minerals. The livestock products provide almost one third of protein intake by the people. However, keeping in view the growing population, the animal protein availability has to increase at least three fold (Table 3) for maintaining the nutritional level of growing children and nursing mothers in India. By increasing the production, it will serve as potential remedy for widely prevalent malnutrition in children and pregnant and nursing mothers. The price of meat, chicken, and eggs has also gone very high in the domestic market. This shows that there is significant short supply of these items in the market which is due to spurt in demands owing to increased purchasing power and growing nutrition consciousness in people(Agnihotri,2013).

**Demand Projections by 2020**

Assuming that national economy would continue to grow above 7% GDP (High income growth), Dastagiri (2004) estimated that by 2020 country would require about 227.17 million tons of milk, 47.37 million tons of mutton and goat meat, 1.45 million tons of beef and buffalo meat, 1.23 million tons of chicken and 79.10 billion of eggs. Considering 1993 as base year, during 1993-2020, the demand will grow at the annual compound growth rate of 6.71% for milk, 20.01% for mutton and goat meat, 4.41% beef and buffalo meat, 6.47% for chicken, and 8.48% for eggs (Table-7). The demand for mutton and goat meat will grow much faster among livestock products followed by eggs.
Meeting the growing demand could be possible only through improving the productivity rather than number of livestock. Increasing productivity per unit time rather than their numbers is also important from environmental view point as livestock is considered as one of the important contributors of greenhouse gases (Agnihotri and Rajkumar, 2008).

Meeting the growing demand is a challenge as well as opportunity. It could be possible through improving the productivity rather than number of livestock, improving infrastructure for handling, value addition, processing, and marketing. Increasing productivity per unit time rather than their numbers is important from environmental view point also.

**Supply Chain**

Production and supply of meat for local consumption is one of the most neglected and poorly organized sectors in the country. The local slaughterhouses operate as service abattoirs where butchers slaughter the animals for a fee/wages or get some edible/inedible by-products as a part of daily remuneration.

Meat produced in municipal slaughterhouses following ante and post-mortem inspection and declared fit for human consumption is transported to shops and sold “hot” for local consumption. The consumers prefer fresh carcass meat instead of chilled or frozen due to lack of confidence on cold chain maintained during transit and storage. Though the consumption of meat and meat products is on rise, hygiene, safety and quality aspects have not changed much. Except poultry (20%), less than 1% of meat produced from buffalo, small ruminants, and pigs is under organized sector.

In private sector, there are 37 modern integrated approved abattoirs-cum-meat processing plants where quality de-boned frozen meat is produced for exports adopting OIE guidelines and international quality standards. These plants follow all the sanitary and phytosanitary (SPS) measures required by the International Animal Heath Code of OIE. In addition, 40 meat processing and packaging units that receive dressed carcasses from approved municipal slaughter houses across the states are also licensed under APEDA for exports.

**Exports**

There is a preference for meat exported from India because of certain inherent merits such as its lean character, relative freedom from toxic feed additive residues, OIE ‘negligible risk’ classification for BSE, eradication of RP and CBPP, cheap, and near organic meat production. Currently India has been exporting meat to more than 60 countries. Buffalo meat is exported in frozen bone-less and de-glanded form and is free from FMD virus due to its ageing for minimum of 24 hrs at 2°C to bring down the meat pH below 6.0. Meat is available at very competitive prices. The Indian buffalo and lamb meat has established itself in the markets’ of South-East Asia, Middle-East and African countries.

India is the 6th largest producer of meat. The top five meat producing countries are China (80.75 million tons, 27.37 %), USA (42.17 million tons, 14.29%), Brazil (23.45 million tons, 7.95%), Germany (8.22 million tons, 2.78% and India (6.18 million tons, 2.00%)

The world total buffalo meat production is about 3.32 million tons. Out of that, India contributes about 1.50 million tons. With this much production of buffalo meat, India ranks first, followed by Pakistan (0.68 million tons) and China (0.31 million tons).

Country is 5th largest exporter of bovine (buffalo) meat out of the 9.45 million tons bovine meat traded internationally. The top five bovine meat exporting countries are Brazil (1.96 million tons), Australia (1.28 million tons), USA (0.60 million tons), Ireland (0.53 million tons) and India (0.48 million tons).

Buffalo meat is one of the major commodities, among livestock products, exported from the country. Among the animal products exported from India, meat and meat products account for more than 90% of the total exports volume. Remaining 10% are dairy products and honey (Table 5).
Export Potential

The meat exported from the country is simply chilled, de-boned, packed, and frozen for exports. In real terms there is no value addition as hardly 3% of the total meat produced is processed and converted to various value added ready-to-eat/ready-to-cook products such as cured and canned products, sausages, ham, bacon, burgers, tikka, patties, kebabs, pickle, cutlets etc. The consumption pattern of meat and poultry is quite promising for the processing industry but lacks proper organization and quality assurance. Under MFPO, 1973 there were 330 licensed meat processing units in the country as on 31-12-2010. The meat processed in these units mainly comprised of cured products, sausages and canned products. Several traditional meat products like meat kebab, chicken biryani, tandoori chicken, meat curry, etc. are already quite popular. Nowadays other products like samosa, meat tikka, meat kofta, meat pickle are also in demand. Various region specific meat products like Nihari (Delhi), Goa sausage (Goa), pork pickle (HP), Gustaba and Nate Yakhni (Kashmir), Rapka (Arunachal Pradesh) are gaining wider acceptability. Western meat products like cured ham, bacon, sausages, frankfurters, hotdog, luncheon meat etc. have also registered an increase in demand in big cities. In view of the demand of Indian delicacies across the world, processing and export of processed meat products should be emphasized rather than export of live animals and fresh meat to fetch better profit and generating more employment.

Major meat production centers in the country for exports are- Aurangabad, Nanded, Mumbai and Satara in Maharashtra; Goa; Zaharabad and Medak in Andhra Pradesh; Derabassi in Punjab; Barabanki, Unnao, Aligarh, Meerut, Saharanpur, Noida and Ghaziabad. in UP; Mourigram in West Bengal and Gurgaon in Haryana. State of UP is the largest producer and exporter of buffalo meat.

Malaysia, Philippines, Saudi Arabia, Egypt, Angola, Jordan, UAE, Kuwait are major destinations for buffalo meat. Though there is no religious biasness for buffalo meat, its demand in domestic market is very limited due to limited consumer preference. Therefore, buffalo meat offers great potential for exports especially from male buffalo calves. During 2011, on carcass weight equivalence basis, around 1.15 million tons of buffalo meat was exported.

The EU is expected to import large volume of bovine meat, over the next few years, as abolition of subsidies might lead to rapid decrease in production of livestock products. The Central America and the Caribbean, Russia, Middle East, East Asia and most of the African countries are the bovine meat deficit regions. The major demand for bovine meat is expected to come from these areas and also from the EU. South America, Oceania (Australia and New Zealand) and India are emerging as the major bovine and buffalo meat surplus countries. The continuous drought has been affecting Australia’s herd building during the last few years and BSE issue limits the potential of North America. There are also serious concerns that Brazilian bovine meat supply may not be able to keep pace with sharply increased export projections for bovine meat. This offers a great opportunity for India, to grow its international trade volume in meat.

Rules and Regulations for Export

The abattoirs and meat plants engaged in export production are required to be registered with APEDA, which is compulsory vide DGFT notification No. 12/ (2004-2005) dated 21st December, 2004. The plants are periodically inspected by a committee consisting of officials of various Government departments.

(a) Standards have been laid down for export of meat and meat products under Export (Quality Control and Inspection) Act, 1963. The export of Raw Meat (Chilled and Frozen) shall be allowed subject to the provision specified to the Gazette Notification 1993 on Raw Meat (Chilled and Frozen) under Export (Quality Control and Inspection) Act, 1963. The notification lays down the Standards for abattoirs, meat processing plants and the products. Offal’s too are subject to the same conditions of quality control and inspection.

(b) All consignments of Raw Meat (Chilled and Frozen) and export of canned meat products need to accompany with a Pre-shipment Inspection Certificate for which the Govt. has designated three agencies (i) All State Directorates of Animal Husbandry (ii) Export Inspection Agency (iii)
Directorate of Marketing and Inspection/FSSAI, Government of India, to carry out the inspection in accordance with either the standards prevalent in the exporting country or standards prescribed under the Meat Food Products Order, 1973 under Export (Quality Control and Inspection) Act, 1963 or orders made there under.

(c) Export of meat and meat products will be allowed subject to the exporter furnishing a certificate to the Customs at the time of export that the above items have been obtained/sourced from an abattoir/ meat processing plant registered with APEDA.

Export Policy

Export of Meat and Meat Products from India is, inter-alia, governed by the provisions of following Acts, Rules and Orders (Source: Indian Meat Industry: Red Meat Manual, APEDA) issued by Government of India:

(a) The Export (Quality Control and Inspection) Act, 1963;
(b) The Export of Raw Meat (Chilled/Frozen) (Quality Control and Inspection) Rules, 1992;
(c) Provision contained in Government of India Order issued vide S.O. 203, dated 15-01-1993;
(d) ITC (HS) Classification of Import and Export Items;

Import Regulations under EXIM Policy

The effective animal Quarantine & Certification services are necessary to ensure that international trade in animal products takes place without incurring unacceptable risk to human and animal health. The animal disease situation in the exporting country, in the transit country and importing country must be in compliance with the SPS requirements under WTO, OIE, and International Trade. Therefore, proposals for exports/imports need to be critically examined for permissions.

The import of pig fat, poultry fat, fats of bovines, ovines, caprines etc. is totally ‘prohibited’ whereas meat of bovines, their edible offal’s, tongue, liver is ‘restricted’ for imports including guts, bladders, stomachs of bovine, caprine, ovines. The import is permitted against a license from DGFT after recommendation from Department of Animal Husbandry, Dairying and Fisheries (DADF). The meat & products, edible offal’s of swine, caprine, ovine, poultry, rabbit, liver, tongue etc of swine, caprine, ovine, pig bristles, hair, yak tail comes under the ‘free’ items and import is permitted against a Sanitary Import permit from DADF, Ministry of Agriculture, GOI.

Export Regulations under EXIM Policy

The export of beef of cows, oxen and calf and offal’s (includes heart, liver, tongue, kidneys and other organs) is ‘prohibited’ and not permitted to be exported. Meat of buffalo (both male and female) and offal of buffaloes except gonads and reproductive organs is ‘free’ to export subject to the condition that exporter has to produce a certificate from the designated veterinary authority of the State, from which the meat or offal’s emanate, to the effect that the meat or offal's are from buffaloes not used for breeding and milch purposes. The quality control and inspection under (a) and (b) and (c) stipulated above under Rules & Regulations for Export are required to be fulfilled. The meat and offal’s of Indian sheep and goat is ‘free’ for export subject to fulfilling the conditions (a) and (b) and (c) stipulated above. Tallow, fat and/or oils of any animal origin excluding fish oil are ‘prohibited’ for export.
Meat Food Safety Regulations and Need for Harmonization

The following regulations are in vogue in India which are to be addressed starting from transportation of animals, for setting an abattoir/processing plant, humane slaughter, quality control & hygiene, packaging & labeling, storage, sale and exports:

(i) The Food Safety and Standard Act 2006 (Food Safety and Standards Authority of India-FSSAI)
(ii) Meat Food Product Order, 1973
(iii) Water (Prevention and Control of Pollution) Act, 1974
(iv) Air (Prevention and Control of Pollution) Act, 1981
(v) Prevention of Food Adulteration Act, 1954
(vi) The Prevention of Cruelty to Animal Act, 1960
(vii) Export (Quality Control and Inspection) Act, 1963
(viii) Export (Quality Control and Inspection) Rules, 1964
(ix) Export of Raw Meat (Chilled/Frozen) Rules, 1992
(x) The Food Safety and Standards Authority of India (FSSAI): FSSAI has notified the following regulations which came in force from 5th August, 2011:

(1) Food Safety and Standards (Licensing and Registration of Food Business) Regulation, 2011- Specific Hygiene and Sanitary Practices to be followed by Food Business Operators engaged in manufacture, processing, storing, and selling of Meat and Meat Products are listed in it.

(2) Food Safety and Standards (Packaging and labeling) Regulation, 2011.

(3) Food Safety and Standards (Food product standards and food additives) Regulation, 2011.

(4) Food Safety and Standards (Contaminants, toxins and residues) Regulation, 2011.

(5) Food Safety and Standards (Prohibition and restriction on sale) Regulation, 2011.

(6) Food Safety and Standards (Laboratory and sampling analysis) Regulation, 2011.

The regulations at Sl. No. (5) and (6) above does not specify the meat & meat products. MFPO 1973 regulations cover these items.

(xi) Local bye laws as slaughtering is a state subject and slaughterhouses are controlled by Local Bodies.

(xii) State animal preservation acts, rules and bye laws.

(xiii) BIS standards and specifications

Except BIS specifications, rests are mandatory in nature.

There is need for their harmonization so as to meet Codex standards for exports as well as to have single window system.

The processed meat sector, formerly regulated by the Ministry of Food Processing Industries (MOFPI), is now regulated by the Food Safety and Standards Authority of India (FSSAI) under the aegis of Ministry of Health and Family Welfare through the Food Safety and Standards Rules and Regulation 2011. These regulations were enforced nationwide with effect from August 5, 2011, repealing the Meat and Meat Products Order (MFPO), 1973. It requires registration and licensing of meat processors and other food operators in the meat value chain. It also enforces sanitary maintenance and controls at all stages of meat (including fish and poultry) products production. These standards equally apply to domestic and imported meat and meat products.

FSSAI is mandated with laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food for human consumption and for matters connected therewith or single Act for
Consolidation of Laws relating to PFA 1954, MFPO 1973 and other food laws by moving from multi-level, multi departmental to a single line of command and control.

With the introduction of National Food Safety and Standard Authority under the provision of the Food Safety and Standards Act 2006, production, packaging, transportation, supply, marketing and storage of meat and food products under strict hygienic condition has become essential to safeguard human health.

Livestock Sector Faces Many Challenges

Due to shrinkage in pasture and grazing land, dwindling feed and fodder resources, use of course grains (which are generally used as feed ingredients) for ethanol production (for bio-energy), emerging new diseases, decreasing profitability in farming, indiscriminate use of pesticides/insecticides in various crops and their presence at higher level in various crop residues and agro industrial by-products, biotic and abiotic stress due to various other factors and climatic changes, livestock sector including meat sector faces many challenges to attain the optimum growth rate and increase the livestock products production (Agnihotri, 2011).

Today livestock is alleged as major player responsible for atmosphere and climate change by way of emitting 18% of the greenhouse gases (most of that from enteric fermentation); land degradation & deforestation by overgrazing; increasing problem of freshwater shortage, scarcity, depletion and water pollution; threat to biodiversity and to a certain extent for the shortage of food grains (use of course grains as an ingredient in livestock feed). For sustainable meat production and to get safe and quality products, it is essential to improve the resource use efficiency (water, feed, energy) of livestock production and product processing. This would not only reduce the environmental impacts but also increase the profitability and availability of livestock products to fight the malnutrition and hidden hunger on sustainable basis.

Major Bottlenecks in Meat Sector

(i) Poor productivity of livestock, shortage of quality feed and fodder, wide livestock diseases prevalence and poor access to modern livestock services to counter them.
(ii) Poor hygiene and lack of proper infrastructure for transportation, processing, packaging, and storage.
(iii) Meat Food safety and traceability
(iv) Least priority given by the states due to controversies involved with the subject (meat, poultry slaughter).
(v) Inadequate cold chain and marketing support
(vi) Lack of credit and extension support and quality human resource
(vii) Slow results from livestock development schemes.

Approaches for Sustainable Production

Meat production from buffaloes in India is a by-product of dairy farming by utilizing spent animals after finishing their productive/reproductive life except in species like sheep, goat, pig, and poultry. Meat sector is not very well organized and is generally managed by uneducated people who have no knowledge of animal rights, hygiene and meat food safety aspects. The sector is growing because of market pressure alone. The schemes for the modernization of slaughterhouses and setting up of carcass utilization centre have been going on for about last four decades in one or the other forms but due to poor response from the states to avail the funds available for this activity, not much could be done. The sector involves public sentiments associated with slaughter of meat animals and that is why not much political and administrative will has been applied for the development of proper infrastructure and facilities for slaughter, meat processing, quality control, and by-products utilization to address the animal welfare and environmental concerns.
(i) Salvaging male buffalo calves for meat production and export

Buffalo population trends, over the years, reveal positive growth. Primary importance of buffalo in the country is largely for the purposes of milk and to a small extent for meat and draft purposes. It is estimated that about 8-10 million male buffalo calves are removed annually from buffalo production system due to inadequate feeding by the farmers to save milk and feed leading to parasitism, stunted growth and untimely deaths. Livestock owners do not consider raising males to be remunerative; as a result, the country suffers a loss of about Rs.750 million per annum on this account.

In a study conducted by Central Leather Research Institute (CLRI), Chennai, high incidence of mortality ranging from 42-88% in buffalo calves has been reported (Table 8). These calves could otherwise be salvaged for meat production and recovery of hides, skins, bones etc. thereby improving economic condition of farmers and fetching quality meat for exports and domestic consumption. Raising these male buffalo calves will also generate additional employment in rural areas. In most of the states only culled, old and unproductive animals are permitted to be slaughtered. It requires change in the state’s policy so that such male calves when reared for meat purposes are allowed to be slaughtered.

Thus for enlarging raw material base and improving leather availability and boost buffalo veal export, there is great potential to salvage and rear male buffalo calves especially from breeds like Murrah, Jaffarabadi (Table 11). Considering their economic value, DADF, GOI has launched a scheme to assist farmers, NGOs, professionals, and corporate bodies to rear male buffalo calves for meat production.

(ii) Focus on sheep and goat slaughter and carcass weight

There is large potential for improvement in slaughter and carcass weight in small ruminants through improved feeding and management. Among livestock products, the small ruminants meat demand is growing faster and is projected to be 47.37 million tons (Dastagiri, 2004) by the year 2020. With current production of 0.87 million tons, even with highest small ruminants population and average annual growth rate of more than 3.4%, it is impossible to achieve the projected production unless some stringent measures to check the slaughter of immature animals (25% sheep and goats slaughtered are below 6 months age), improvement in slaughter and carcass weight (through improved feeding and management), adequate health coverage to check very high mortality (range 2.70-77.33%), feed and fodder resource development are taken. The Sirohi, Marwari, Kutchi goat breeds under extensive system could attain an average body weight of 20 kg at 12 months but under intensive system the same breed animals attained body weight of 37, 35, 33 kg, respectively at 9 months of age and 45,41,40 kg at 12 months of age. The percent increase in body weight varied from 27-115% (Agnihotri, and Rajkumar, 2001) (Table 10). The Muzaffarnagari lambs, which could attain about 15 kg body weight at 6 months of age and 25 kg at 12 months, under intensive system, could attain slaughter weight of 31 kg at 6.5 months of age. Therefore, by improving feeding and management alone of indigenous goat and sheep breeds, meat production can be increased many fold. A focused approach with higher investment and developing marketing and processing facilities would provide required boost to the sector.

(iii) Thrust on pork production

Off total pig population in the country, about 15% are graded and exotic variety. There are about 158 pig breeding farms in the country run by the State Governments / Union Territories. Exotic breeds like Large White Yorkshire, Hampshire and Landrace are maintained at these farms.

The demand for pork is higher in North–Eastern states and to certain extent in Punjab. About 80% of the total pork production in the country is consumed by people of NE region. There is mismatch between the consumptions and production centers. While the major demand for pork is in NE region, the production is limited to areas in UP, MP, Jharkhand, and Andhra Pradesh. The high transportation cost adds to finished product cost, making it costlier. This is happening because of lack of feed and proper rearing and processing facilities. The NE region has one of the most prolific pig
breed “Ghungroo” which could give up to 17 piglets in one farrowing. This needs to be exploited by making large investments involving community participation/self help groups.

(iv) Emphasis on backyard poultry production

Although the poultry sector has been completely commercialized, backyard poultry offers scope in the rural areas for providing not only income to weaker section of the society but also for providing much needed animal protein in the form of egg and chicken.

(v) Focus on feed and fodder development

In India, the livestock continues to be raised on crop residues and agricultural by-products. The area under cultivated fodder production is limited only to 4.60% of the total cultivable land. The schemes and programmes relating to feed, fodder and pasture development have recently gained momentum.

Judging by the present requirement and availability of fodder, the deficit in terms of dry matter (DM) for dry fodder, green fodder and concentrates is 39, 36 and 56 percent, respectively which may persist and even aggravate unless adequate measures are undertaken to augment their resources. Unless feed and fodder situation in the country improves, the livestock development efforts may not give the desired results.

(vi) Need for modernization

Legally, slaughter of animals should be done only in licensed slaughterhouses. However, illegal slaughter is common. There are 2336 registered slaughterhouses and almost equal number of unregistered slaughterhouses operating in the country (Basic Animal Husbandry Statistics report 2012, DADF, GOI). Operating authorities are also responsible for giving licenses and therefore, there is laxity in adhering to operating standards.

To regulate the illegal slaughter and improve hygiene and sanitation, modernization of existing slaughterhouses is needed at a larger scale. Environmental and animal right’s issues, through modernization of slaughter, could be addressed more effectively.

To provide safe and wholesome meat to the consumers, in smaller towns, cities and the rural areas and provide pollution free environment, Department of Animal Husbandry, Dairying and Fisheries is implementing Centrally Sponsored scheme on ‘Establishment/modernization of rural slaughterhouses’. The scheme aims to address those areas not specifically and fully covered by existing scheme of Ministry of Food Processing Industries (MFPI). Issue is of Public health importance with larger dimensions and the task is huge. Therefore, a multi-pronged approach is required to supplement the efforts of the States.

(vii) Improvement in Meat food safety, quality control, and hygiene

In the interest of food safety and consumer protection, increasingly stringent hygiene measures are required at national and international trade levels. The key issues in this respect which need to be addressed are Food Traceability, Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) schemes.

Extensive knowledge of hazards, other than that of microorganisms, is indispensable in modern animal products processing. Thus, along with animal products processing hygiene including causes for product spoilage and food borne illnesses, checking the entry of harmful residues in product chain is equally important.

For the purpose of consumer protection and quality control, simple test methods need to be made available that can be carried out at the small enterprise level without sophisticated laboratory set-ups.

To remain competitive in the world market, animal products have to be produced adopting OIE and Codex guidelines and quality standards. While the mandate of the Codex is to develop international standards, guidelines and recommendations on food safety for international trade, OIE (World Organization for Animal Health) has the mandate to develop international standards,
guidelines, and recommendations on animal health including zoonoses.

(viii) Human Resource Development needs

Though the country has achieved the self-sufficiency in food grain production, for the burgeoning human population, equitable distribution and supply of animal products at an affordable price is going to be major challenge. The demand projections for high value livestock product commodities by 2030 indicates that to meet the challenge of providing balanced and nutritious diet to a large population, the animal products viz. meat, milk, fish, eggs etc, the current production has to be increased by about three times. This is going to be a mountainous task, yet possible to achieve through renewed thrust on animal products production.

Shortage of manpower in the animal husbandry sector is a major concern. As per an estimate done by the Planning Commission, against the requirement of 67,000 veterinarians, only 34,500 are available. Similarly, against the requirement of 7,500 veterinary and animal science specialists for teaching and research, only 3,050 are available. Availability of para-vets and other supporting staff is only 52,000 against the requirement of 2,59,000. Leaving aside the other sub-sectors, and assuming that the demand of veterinarians would grow at the same rate as GDP, 2,647 veterinarians would be required by 2019-20 for meat processing sector (Rama Rao et al., 2011). This would not only require the enhanced resource allocation for the development of livestock sector but also on human resource development to take up the challenge of delivering the livestock services at grassroots level (Agnihotri and Rana, 2012).

Some other issues to be addressed

- The climate change and effect of biotic and abiotic stresses on livestock specially buffalo meat production performance needs special attention for the research. In most of the states only culled old and unproductive buffaloes are permitted to be slaughtered. It requires change in the state’s bovine preservation policy so that male buffalo calves when reared for meat purposes are allowed to be slaughtered. There is an urgent need for strengthening of quality testing and monitoring laboratories to carry out the testing and monitoring of animal product samples for pesticide residues, toxic metals, veterinary drug residues and mycotoxins etc. More number of food and feed testing laboratories should be accredited with the laboratories of the developed countries to facilitate the export as well as supply of safe and wholesome products to the domestic consumers. To remain competitive in the world market, information about trade linked animal diseases, veterinary drug residues and other harmful residues in feed and products of animal origin, their maximum permissible limits set by Codex and consequences of their presence beyond permissible limits to human health, trade and industry needs to be disseminated to farmers, feed manufacturers, field veterinarians, animal product processors and the consumers by the States. Processing of meat into value added products, establishment of cold chains, modern storage facilities and packaging of products in durable and attractive packaging materials are the other important areas which will require greater attention by the industry in near future to meet the growing demand of quality ready-to-eat products. Not more than 3% of the total meat produced is processed into value added meat products. Exports mainly comprise of chilled/frozen meats. The industry should seriously think of establishing backward linkages at primary livestock production level with the farmers and processing meat in to value added products to achieve real “pink” revolution. National Meat and Poultry Processing Board may take lead to act as facilitator between meat industry and research organizations, assist in technology transfer and adoption and organize customized training programmes for the meat workers to observe proper sanitation and hygiene while handling meat in slaughterhouses, processing plants and consumer/retail outlets. To minimize the multiplicity of food laws, there is need for harmonization of meat and meat food standards with the CODEX standards.

References

Agnihotri, M.K., and Rajkumar V. (2001). Carcass traits of sheep and goats under various feeding/managerial regime. The proceedings of IV-National Seminar of Indian society for...
sheep and goat production and utilization (ISSGPU) November 9-11, 2001 held at Bharathiar University, Coimbatore, pp.36-45.


**Table 1: Livestock and poultry resources in the country**

<table>
<thead>
<tr>
<th>Species</th>
<th>Popln. in 2003 (millions)</th>
<th>Popln. in 2007 (millions)</th>
<th>Av. annual growth rate (%)</th>
<th>% of world population</th>
<th>World ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>185.20</td>
<td>199.10*</td>
<td>1.83</td>
<td>14</td>
<td>2nd</td>
</tr>
<tr>
<td>Buffalo</td>
<td>97.90</td>
<td>105.30</td>
<td>1.84</td>
<td>57</td>
<td>1st</td>
</tr>
<tr>
<td>Sheep</td>
<td>61.50</td>
<td>71.60</td>
<td>3.87</td>
<td>6</td>
<td>3rd</td>
</tr>
<tr>
<td>Goat</td>
<td>124.4</td>
<td>140.50</td>
<td>3.10</td>
<td>16</td>
<td>2nd</td>
</tr>
<tr>
<td>Pigs</td>
<td>13.50</td>
<td>11.10</td>
<td>(-)4.74</td>
<td>1.50</td>
<td>-</td>
</tr>
<tr>
<td>Total Livestock</td>
<td>485.0</td>
<td>529.70</td>
<td>2.23</td>
<td>11.00</td>
<td>-</td>
</tr>
<tr>
<td>Poultry</td>
<td>489.0</td>
<td>648.0</td>
<td>7.33</td>
<td>-</td>
<td>5th</td>
</tr>
</tbody>
</table>
Table-2: Production* of Major Livestock Products

<table>
<thead>
<tr>
<th>Year</th>
<th>Milk (million tons)</th>
<th>Eggs (billion Nos.)</th>
<th>Meat (million tons)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999-2000</td>
<td>78.30</td>
<td>30.44</td>
<td>3.99</td>
</tr>
<tr>
<td>2010-2011</td>
<td>127.30</td>
<td>65.5</td>
<td>6.18</td>
</tr>
<tr>
<td>Rank in World</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Growth rate in 2009-10 over 1999-2000</td>
<td>63.0</td>
<td>115.0</td>
<td>28.0</td>
</tr>
</tbody>
</table>

Extracted from Basic Animal Husbandry Statistics Report (2012), DADF. *Anticipated ** plus poultry

Table-3: Demand projections for high value livestock product commodities by 2030 (in million tons)

<table>
<thead>
<tr>
<th>Commodities</th>
<th>Year</th>
<th>Expected increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000</td>
<td>2030</td>
</tr>
<tr>
<td>Meat</td>
<td>4.5</td>
<td>15</td>
</tr>
<tr>
<td>Fish</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Eggs</td>
<td>17</td>
<td>57</td>
</tr>
<tr>
<td>Milk</td>
<td>76</td>
<td>182</td>
</tr>
</tbody>
</table>

Source: Vision 2030, ICAR (2011)

Table-4 : Growth rate in meat sector during XI plan

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Meat</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo meat</td>
</tr>
<tr>
<td>3</td>
<td>Milk</td>
</tr>
<tr>
<td>4</td>
<td>Eggs</td>
</tr>
</tbody>
</table>

Table-5: Export of animal products from India (Quantity in M.Ts, Value in Rs. Crore )

<table>
<thead>
<tr>
<th>Products</th>
<th>Qty.</th>
<th>Value</th>
<th>Qty.</th>
<th>Value</th>
<th>Qty.</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008-09</td>
<td>2009-10</td>
<td>2010-11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buffalo meat</td>
<td>462749</td>
<td>4839.70</td>
<td>495019</td>
<td>5480.60</td>
<td>709437</td>
<td>8412.68</td>
</tr>
<tr>
<td>Sheep and goat meat</td>
<td>37790</td>
<td>493.37</td>
<td>52868</td>
<td>747.20</td>
<td>11908</td>
<td>253.18</td>
</tr>
<tr>
<td>Poultry products</td>
<td>1057016</td>
<td>422.05</td>
<td>1016783</td>
<td>372.11</td>
<td>619150</td>
<td>301.32</td>
</tr>
<tr>
<td>Animal casings</td>
<td>1823</td>
<td>8.84</td>
<td>2020</td>
<td>31.52</td>
<td>1809</td>
<td>35.14</td>
</tr>
<tr>
<td>Processed meat</td>
<td>857</td>
<td>10.14</td>
<td>716</td>
<td>9.58</td>
<td>1366</td>
<td>21.05</td>
</tr>
<tr>
<td>Swine meat</td>
<td>817</td>
<td>9.17</td>
<td>1117</td>
<td>10.34</td>
<td>1115</td>
<td>10.51</td>
</tr>
<tr>
<td>Dairy Products</td>
<td>70146</td>
<td>980.96</td>
<td>34380</td>
<td>402.68</td>
<td>36867</td>
<td>533.89</td>
</tr>
<tr>
<td>Natural Honey</td>
<td>15587</td>
<td>148.96</td>
<td>13311</td>
<td>146.65</td>
<td>31675</td>
<td>249.58</td>
</tr>
<tr>
<td>Total</td>
<td>1646790</td>
<td>6913</td>
<td>1616216</td>
<td>7201</td>
<td>1413330</td>
<td>9817</td>
</tr>
</tbody>
</table>

Source: APEDA 2012
Table 6: Meat yield from various livestock in India

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species</th>
<th>Average carcass weight/ per animal (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>India</td>
</tr>
<tr>
<td>1</td>
<td>Cattle</td>
<td>103</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo</td>
<td>138</td>
</tr>
<tr>
<td>3</td>
<td>Sheep</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Goat</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Pig</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 7: Demand projections for livestock products (in million tons) in India by 2020

<table>
<thead>
<tr>
<th>Livestock Product</th>
<th>Year</th>
<th>Growth rates (%) from 1993 to 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1993</td>
<td>2020</td>
</tr>
<tr>
<td></td>
<td>45.02</td>
<td>277.17</td>
</tr>
<tr>
<td></td>
<td>6.71</td>
<td></td>
</tr>
<tr>
<td>Mutton &amp; Goat meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.78</td>
<td>47.37</td>
</tr>
<tr>
<td></td>
<td>20.01</td>
<td></td>
</tr>
<tr>
<td>Beef &amp; Buffalo meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.49</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>4.41</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>6.47</td>
<td></td>
</tr>
<tr>
<td>Egg *</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.30</td>
<td>79.10</td>
</tr>
<tr>
<td></td>
<td>8.48</td>
<td></td>
</tr>
</tbody>
</table>

* Billion numbers in case of egg. Growth rate of total is weighted average growth rate. 1993 is considered as base year.

Table 8: Percentage of young stock to total deaths in bovines

<table>
<thead>
<tr>
<th>S. No.</th>
<th>State</th>
<th>Cattle</th>
<th>Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total death (Nos.)</td>
<td>Calf mortality (Nos.)</td>
<td>% of calf death to total deaths</td>
</tr>
<tr>
<td>1</td>
<td>Andhra Pradesh</td>
<td>146</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>Bihar</td>
<td>251</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>Chhattisgarh</td>
<td>254</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>Jharkhand</td>
<td>128</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>Kerala</td>
<td>191</td>
<td>89</td>
</tr>
<tr>
<td>6</td>
<td>Madhya Pradesh</td>
<td>197</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Maharashtra</td>
<td>304</td>
<td>84</td>
</tr>
<tr>
<td>8</td>
<td>Punjab</td>
<td>195</td>
<td>146</td>
</tr>
<tr>
<td>9</td>
<td>Rajasthan</td>
<td>747</td>
<td>234</td>
</tr>
<tr>
<td>10</td>
<td>Tamil Nadu</td>
<td>153</td>
<td>61</td>
</tr>
<tr>
<td>11</td>
<td>Uttar Pradesh</td>
<td>276</td>
<td>95</td>
</tr>
<tr>
<td>12</td>
<td>Uttarakhand</td>
<td>281</td>
<td>77</td>
</tr>
<tr>
<td>13</td>
<td>West Bengal</td>
<td>410</td>
<td>179</td>
</tr>
<tr>
<td>Total</td>
<td>3533</td>
<td>1328</td>
<td>38</td>
</tr>
</tbody>
</table>


Note: Field study was conducted adopting standard statistical analysis methods. The mortality reported is for young stock, not sex-wise. The above annexure provides state wise data on percent of calf deaths to the total deaths in a sample population selected for the study.
Table 9: Body weight, carcass weight and dressing percentage of goats

<table>
<thead>
<tr>
<th>Breed</th>
<th>Body wt., kg</th>
<th>Carcass wt. kg</th>
<th>Dressing %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirohi</td>
<td>21.00</td>
<td>9.24</td>
<td>44.00</td>
</tr>
<tr>
<td>Marwari</td>
<td>19.29</td>
<td>8.68</td>
<td>44.46</td>
</tr>
<tr>
<td>Kutchi</td>
<td>22.83</td>
<td>11.41</td>
<td>49.98</td>
</tr>
<tr>
<td>White Bengal</td>
<td>13.35</td>
<td>5.71</td>
<td>42.80</td>
</tr>
<tr>
<td>Brown Bengal</td>
<td>14.49</td>
<td>6.01</td>
<td>41.54</td>
</tr>
<tr>
<td>Black Bengal</td>
<td>13.14</td>
<td>6.29</td>
<td>47.90</td>
</tr>
<tr>
<td>Osmanabadi</td>
<td>18.80</td>
<td>9.20</td>
<td>49.10</td>
</tr>
<tr>
<td>Jakharna</td>
<td>20.90</td>
<td>9.40</td>
<td>44.50</td>
</tr>
<tr>
<td>Chegu</td>
<td>16.64</td>
<td>6.55</td>
<td>39.36</td>
</tr>
<tr>
<td>Angora Local</td>
<td>16.50</td>
<td>7.73</td>
<td>46.90</td>
</tr>
<tr>
<td>Gaddi*</td>
<td>17.71</td>
<td>6.78</td>
<td>38.80</td>
</tr>
<tr>
<td>Beetal</td>
<td>20.30</td>
<td>4.30</td>
<td>46.20</td>
</tr>
<tr>
<td>Barbari</td>
<td>16.66</td>
<td>6.96</td>
<td>41.80</td>
</tr>
<tr>
<td>Jamunapari</td>
<td>22.50</td>
<td>10.4</td>
<td>45.50</td>
</tr>
<tr>
<td>Sangamaneri</td>
<td>12.60</td>
<td>5.80</td>
<td>46.14</td>
</tr>
</tbody>
</table>

* At 17 months of age. At 12 months of age for other breeds

Table 10: Scope for improvement in performance of goats.

<table>
<thead>
<tr>
<th>Breed</th>
<th>System</th>
<th>Slaughter wt., kg*</th>
<th>Improv., %</th>
<th>Carcass wt., kg</th>
<th>Improvement, %</th>
<th>DP %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirohi</td>
<td>I</td>
<td>45.15</td>
<td>115</td>
<td>20.08</td>
<td>117.31</td>
<td>48.91</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>34.6</td>
<td>64.76</td>
<td>16.73</td>
<td>81.06</td>
<td>48.47</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>29.7</td>
<td>41.42</td>
<td>14.00</td>
<td>43.98</td>
<td>42.21</td>
</tr>
<tr>
<td>Barbari</td>
<td>I</td>
<td>21.14</td>
<td>26.89</td>
<td>11.07</td>
<td>59.05</td>
<td>52.23</td>
</tr>
<tr>
<td></td>
<td>SI</td>
<td>19.97</td>
<td>19.86</td>
<td>9.15</td>
<td>31.46</td>
<td>46.43</td>
</tr>
<tr>
<td>Marwari</td>
<td>I</td>
<td>41.25</td>
<td>114.28</td>
<td>19.99</td>
<td>135.29</td>
<td>48.64</td>
</tr>
<tr>
<td>Kutchi</td>
<td>I</td>
<td>40.38</td>
<td>76.87</td>
<td>21.02</td>
<td>84.22</td>
<td>49.98</td>
</tr>
</tbody>
</table>

I = Intensive; SI = Semi-intensive. * At 12 months age

Table 11: Adult body weight of male buffaloes and potential for the improvement

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Breed</th>
<th>Average body weight(kg)</th>
<th>Highest body weight recorded(Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bhadawari</td>
<td>475</td>
<td>540</td>
</tr>
<tr>
<td>2</td>
<td>Jaffarabadi</td>
<td>1,000</td>
<td>2,000</td>
</tr>
<tr>
<td>3</td>
<td>Marathawada</td>
<td>370</td>
<td>400</td>
</tr>
<tr>
<td>4</td>
<td>Mehsana</td>
<td>565</td>
<td>602</td>
</tr>
<tr>
<td>5</td>
<td>Murrah</td>
<td>567</td>
<td>800</td>
</tr>
<tr>
<td>6</td>
<td>Nagpuri</td>
<td>400</td>
<td>522</td>
</tr>
<tr>
<td>7</td>
<td>Nili-Ravi</td>
<td>567</td>
<td>700</td>
</tr>
<tr>
<td>8</td>
<td>Pandharupu</td>
<td>416</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Surti</td>
<td>500</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Toda</td>
<td>380</td>
<td>-</td>
</tr>
</tbody>
</table>
Manipulating the Postpartum Physiological and Metabolic Adaptation to Augment Milk Production in Bovines

Mahendra Singh
Principal Scientist (Animal Physiology), National Dairy Research Institute, Karnal-132001 HR

The production performance of dairy animals, besides proper nutrition largely depends on their appetite, digestion and metabolization of feed. The cow starts producing milk after calving which gradually increases and reaches the peak level in 40-50 days. Thereafter, milk production declines slowly and is completely stopped in around 300 days. Beside dairy cows undergo tremendous changes during the transition from late gestation to early lactation. The importance of this period in determining health, productivity, and profitability has been underscored by the intense interest in nutrition and management of exotic dairy cows during the transition period over the last several years. After parturition, nutrient demand of the mammary gland is not able to be met through feed intake alone because the rate of increase in dry matter intake (DMI) is slower than the rate of milk energy output. During early lactation, milk production increases more rapidly than DMI, and dietary intake lags behind the increase of nutrient needs (Bell, 1995). To meet the enhanced nutritional demands of milk synthesis, dairy cows mobilize body reserves causing negative energy balance (NEB) until nutrient intake covers the demands (Nebel and McGilliard, 1993; Hattan et al., 2001). Circulating ketones [β-hydroxybutyrate BHB]) increase during periparturient lipolysis and may result in metabolic disturbances (Herdt, 2000; Walsh et al., 2007). The nutrient and energy deficits after parturition are met by not only by mobilization of body reserves but by decreasing nonessential use of glucose in non-mammary tissues. Such metabolic changes also referred as metabolic adaptations are of more severe nature as compared to the indigenous cows which are low and medium producers. However with the use of modern feeding and management interventions the progressive farmers in particular and dairy farmers in general are taking the dairy profession as a business entrepreneur and rearing elite cows producing milk yield of 15-20 kg is a common phenomenon. We are working on the metabolic adaptation in cows and buffaloes to find out the extent of lipolysis and consequently adopted measures to augment the milk production during different climatic conditions.

Body Condition

The body weight of the cows gives a picture of the management practice followed by the dairy herd or a farmer. Dairy cows undergo a normal cycle of body energy storage and mobilization, with increased body fat storage during mid-gestation and increased body fat mobilization during early lactation. Over the long term period, dairy cows appear to “strive” to maintain some normal degree of body fatness (Friggins, 2002). The lactating cows eventually converge to a similar body condition score by 4 to 8 wk after calving (Garnsworthy and Topps, 1982) when feed intake is optimum.

Role of adipose tissue in metabolic adjustment

A reduction in the ruminal production of propionic acid, the main precursor of glucose in ruminants, results in hypoglycaemia. This leads to a mobilization of free fatty acids and glycerol from the fat stores (Holtenius and Holtenius, 1996; Andrews, 1998). The level of increased serum concentrations of NEFA appears to be causally linked to these problems, and feeding strategies to decrease or avoid this dramatic increase are desirable for optimal health and performance in dairy cows (Gerloff, 2000). In case of excess NEFA associated with an elevated formation of acetyl-coenzyme A, fatty acids are used for ketogenesis. Extreme lipid mobilization from adipose tissue exceeds the metabolizing capacity of the liver, leading to an increased accumulation of triglycerides (Herdt, 1988). The affected cows display fat infiltration and degeneration of the liver (Moore and Ishler, 1997). The adipose tissue thus has a critical role in the development of fatty liver and ketosis. These changes in liver composition and metabolism arise both from excessive lipolysis in adipose
tissue and altered secretion of adipose tissue-derived hormones, which modulate hepatic metabolism (Vernon, 2005). The postpartum mobilization of fat and loss of body weight was associated with increasing milk fat and decreasing milk protein contents as a consequence of the energy deficit. The fat: protein ratio allows for an identification of the cows with elevated ketone body concentrations. Cows with fat: protein ratios >1.3 in the first week of lactation are at risk for ketosis or are already affected with it (Heuer et al., 1999).

**Use of hormones in metabolic adjustments**

To augment milk production, lactating animals have been supplemented with hormonal preparations like bovine somatotropin (Ludri et al., 1989; Bachman et al.,1992) but it has limitations due to residual effect in milk causing an increased risk of breast and prostate cancer (Prosser, 1989, Ludri and Singh, 1989). In addition, use of bST hormone adversely influences estrous activity and lengthens the calving interval, affecting reproductive efficiency of dairy animals. The other risks associated with the use of bST in dairy animals are changes milk protein composition which might favor allergic reactions (Daughday and Barbano, 1990; Prosser,1988) besides increased incidence of mastitis (Judega,1997). Though bST or GH is related to partitioning of nutrients and injections of BST enhances milk yield in cows and buffaloes (Jyotsna and Singh, 2010). EU Scientific committee on Animal Health and Animal Welfare has stated the use of bST substantially increases foot problems, mastitis, injection site reactions and reproductive disorders in dairy animals, therefore from the point of view animal health and welfare bST should be not be used (Nott, 1993). However experiment carried not on Murrah buffaloes and cows in field conditions at NDRI, Karnal revealed no adverse effect on health, conception role, pregnancy and health of new born cows. In addition to this, use of other hormones and iodinated casein to augment lactation has not been successful due to adverse effect on animal health. During the last one decade the non hormonal compounds and the herbal feed additives have been tried from time to time to enhance milk production of dairy animals (Singh and Dang, 2004).

**Role of Supplemental Fat**

Supplemental fat is often used to increase the energy density of lactating cow rations. However, added fat in transition cows has resulted in inconsistent and often contradicting results due to the level of fat feeding relative to body condition. The variable results on account of fat feeding are also due to propensity for adipose tissue mobilization, palatability of the fat source, form of fat including calcium salts, timing of fat introduction relative to calving and fatty acids profile. The addition of fat in rations could be practiced until 30 days postpartum and the dietary fat should not exceed 5-6% of the ration. Recently the profile of fatty acids has emerged as a potential factor in regulating the metabolic capacity of liver. Cows that are overfed to induce fatty liver at calving had twice the values of plasma NEFA and liver triglycerides than control values (Rukkwamsuk et al., 2000).

Thus the high energy demand during the postpartum period could be met by supplementation of additional energy in the diet which could minimize the mobilization and helps the cows to adapt in early stages. The feeding of prilled fat (@75g/d) in lactating cows and buffaloes showed that the additional energy supplementation to an extent prevent decline in body weight and improved milk yield by about 1kg/day. The milk yield of cows and buffaloes has positive association with the body weight; therefore fed cows with prilled fat have lesser declines in body weight and more milk yield (figure). However long term studies needs to be carried out to find and out the effect in successive lactations.

**Advantages of prilled fat supplementation**

- No adverse effect on DMI in cows and buffaloes
- Increase in milk yield
- Improved conception rate
Marginal increase in plasma HDL

Improved body weight

Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones. Supplementation of prilled fat increases DMI concomitant to rise in thyroid and Ghrelin and plasma glucose level (Rajesh et al., 2013). Growth hormone (GH) is increased around parturition and in early lactation (Grum et al., 1996) which increases responsiveness of adipose tissue to lipolytic signals such as nor epinephrine. The resulting increase of nonesterified fatty acids (NEFA) from adipose tissue are used as an alternate fuel for much of the rest of the body, and are also converted by the liver to ketone bodies. The ketones serve as alternate water-soluble fuels that can replace glucose in many tissues, thus conserving glucose for milk synthesis. The total intake of energy by cows after calving usually is less than energy requirements, even in healthy cows (Bell, 1995). The high ratio of growth hormone to insulin in blood of cows allows mobilization of long-chain fatty acids from adipose tissue (body fat) to attempt to make up the deficit between intake and requirements (i.e., negative energy balance). Fatty acids released from adipose tissue circulate as NEFA, which are a major source of energy to the cow during this period. However supplementation of bypass fat/prilled fat restricts mobilization (marker NEFA) and improves the health status of the lactating animals. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization (Pullen et al., 1989) which decreases following prill fat feeding. Therefore, as negative energy balance increases, more NEFA are released from body fat and the concentration of NEFA in blood increases. The mobilization of body fat is more in high producing cows than the low producers. Once taken up by the liver, NEFA can be i) completely oxidized to carbon dioxide to provide energy for the liver, ii) partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues, or iii) reconverted to storage fat (triglycerides). If the metabolic adaptation around parturition is impaired by environmental influences or based on the animal genetics, cows develop periparturient illnesses (Drackley et al., 2005). However, there is considerable individual variation of the adaptive ability of cows during early lactation based on a variety of metabolic and endocrine variables. Supplemental fats are frequently added to the diet of lactating dairy cows to improve energy intake, especially during the summer when heat stress depresses intake. Previous research has demonstrated that calcium salts of long chain fatty acids can lower DM intake in some cases, but milk yield is sustained. Fats have a lower heat increment and theoretically should reduce the total amount of heat generated by the cows which would reduce heat stress, but this has not been documented and needs to be investigated (Mohanta et al., 2010; Harvatine and Allen, 2005).

Use of mist and fan to enhance milk productivity

Traurion cows require more comfort to channellize the nutrients towards mammary gland for lactogenesis. Summer climates cause the stress in post partum dairy cows resulting in milk production depression. Heat stress occurs when the ambient temperature is higher than that of the animal’s thermal neutral zone. The heat load is greater than their ability to lose heat (Soch et al., 1997; Dolejs et al., 2000). The potential for heat stress exists when the air temperature rises above the comfort zone, particularly if humidity is also high. Therefore, THI is commonly used to indicate the degree of stress in dairy cattle. THI values suggest that within the normal range up to 70, cattle show optimal performance. In the warming range of THI values 70–72, dairy cow performance is inhibited and the cooling of animals becomes desirable. Critical THI values are 72–78, when milk production is seriously affected. The dangerous category is at the THI values 78–82 (Du Prezz et al., 1990). The provision of mist-and fan helps to alleviate the heat stress in cows and buffalos. In later case cooling by wallowing was more efficient in reducing heat stress and restores the physiological responses to normal values without affecting quality of milk and health of animals (Aggarwal and Singh, 2010). This is due to the fact that heat stress increases maintenance energy requirements and lowers dry matter intake, making it difficult to meet energy needs. Therefore, feeding management and composition of feed become more important. Feed rations should be changed gradually, and sufficient space for feeding should be provided to the animals. Appropriate housing facilities and equipment to
protect dairy cows from climatic extremes are of significance for production maintenance. The cooling of cows and buffalos by misting combined with air movement should be used to alleviate the heat stress. Evaporative cooling is the best for protection against high temperature stress.

**Nutritional Management during the Transition period**

In view of the dynamic nature of physiological changes during the transition period the following points can help the cows and buffaloes to adapt rapidly in postpartum period:

- Maximize the appetite of the cow/buffaloes at and after calving.
- Provide a palatable, well-balanced, and highly digestible diet to allow the cow to attempt to meet her nutritional requirements.
- Maintain (or enhance) immune function (vitamin E supplementation @500-1000IU).
- Minimize the extent of body fat mobilization around calving to that which is “normal” for the cow and buffaloes.
- Provide adequate metabolizable protein to meet amino acid requirements for maintenance, fetal or milk requirements, and immune function.
- Maintain blood level of calcium and magnesium at and after calving.
- Increased nutrient density allows maintenance of the same intake amounts before 7 days of parturition.

**Postpartum Feed Efficiency**

Feed efficiency (FE; sometimes called dairy efficiency or dry matter intake efficiency) is a simple measure to determine the relative ability of cows to turn feed nutrients into milk or milk components. In the simplest terms it is the kilogram of milk produced per kilogram of dry matter consumed. This measure should always be a consideration of dairy diets and becomes increasingly important during times of decreased profit margins (high input and low returns). An added benefit to increasing cows’ feed efficiency is that fewer nutrients will be excreted in manure, so feed efficiency affects both economic and environmental efficiency. It has been found that summer season adversely affect feed efficiency of lactating cows (Singh et al., 2013). There are two ways to improve feed efficiency. One is to increase milk yield with the same dry matter intake, and the other is to decrease dry matter intake and maintain the same milk yield. Many diet modifications that increases milk yield will also increase feed efficiency. In general, as the cow produces more milk the proportion of nutrients used for maintenance becomes smaller. In other words the fixed cost of the animal are spread out over more kilogram of milk, making the animal energy efficient. It is important to optimize rather than maximize dry matter intake in the freshly calved dairy cows maintained in adverse climatic conditions. Use of mist and fan during the harsh weather condition in summer increases dry matter intake of cows and buffaloes by about 10-20% resulting in more milk yields (Aggarwal and Singh, 2009; 2010). The physiological responses are also restored due to effective cooling of the skin which also leads to decline in stress hormone like prolactin and cortisol.

**Use of Shatavari to Augment Productivity**

Indian herbs play significant role in improving milk production, reproduction and growth in cattle and buffaloes. Preliminary research work has been carried out during the last 5 years on shatavari effect in dairy animals. Postpartum *Asparagus racemosus* root powder supplementation in KF cows (@200mg/kg live body weight) increased daily milk yield significantly at during winter season, however with this dose level the effect of *Asparagus racemosus* root powder on reproduction and milk composition (fat, SNF and total solids) was not found significant. It has also been found that *Asparagus racemosus* root powder supplement has beneficial effect on udder health of supplemented cows and helps in metabolic adjustments by improving the feed intake, digestibility and rumen fermentation pattern. Postpartum supplementation of *Asparagus racemosus* root powder reduced/litre cost of milk production and increased net return/day/animal. In addition its supplementation during different stages of lactation improved the reproductive performance and nutritional status of dairy
cows. Mean plasma triglycerides, LDL cholesterol, HDL cholesterol, glucose, and nonesterified fatty acid (NEFA) remain unaffected by the treatment. Plasma prolactin and cortisol concentrations significantly (P<0.01) increase due to Shatavari feeding and enhance milk yield. On day of parturition, plasma prolactin, cortisol, LDL, and plasma total cholesterol were also higher (P<0.01) in treatment group buffaloes in comparison to control group. A. racemosus feeding significantly (P<0.01) increased plasma prolactin, cortisol (P<0.01) and milk fat cholesterol (P<0.05) without affecting total cholesterol, HDL, LDL, glucose, and NEFA concentrations. The buffaloes fed with Shatavari also produce more milk (@0.526 kg/animal/day) suggesting thereby that A. racemosus is galactopoietic.

Based on many trials conducted in cows and buffaloes, it can safely be concluded that its use is beneficial and could serve as potential management tool to improve reproductive performance in crossbred dairy cows and buffaloes however, its potential use to ameliorate the temporary cold heat stress and heat stress seems to be further studied. The beneficial effect of shatavari has also been studied in transient cows. ARS (shatavari) supplanted group cows (@100 mg/kg live body weight once in the morning) from day -60 till parturition, produced significantly more (P<0.01) milk over the control group. Such cows colostrum have more protein, total solids, SNF (P<0.05) and total immunoglobulin level which can be used for the pharmaceutical purpose (Kumar et al., 2013). The reproduction of cows is also improved by ARS feeding as cows took less time to expel placental membranes (P<0.05), had less service period and service/conception (P<0.05). Thus prepartum supplementation of Shatavari significantly increased milk yield, colostrums total immunoglobulin and reduced total milk cholesterol, service period and service/conception in ensuing lactation.

Effect of milkofeed to augment milk production

The efficacy of herbal feed supplement ‘MilkoFeed’ for augmenting milk production in dairy cows having average milk yield of 16 kg/day around 66-67 days post partum in summer season have been reported (Singh and Dang, 2006). Supplementation of Milkofeed in cows@ 10 gm per day for period of 10 weeks increased milk yield over the un-supplemented control group cows with an average of 7.3 % over the control (1.12 kg/animal/day). EEC feeding did not influence udder health inspite of increased milk production. The increased milk yield was found to be due to rise in plasma glucose levels.

Effect of encapsulated choline chloride on milk production

Feedstuffs for dairy cattle contain free choline and phosphatidyl choline or relatively small quantities while ruminal degradation of choline and phosphatidyl choline is extensive (Sharma and Erdman,1988). The choline requirement of dairy cows is still unknown (NRC, 200), but milk fat and yield increase with protected choline supplementation either in feed (Atkins et al.,1988; Pinotti et al. 2003 ; Overton and Waldron, 2004) or by infusion into abomasums (Erdman and Sharma, 1991). Due to this season the effect of choline chloride feeding on milk production and composition in crossbred cows have been reported. The use of ECC revealed that it could be used to augment the milk production and was cost economic also. Lactating cows fed ECC (@120 g/animal/ day) for a period of 8 weeks produced significantly more milk over the control by 7.3%. Milk fat and lactose were more in
experimental (P<0.01) than the control group cows, while NEFA and protein remained unaffected by ECC feeding. The increase in milk yield (P<0.01) was due to increased feed intake (P<0.01) which led to rise in plasma glucose level. Hence milk production and composition of crossbred cows can be augmented by feeding of encapsulated choline chloride without any effect on udder health.

**Role of vitamin E in Metabolic Adjustment**

Preliminary research studies have indicated that feeding of vitamin E in concentrate mixture @1mg/d/ animal improves the udder health and immunity. Such animals produce more milk in the ensuing lactation due to improved immunity of the udder. The feeding of Vitamin E have beneficial effect on milk yield also which have been found to increase ranging between 8-15 % in many studies. However its application in routine has not been advocated. The vitamin E use also has been tried in curing the mastitis incidence in dairy cows (Manju and Singh, 2009) in conjunction with frequent milking of the udder of cows and buffaloes. Certainly Vit. E supplementation in routine in the minimal dose could help to bear the metallic stress of udder in the freshly calved cows. More investigation in this area needs to be carried out.

**Effect of BT cotton feeding on milk production**

India is one of the leading cotton producers, introduced the Bt cotton cultivation in the year 2002. Insertion of Bacillus thuringiensis into the DNA of local cotton varieties gives them resistance towards lepidopteran insect pests. Cottonseed is a traditional protein and energy supplement in the ration of lactating cows and buffaloes. Reports about the absence of transgenic proteins in milk and blood in ruminants (Phipps et al., 2003, Faust et al., 2007, Huls et al., 2008) and in tissues of pigs and poultry (Jennings et al., 2003, Elangovan et al., 2006) following the feeding of GM cottonseed based rations suggested that these are safe for animals and the consumers of products from such animals. Incorporation of transgenic crushed whole delinted cottonseed @ 10.7% of total DMI in the lactating dairy cows had shown similar DMI, BW gain, nutrient digestibility, milk yield, milk composition and blood biochemical parameters to non-transgenic cottonseed based rations under similar feeding conditions for 4 weeks. Bollgard II cottonseed contains the Cry1Ac and Cry2Ab insecticidal proteins that protect cotton plants from feeding damage caused by certain lepidoteran insects. Thus, cottonseed expressing Cry1Ac protein is as safe and nutritious as conventional cottonseed when fed to lactating dairy cows (Mohanta et al., 2010). Lactating multiparous cows were fed diets containing Bollgard II® cottonseed (BGII) or a control non-genetically modified isogenic cottonseed (CON) and its effect on milk yield and milk and blood plasma were investigated for Cry1Ac and Cry2Ab proteins. Feeding of BT cotton did not alter DMI, BW and milk components. The 4% fat corrected milk (FCM) production and FCM/Kg DMI for cows in the BGII treatment (14.0 Kg/cow per day, 1.12 Kg/Kg) were significantly improved compared with cows of control treatment (12.1 Kg/cow per day, 0.97 kg/kg). Gossypol contents in BGII cottonseed and conventional cottonseed were similar. Cry1Ac and Cry2Ab2 proteins in Bollgard II cottonseed were 5.53 and 150.8 µg/g, respectively and were not detected in the milk or plasma samples. Thus Bollgard II cottonseed can replace conventional cottonseed in dairy cattle diet with no adverse effects on performance and milk composition.
Dairy cows undergo a tremendous set of metabolic adaptations as they go from late pregnancy to early lactation. These changes normally are exquisitely coordinated by hormonal changes to support the new physiological state of lactation, the concept known as homeorhesis. However, these adaptive processes fail or are overcome by environmental influences in too many cows, resulting in periparturient illness. Though feeding of the herbal feed supplements help to sustain and augment milk production and reproductive performance in dairy animals, but at the same time research needs to continue to increase our understanding of the adaptive processes and how they are affected by precalving nutrition and environmental influences. This will undoubtedly improve our management capabilities for dairy animals during the transition period.

**Role of Hormones in Metabolic and milk secretion**

Using the alkaloid drugs like bromocryptine, the role of prolactin in initiation of lactation and milk secretion is well documented in cows buffaloes and goats (Prasad and Singh, 2010, Prasad and Singh 2010; Singh and Ludri, 200). Similarly, role of GH in maintenance of milk production have been clearly established in bovines (Ludri and Singh, 1989, Ludri et al, 1989). Abolition of prolactin peak around parturition stimulates lactogenesis in cows and buffaloes (Figure) but during early lactation the treatment have no effect on milk production and composition of buffaloes (Saha and Singh, 1998). The herbal feed like Shatavari also increase milk production by stimulating the release of GH and prolactin in periparturient cows. It also causes release of less cotisol due to its calmness effect. Thus hormonal manipulation could be achieved though feeding of herbal supplements rather than injecting hormone as such.

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Stress and Lactation: An Overview

Sarvejot Yadav and Mukul Anand

Department of Physiology, College of Veterinary Science & A.H., DUVASU, Mathura

Introduction

The livestock sub sector has emerged as one of the key components of agricultural growth in developing countries in recent years. The Indian livestock system is the endeavor of small holders and it is a centuries of old tradition. As a result of gradual transition from subsistence to market system, the economic dimensions of livestock keeping have assumed increasing significance in household behavior. Over 70% of the rural households in India depend on livestock farming for supplementary income. The sector is highly gender sensitive and over 90 per cent of activities related to care and management of livestock are carried out by family’s women folk.

India has the largest population of livestock in the world and stands first in the milk production. The emergence of white revolution has dramatically improved the milk production status of India. India is now dreaming for second white revolution to cater its need for milk production. The major concern about the second white revolution is global climate change and an impending thermal stress. Stress has been emerged as the most powerful constraint in milk production as well as wellbeing of the female health. Stress has not only altered the hormonal milieu of the blood but also questioned the overall lactation of the animals. As a result of this, both milk production as well as the quality of milk has been questioned. Side by side to this, another significant problem is the emergence of pathogens of newer serotypes and pathogenic ability which are detrimental to animal health.

Environmental physiologists have evidence that temperate environments are optimal for production if management and feed practices are appropriate. We know that tropical environments, whether they are hot-humid or hot-dry, limit milk production to a great extent of the year. The effects of internal parasites, ticks, flies, feed quality and quantity, water, housing, management during periparturient periods and many other environmental factors may limit milk production.

With development and advances in modern physiology and biometeorology, we are gradually gaining a better understanding of factors such as temperature, humidity, air velocity, radiation, parasites, crowding, transport, management can influence the behavior of other animals including man, and may affect a dairy animal's physiological functions. In most areas, the external environment of a cow is changing continuously, and the cow's internal environment is adjusting and compensating to these external changes simultaneously as processes of body maintenance, lactation, reproduction, growth. When temperate-evolved domestic animals are subjected to climates ranging from the arctic to the tropics, external stressors vary due to range of temperature, humidity, radiation, wind, light, or other meteorological factors directly affecting thermal balance. These environmental stimuli or depressants incite compensations in energy, thermal, hormonal, and water regulatory mechanisms which may not be conducive to optimal growth, milk secretion, or associated reproductive functions of the animal. All animals are either in a state of equilibrium with their environment or are making compensatory physical and physiological changes that allow them to function in the most effective manner. Among other homeothermic and homeostatic functions of cattle, the neuroendocrine system may be a good indicator of an animal's equilibrium under its existing state of thermal, energy, and water balance.

With this background information, the present paper recognizes and discusses some of the potential stressors those which affect the overall health of the animal and in specific the lactation. The
paper discusses the neuroendocrine axis as well as the hormones that are associated with the functions of mammary gland causing milk synthesis and regulation of lactation.

In the broadest sense, and in the way it was initially defined by Selye, a stressor is any stimulus able to alter homeostasis. The term homeostasis was coined by Walter Cannon, to refer to the set of physiological mechanisms that allow the organisms to adapt to external (physical and biological) challenges and to maintain within a narrow range certain critical parameters (i.e. glycaemia, osmolality, core temperature).

Stress is the response of the organism, evolved in the course of the phylogeny, to agents actually or symbolically endangering its integrity”. This definition includes stressors, which represent a potential, but not actual, danger for the organisms (i.e. the presence of a predator).

A stressor is any environmental change that disrupts homeostasis, and stress refers to a state in which the homeostasis of the animal is threatened or perceived to be threatened. Neutral stress results in responses that are neither harmful nor beneficial to the animal. Distress causes the animal to respond in a way that can negatively affect its well-being or reproduction and which may cause pathological damage. The response to stress may have evolved as a mechanism for coping with threats to the survival and well-being of the animal by adjusting several systems within the body to maintain homeostasis. When these adjustments fail to compensate for the stress, or when the response is excessive or inappropriate, pathological changes and damage to the animal can occur.

The concept of allostasis illustrates the idea that a quite strict stability (the actual meaning of homeostasis) is needed only for certain critical parameters and that this stability is maintained because of the action of a wide range of physiological factors (i.e. hormones) that are continuously changing to maintain the stability of the critical variables despite environmental challenges. Therefore, allostasis may be a better term than homeostasis to define physiological mechanisms developed to face external perturbations. The price organisms pay to maintain (or try to maintain) the stability of a few set of critical parameters is called allostatic load. If such allostatic load is excessive and prolonged, pathological consequences could ensue.

STRESS AXIS AND RESPONSE OF STRESS AXIS TO STRESSORS

The brain region most critically involved in the control of the HPA axis is the parvocellular region of the PVN, a nucleus that occupies a medial and dorsal position within the hypothalamus and therefore lines the third ventricle. The PVN is a functionally complex nucleus involved in the regulation of both neuroendocrine and vegetative functions. On the basis of morphological and functional criteria it is subdivided into several regions: magnocellular, dorsal cap, medial parvocellular (mp) and submagnocellular.

The magnocellular region PVN comprises vasopressin (arginin- vasopressin, AVP, in most mammals) and oxytocin (OT) neurons, which send their axons through the internal median eminence to the neurohypophysis and are involved in the control of several well-known physiological responses such as antiuresis (AVP) and smooth muscle contraction (OT). It should be noted that another distinct hypothalamic nucleus, supraoptic (SON), is also formed of magnocellular neurons synthesizing AVP and OT and projecting to the neurohypophysis.

The dorsal cap and submagnocellular regions of the PVN are important areas for the control of vegetative functions through projections to brainstem and spinal nuclei controlling sympathetic and parasympathetic activity. Finally, the mpPVN is the origin of neurons sending axons to the external
zone of the median eminence, where they release hypothalamic hypophysiotropic factors into the primary capillaries of the pituitary portal vessels. These capillaries coalesce to form the long pituitary portal vessels and later the veins that will penetrate the anterior pituitary and distribute the hypothalamic factors within the gland. These hypothalamic factors act on the corticotrope cells of the anterior pituitary to control the synthesis and release of the adrenocorticotropic hormone (ACTH), that is formed from a larger precursor protein called pro-opiomelanocortin (POMC). Following figure depicts the response of HPA axis to stress.

**Fig-1** HPA axis response to stress

![HPA axis response to stress](image)

After exposure to acute stress, changes at all levels of the HPA axis are detected, although time is a critical factor. Thus, the activation of parvocellular PVN neurons can be rapidly detected by an enhanced expression of IEGs such as c-fos that cannot be consistently observed before 15 min after initial exposure to the stressor, but reach a maximum at about 30 min. A fast increase in CRF gene expression in the PVN is also observed after stress using intronic probes that measure recently formed RNA in the nucleus of the cells (heteronuclear, hnRNA). Therefore, the intronic CRF probe is a much better tool to study central HPA response because resting levels of CRF hnRNA are almost undetectable and a marked increase is observed in response to stressors of different intensities. In contrast, increases in CRF mRNA are only consistently observed after 3-4 h of initial exposure to the stressors, and this increase is not usually observed after exposure to mild stressors, because of the already high resting levels of CRF mRNA levels. The activation of transcriptional activity of AVP gene in parvocellular PVN neurons is much slower than that of CRF, with peaks of AVP hnRNA levels at about 1 h after initial exposure. Stress also can increase transcriptional activity of POMC cells in the anterior pituitary, although the effect is only noted with more severe stressors.
Basal levels of ACTH are between 20 and 100 pg/ml depending on the assay procedure used. In our hands, ACTH levels after 15 min exposure to novel environments is about 600 pg/ml, whereas maximum plasma ACTH levels are achieved after exposure to IMO and are usually within the range of 2000-4000 pg/ml. Exposure to stressors causes a prompt release of ACTH that is clearly observed at 5 min after the start of exposure. If such exposure continues, maximum ACTH secretion is usually observed at 15 min and plasma levels are maintained for about 30-60 min. After that, a progressive decline is observed which may result in modest increases or apparently resting ACTH levels (depending on the severity of the stressor) after 10-24 h of continuous exposure. This progressive decline is observed even after exposure to severe stressors.

Following figure depicts the response to stress.

**Fig 2: Response to stress**

Therefore, evaluation of plasma ACTH levels after the first hour of initial exposure to the stressor is not appropriate to evaluate the actual response to the stressor and can lead to misleading interpretation. The reason for this progressive decline is not entirely clear, but may be due at least in part to the combination of negative glucocorticoid feedback and an incapability of the corticotropes to maintain for hours ACTH release at a high level. That reduced ACTH release is not due to an adaptation of the animal to the stressful situation thus resulting in a progressively lower emotional activation is demonstrated by the finding that ACTH release is not observed under these conditions by exposure to a novel stressor.

Following figure depicts the response of sympathetic nervous system to stress.
Fig 3: Sympathetic nervous system response to stress

STRESSORS AND THEIR EFFECTS

Environmental factors causing stress in the organisms are known as stressors. The stressors affect the HPA and modulate the functions of HPA. Altogether these lead to a plethora of physiological responses that either cause homeostasis or may lead to imbalance in the internal balance. The following figure depicts the stressors and their effects on nutrient utilization and portioning of the nutrients.

Fig 4: Effect of Stress on nutrient utilization and portioning of nutrients
IMPACTS OF HEAT STRESS ON HORMONES AND MILK PRODUCTION

An animal communicates with its external environment through the neuroendocrine and neural systems which in turn influences lactation. Effect of environmental temperature on Hypothalmic-pituitary-thyroid, Hypothalmic-pituitary-Adrenal and Hypothalmic-pituitary-ovarian axis may influences the mammogenic, lactogenic and galactopoietic functions of mammary gland and ultimately influences the milk production in dairy animal. Hormones involved in the lactational processes, such as thyroxine, glucocorticoids, prolactin, and growth hormone may assist the animal in efficiently meeting the greater energy demands of lactation, and these are influenced by environmental temperature. However, information on stressor effects is lacking on glucagons, insulin, vasopressin, calcitonin, and oxytocin.

The below given figure shows lactation curve of a dairy cow. Stress can affect any part of the axis.

Fig. 5 Lactation Curve of a cow
Thyroid Hormone

Thyroid hormones are galactopoic. They mediate and modulate the functions of growth hormone and prolactin. Injection of thyroid hormone into cows increases milk production for a short period of time (several weeks). Thyroprotein (iodinated casein) is a commercial product that increases milk yield in cows by about 10% in early lactation and by 15-20% in late lactation. However, the positive effect only lasts for 2-4 months and subsequent yield is below normal. Thyroid function in lactating animals has shown a general depression in the summer months and is normal or elevated during winter months. There are many environmental variables, including: (a) time, (b) duration, (c) severity of temperature, (d) wind, (e) radiation, (f) humidity, (g) quality and quantity of feed, and (h) parasites, etc. Such factors may influence the extent of thyroid functional change; and of course, there are differences between species and breeds in climatic adaptability or acclimatization.

During lactation there was decreased conversion of thyroxin to tri iodothyronine in liver and kidneys and increased conversion in mammary gland. Further more, during lactation the mammary gland is in euthyroid state and rest of the body is hypothyroid. These conditions would enhance the metabolic priority of the mammary gland.

Growth Hormone

Growth hormone is the major regulator of mammary gland growth and milk synthesis. It is galactopoic in nature and promotes the synthesis of alpha casein and increases the uptake of amino acids by the mammary epithelial cells. GH mediates its function through the IGF axis and in specific through the IGFI and IGF III. Growth hormone is important to growth and lactation intensity in cattle because of effects on positive nitrogen balance and increased protein synthesis. By improving efficiency, this hormone may be especially important for high intensity lactation. Acute exposure to environmental heat or other external or emotional stimuli causes immediate release of growth hormone from the pituitary. However, prolonged exposure of days and weeks to an environmental stressor lowers the growth hormone in cattle. Accompanying lowered growth hormone are depressed growth rates, less nitrogen retention, and poor lactation performance in cattle.
A substantial literature supported the notion that growth hormone induced secretion of IGF-I, from either the liver or from cells in the mammary stroma and it may be IGF-I that mediates the mammogenic action of growth hormone via endocrine, paracrine or autocrine mechanism. Mechanism of action of growth hormone is probably located outside the mammary gland, and they must be very sensitive to small changes in secretion of growth hormone. It was postulated that growth hormone was involved in coordinating the partitioning of nutrients towards the mammary gland during lactation. For example more energy from fat is made available to the mammary gland during lactation, especially early lactation. During these physiological state adipocytes become less sensitive to insulin, and growth hormone receptors located on the adipocytes were probably involved in mediation of this action. Growth hormone also binds to receptors on hepatocytes, which stimulated increased secretion of IGF-I, IGF-I increased proliferation or survival of mammary cells.

**Glucocorticoids**

Glucocorticoids are the potential regulator of lactation. In physiological circulating levels, these mediate galactopoic effects, whereas, in higher levels they are inhibitory to lactation. The glucocorticoids mediate casein biosynthesis and promotes glucose uptake by the mammary epithelial cells. Glucocorticoids Induce the formation of ultra structural components necessary to support milk synthesis and secretion, including rough endoplasmic reticulum and tight junction sealing; regulate milk protein gene expression, and prevent the second phase of involution, possibly by preventing the breakdown of the extracellular matrix. Stress activates the HPA axis to cause the secretion of glucocorticoids leading to suppression of galactopoiesis. Prolonged exposure (24 days) to high environmental temperature resulted in lower than normal glucocorticoids. Glucocorticoids may limit milk secretion during advanced lactation. Corticoids are significantly higher in lactating cows as compared to nonlactating. Glucocorticoids and milk production were significantly correlated (.31) at 15°C (P<.004) and significantly negatively correlated (-.49) at 30°C (P<.0001). (Vanjonack and Johnson,1975) suggest that the shift from a positive relationship between glucocorticoid and milk yield at 15°C to a negative relationship at short term (18 h) thermal exposure (30°C) is because the high producing cows were stressed more by thermal exposure due to the greater energy intake associated with high lactation.

**Prolactin**

Prolactin has been the most intensely studied hormone related to mammary function. It directly increased lobule-alveolar development of the mammary epithelium during pregnancy. Prolactin was discovered to be critically important for initiation of lactation in the periparturient period in several species, including cattle. It is galactopoic in ruminants and specifically involved in the synthesis of milk protein caseins.

**Catecholamines**

The major regulators of stress and are the potent regulators of stress axis. With increase in stress, the levels of secretion of catecholamines dramatically increases and inhibit the secretion of milk. These are also considered as inhibitors of milk synthesis. With the advent of stress the degree of milk yield decreases and the cows also suffer from negative energy balance.

**Impacts of Heat Stress on Milk Production**

All animals have a range of ambient environmental temperatures termed the thermo neutral zone. This is the range of temperatures that are conducive to health and performance. The upper critical temperature is the point at which heat stress effects begin to affect the animal. There are a number of environmental factors that contribute to heat stress. These include high temperature, high
humidity and radiant energy (sunlight). Heat stress can be simply defined as the point where the cow cannot dissipate an adequate quantity of heat to maintain body thermal balance.

The environmental conditions that induce heat stress can be calculated using the temperature humidity index (THI). There are a number of equations that have been used to calculate THI. These equations usually include temperature and humidity to calculate THI. One equation is: \( \text{THI} = (\text{Dry bulb temperature} \, ^\circ\text{C}) + (0.36 \times \text{dew point temperature} \, ^\circ\text{C}) + 41.2 \). A THI > 72 is the point at which dairy cow start to decrease Productivity

The biological mechanism by which heat stress impacts production and reproduction is partly explained by reduced feed intake, but also includes altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements resulting in a net decrease in nutrient/energy availability for production. This decrease in energy results in a reduction in energy balance (EBAL), and explains why cows lose significant amounts of body weight when subjected to heat stress. Reductions in energy intake during heat stress result in a majority of lactating cows entering into negative energy balance (NEBAL), and this is likely stage of lactation independent.

Essentially, because of reduced feed and energy intake, the dairy cow is putting herself in a bioenergetic state, similar (but not to the same extent) to the NEBAL observed in early lactation. The NEBAL associated with the early postpartum period is coupled with increased risk of metabolic disorders and health problems, decreased milk yield and reduced reproductive performance. It is likely that many of the negative effects of heat stress on production, animal health and reproduction indices are mediated by the reduction in EBAL (similar to the way it is during the transition period).

A number of physiological alterations are seen in the animals as a result of stress. The most common are elevated body temperature – Body temperatures > 102.5 \(^\circ\text{F}\) (normal is 101.5 \(^\circ\text{F}\)); increased respiration rates > 70-80/minute and increased maintenance energy requirement. Dairy cows will activate mechanisms in an attempt to dissipate the excess heat and maintain body temperature. The increased respiration rate is one example. The maintenance energy requirement may increase by 20-30% in animals under heat stress. This decreases the intake energy available for productive functions such as milk production. Blood flow to the skin will increase in an attempt to dissipate heat. At the same time, blood flow to the core of the body will decrease. The following are the some of the other mechanisms by which stress affects the lactation.

a. **Feed nutrient utilization** – An increased loss of sodium and potassium is usually associated with heat stress. This is due to losses associated with the increased respiration rate. This can shift the acid-base balance and result in a metabolic alkalosis. There can also be a decrease in the efficiency of nutrient utilization and hence affects the lactation.

b. **Dry matter intake** – Dry matter intake decreases in dairy cows subjected to heat stress. This depression in dry matter intake can be either short term or long term depending on the length and duration of heat stress. Decreases of 10 to 20% are common in commercial dairy herds.

c. **Milk production** - There is normally a decrease in milk production for cows under heat stress. This decrease can be either transitory or longer term depending on the length and severity of heat stress. These decreases in milk production can range from 10 to >25%. If heat stress lowers milk production in early lactation dairy cows, potential milk production for the lactation will be decreased. Dairy cows in later lactation may recover slowly from the effects of heat stress.

d. **Reproduction** – Heat stress has also been reported to decrease reproductive performance in dairy cows. There are a number of changes in reproductive performance that have been reported. The effects on reproduction can be prolonged and impact the animal for months after the heat stress exposure. These include:
   - The length and intensity of the estrus period

194
- Decreased conception (fertility) rate.
- Decreased growth, size and development of ovarian follicles
- Increased risk of early embryonic deaths.
- Decreased fetal growth and calf size.

Concluding Remarks

Extended periods of high ambient temperature coupled with high relative humidity compromise the ability of the lactating dairy cow to dissipate excess body heat. Cows with elevated body temperature exhibit lower DMI and milk yield and produce milk with lower efficiency, reducing profitability for dairy farms in hot, humid climates. Continued genetic selection for improved DMI and milk yield results in cows that are less heat tolerant, and coupled with the unknowns associated with global warming in the future, suggest that heat stress will become worse for dairies in the future. Improved cooling systems that are more efficient and that can cool cows at night when humidity is high are needed to meet challenges in the future. There is genetic variation in cattle for cooling capability, which suggests that more heat tolerant cattle can be selected genetically, and cross-breeding may also offer opportunities. Continued advances in feeding are needed as cattle are selected for greater milk yield, but are subject to lower intake because of environmental stress. Developing nutritional strategies which support yield but which also address metabolic and physiologic disturbances induced by heat strain will help the cow to maintain a more normal metabolism which should enhance performance.

The marked differences in individual response to dietary factors have led to major controversies in nutrition and puzzled nutrition scientists over the last century. The emerging field of nutrigenomics helps us to understand the basis for some of these differences and also promises us the ability to tailor diet based on individual genetic makeup. Studies based on ethnopharmacology and phytotherapy concepts showed that nutrients and botanicals can interact with the genome causing marked changes in gene expression. This has led to the commercial development of nutraceuticals and functional foods that can modify negative health effects of individual genetic profile bringing the field to the "food/genome" junction. Despite the promise of nutrigenomics to personalize diet, there is skepticism whether it can truly bring about meaningful modification of the risk factors connected to chronic diseases, due to the lack of large scale nutrition intervention studies. In the light of this, nutrigenomic approach is one of the best options for the development of stress tolerance in the dairy cows so as to increase the lactation yield and enhance the internal endocrine status of the dairy cows. Global climate is changing and ever changing climate is also going to be worsening in the near future. That is why it is essential to understand the nutrigenomic strategies those which are key for the improvement of overall health and well being of the dairy cows.

References


Recent Advances In Induced Pluripotent Stem Cells (IPSC) For Health Management

Sadhan Bag, P S Mahapatra and Bhabesh Mili
Division of Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar, Bareilly

Stem cells are the unspecialized precursor cells with unlimited capacity for self-renewal without senescence even after long periods of inactivity and the ability to differentiate into one or more cell types in vivo and in vitro. During early development, as well as later in life, various types of stem cells give rise to the specialized or differentiated cells that carry out the specific functions of the body. It has been proved that different stem cells are not only found in embryo but also in adult organisms and classified as totipotent (can give rise to a fully functional organism as well as to every cell type of the body), pluripotent (capable of giving rise to each type of tissue of all three germ layers but not a fully functional organism), multipotent (more differentiated cells, less plastic and give rise to a limited number of tissues), oligopotent (can differentiate into only a few types of cells, such as lymphoid or myeloid stem cells) and unipotent stem cells (can produce only one cell type, but have the property of self renewal). Pluripotent cells derived from the inner cell mass of the pre-implanted mammalian blastocyst are potential renewable sources of all types of tissues, so termed as ‘Embryonic Stem Cells (ESCs)’ by Gail Martin (Friel et al., 2005). Pluripotent stem cells also include embryonic germ (EG) cells and embryonic carcinoma cells (ECC). ESCs have been intensely studied since their discovery in mouse and observed that once removed from the blastocyst, the cells of the ICM can be cultured under special conditions into ESCs, which maintain an undifferentiated status (Evans & Kaufman, 1981; Martin et al., 1981). These cells are valuable scientifically because of their extraordinary properties of indefinite replication without mutation of the genetic material, genetically normal and differentiate into other cell types in tissue culture with application of right extrinsic and intrinsic signals. ESCs have wide application in regenerative medicine due to their unique property of pluripotency. However, advantages of ESCs cannot be fully exploited due to difficulty in their derivation, ethical consideration, genetic and epigenetic modifications during culture. Therefore, the current focus of research is on reprogramming of somatic cells into ESC like cells and their further differentiation into patient specific cell types. Progress in understanding of the transcriptional network, signalling pathways and signalling molecules responsible for the maintenance of pluripotency in ESCs paves the path for generation of induced pluripotent stem cells (iPSCs) through nuclear reprogramming from somatic cells. First successful generation of iPSCs from mouse fibroblast with ectopic transcription factors (Takahashi and Yamanaka, 2006) has created milestone in stem cell research. Since then iPSCs have been induced from somatic cells of human (Yu et al., 2007) and different domestic species such as pig (Ezashi et al., 2009), dog (Shimada et al., 2010), rabbit (Honda et al., 2010), sheep (Bao et al., 2011), goat (Ren et al., 2011), horse (Nagy et al., 2011), cattle (Han et al., 2011) and buffalo (Bag et al., 2012) using integrating factor methods. iPSCs thus generated are found highly similar to ESCs in various aspects such as morphology, proliferation, gene expression, and in vitro differentiation. Hence, these cells can replace ESCs to avoid ethical and other complicity.

Strategies for pluripotent cells generation using somatic cells:

Till Spemann (1938) it was believed that cellular differentiation is a unidirectional process and the mature cells could never reverse to pluripotent stem cell status. He suggested a method for reversal of pluripotency in somatic cells by isolating the nuclei from progressively older embryos and transferring them into enucleated eggs and the possibility of nuclear transfer from a somatic cell to an enucleated egg. Basing on this hypothesis Briggs and King (1952) established the technique of somatic cell nuclear transfer (SCNT) or “cloning” and probed the developmental potentiality of nuclei isolated from late-stage embryos. Afterwards Gurdon proved that differentiated amphibian cells retain the genetic information necessary to support the generation of cloned frogs which shattered the dogma
that cellular differentiation as an unidirectional process. Wilmut and his co-workers (1997) confirmed that the genome of fully specialized cells remain genetically totipotent and may undergo reversible modifications by producing sheep Dolly, the first domestic animal cloned from mammary epithelial cell. These findings led to genetic manipulation of mammals, but unfortunately cloned animals exhibit phenotypic and gene expression abnormalities which makes them unsuitable for genetic and biochemical studies. Fusion of pluripotent embryonic carcinoma cells (EC cells) with somatic cells (thymocytes) resulting in hybrid cells with properties of EC cells and no features of somatic partner, suggesting presence of soluble factors in EC cells which imparts pluripotent status upon somatic cells (Miller & Ruddle, 1976). The fusion of somatic cells with pluripotent cells of different origins has been successfully re-programming somatic cells of mice and humans into pluripotent cells (Cowans et al., 2005; Do & Scholer, 2004). The major limitation of these hybrid pluripotent cells is the genetic instability with an extra pair of chromosome. Exposure of somatic cell lines to the extracts of oocyte, embryonic germ cell, embryonic carcinoma cell or embryonic stem cell also helps them to regain their pluripotent status. Most recent approach to re-program somatic cells is use of transcription factors involved in determining pluripotency in cells. Cell lineage associated transcription factors help in establishing and maintaining cellular identity during development by promoting the expression of cell specific genes and suppressing lineage-inappropriate genes to re-establish pluripotency when ectopically expressed in other cells. This was first demonstrated by formation of myofibers in fibroblast cell lines by transduction with retroviral vectors expressing the skeletal muscle factor MyoD. In 2006, Shinya Yamanaka re-established pluripotent status by transducing mouse embryonic fibroblasts with retroviral vector containing a set of transcription factors Oct4, Sox2, c-Myc and Klf4 (Known as Yamanaka factors). The resulting cells were called as induced pluripotent stem cells (iPSCs). iPSCs are type of pluripotent stem cell artificially derived from a non-pluripotent cell (an adult somatic cell) by inducing "forced" expression of specific genes. Such cells are similar to natural embryonic stem (ES) cells in many respects, such as the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation as well as potency and differentiability. iPSC cells are artificially derived from adult somatic cell without the use of rare and excellent embryos, so iPSC cells and their derivatives especially germ cells will enable the precise genetic engineering of livestock for improved production traits, act as powerful reproductive tools and could significantly speed up the breeding process. Alternate procedures for iPSC production are adenoviral/ lentiviral transduction, transfection with plasmids carrying the reprogramming factors, use of orphan nuclear receptor Esrrb with Oct4, Sox2 and piggyBac (PB) transposon gene-delivery system to achieve reprogramming without viral integration (Stadtfeld et al., 2008; Okita et al.,2008; Kaji et al.,2009 & Woltjen et al.,2009). Besides these, non-DNA approach in generating iPSCs by using small molecules or chemicals to replace certain transcription factors is also gaining popularity (Huangfu et al., 2008). Reprogramming of mouse embryonic fibroblasts was successfully done using purified proteins of transcription factors with poly-arginine peptide tail to enable their migration through the cell membrane and cultured in medium supplemented with same in presence of histone deacetylase (HDA) inhibitor (Zhou et al.,2009). In recent years, stem cell nanotechnology has emerged as exciting field in iPSCs generation. The iPSCs have been generated from mouse (Lee et al., 2011) and human (Ruan et al., 2011) somatic cells by transfection with nano tagged plasmid vectors of transcription factors. iPSCs has also been generated from mouse neuronal stem cells by delivering reprogramming proteins using titanium oxide nanotubes (Cho et al., 2013). However, attempt to generate iPSCc through nanoparticle-mediated delivery of mature miRNAs resulted in incomplete iPSCs, may be due to insufficient integration (Sohn et al., 2013). Studies conducted in our laboratory has showed that incubation of caprine and buffalo fetal cells in media supplemented with avian egg extract (EE) formed iPSC like cell colonies with the expression of pluripotent markers (Bharadwaj et al., 2013; Mahapatra et al. 2013). However, further research is needed for establishing a pluripotent stem cell line with EE or EE supplemented with other factors.
Induced pluripotent stem cells in domestic species

Recent advances in iPSC technology have overcome the difficulty in establishing pluripotent cell lines in many domestic animal species with use of murine and human factors establishing pluripotent factors don’t have phylogenetic limitation. Human factors used for iPSCs from pig mesenchymal stem cells (MSCs), capable of generating chimeras with germline transmission and same also used to produce iPSCs from avian species, indicating the factors are evolutionarily conserved. So far, the iPS cells have been generated in many livestock species as well as with different somatic cells (Table 1) and provide hope for the future in regenerative medicine with these species. Moreover due to similarity in size, physiology, and immunology, large animals are better models for human genetic or acquired diseases compared to rodents. Because of their longer life span, heterogeneous genetic background they can provide a good model for long-term experiments. In our laboratory buffalo iPSCs have been successfully generated from fetal fibroblast with lentiviral integration method. It has also been observed that the small molecule valproic acid treatment following lenti viral based transduction of ectopic transcription factors enhanced significantly the number of colony formation (Mahapatra et al., 2013). We are able to propagate buffalo iPSC colonies up to 21st passage which is longest ever passage reported so far as per our knowledge.

Table 1: iPS Cells Generated in Domestic Animals

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Species</th>
<th>Transgenes and cells used</th>
<th>iPSC Markers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Pig</td>
<td>hOct4, hSox2, hKlf4, and hc-Myc Fibroblast</td>
<td>Molecular Marker: Oct4, Klf4, Sox2, cMyc, Nanog Immunoproteins: SSEA4, Nanog, Rex1 AP activity: +ve</td>
<td>Esteban et al., 2009 Ezashi et al., 2009 Wu et al., 2009</td>
</tr>
<tr>
<td>3.</td>
<td>Dog</td>
<td>human Oct4, Sox2, c-Myc and Klf4 Fetal and adult fibroblast</td>
<td>Molecular Marker: Oct4, Nanog Immunoproteins: SSEA1, SSEA4, TRA1-60, TRA1-81, and Rex1 AP activity: +ve</td>
<td>Shimada et al., 2010</td>
</tr>
<tr>
<td>4.</td>
<td>Rabbit</td>
<td>hOct4, hSox2, hKlf4, and hc-Myc</td>
<td>Molecular Marker:c-Myc, Klf4, Sox2, Oct3/4, and Nanog Immunoproteins: Oct4, , Nanog, SSEA1, SSEA3 and SSEA4 AP activity: +ve</td>
<td>Honda et al., 2010</td>
</tr>
<tr>
<td>5.</td>
<td>Sheep</td>
<td>Oct4, Sox2, c-Myc and</td>
<td>Molecular Marker: Oct4, Klf4, Sox2, cMyc, Nanog</td>
<td>Bao et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Klf4 Fibroblast</td>
<td>Immunoproteins: Oct4, sox2, Nanog, SSEA4 AP activity: +ve</td>
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<tr>
<td>6.</td>
<td>Goat</td>
<td>Molecular Marker: Oct4, Nanog, Sox2</td>
<td>Ren et al., 2011</td>
<td></td>
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<tr>
<td></td>
<td>Ear fibroblasts</td>
<td>Immunoproteins: SSEA-1, Tra1-60, Tra1-81</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AP activity: +ve</td>
<td></td>
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<tr>
<td>7.</td>
<td>Horse</td>
<td>Molecular Marker: Oct4, Sox2, Nanog, and Rex1, Lin 28</td>
<td>Nagy et al., 2011</td>
<td></td>
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<tr>
<td></td>
<td>murine Oct4, Sox2, c-Myc, and Klf4</td>
<td>Immunoproteins: TRA1-60, SSEA1, and SSEA4</td>
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<tr>
<td></td>
<td>Oct4, Sox2 and Klf4</td>
<td>AP activity: +ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fetal fibroblast</td>
<td>Molecular Marker: Oct4, Nanog, Stat3</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Immunoproteins: Oct4, Nanog, SSEA1 and SSEA4</td>
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<td>AP activity: +ve</td>
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<td></td>
<td></td>
<td>AP activity: +ve</td>
<td>Mahapatra et al., 2013</td>
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**Conclusion:**

Although iPSC lines have been successfully established from a range of livestock species, their promise for biotechnology and medicine remains to be demonstrated. The level and duration of reprogramming factors in the somatic cells is very important in the generating the chimeric-competent livestock iPS cells. So, emphasis should be given to ready manipulation to their genomes and to obtain superior clonability than somatic cells. Care must be taken in generation of iPSCs from animal somatic cells through prescribed pathways in a reproducible manner. Research should also focus on the minimal means needed to establish pluripotency and retention of epigenetic memory of progenitor cells used. Most importantly, the cell lines should not express the reprogramming genes used to create them as their expression can interfere with differentiation. Moreover factors used to maintain human and mouse ESC should be replaced with species derived factors for iPSCs from a farm animal.

**References**

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★★★
Introduction

The mammalian immune system is divided into two types of immunity: innate and adaptive. Adaptive immunity is characterized by specificity, memory and develops by clonal selection from a vast repertoire of lymphocytes bearing antigen-specific receptors. However, it takes time for specific clones to expand and differentiate into effector cells, so to induce immediate responses after encounter with a pathogen, innate immunity forms pre-emptive. Although, innate immune system was first described by Elie Metchnikoff, it has long been viewed as merely a nonspecific response to simple phagocytose pathogens and as something that presents antigens to the cells involved in acquired immunity. However, in 1996, it was demonstrated that the Drosophila protein Toll is required for flies to induce effective immune responses to Aspergillus fumigates. This study helped in understanding that the innate immune system functions as a pathogen detector. The targets of innate immune recognition are conserved molecular patterns of microorganisms called as pathogen associated molecular patterns (PAMPs) or more appropriately microorganism-associated molecular patterns (MAMPs) since they are found not only in pathogenic but also in non-pathogenic microorganisms. The receptors involved in innate immunity are called pattern recognition receptors (PRRs).

Pattern Recognition Receptors

The innate immune system uses a variety of pattern recognition receptors that can be expressed on the cell surface, in intracellular compartments, or secreted into the bloodstream and tissue fluids. The principal functions of pattern recognition receptors include opsonization, activation of complement and coagulation cascades, phagocytosis, activation of pro-inflammatory signaling pathways and induction of apoptosis. Mannan-binding lectin (MBL), C-reactive protein (CRP), and serum amyloid protein (SAP) are secreted pattern recognition molecules produced by the liver during the acute phase response at the early stages of infection. CRP and SAP are members of the pentraxin family, and both can function as opsonins upon binding to phosphorylcholine on bacterial surfaces. CRP and SAP can also bind to C1q and thus activate the classical complement pathway. MBL is a member of the collectin family, which also includes pulmonary surfactant proteins A and D. The collectins are characterized by the presence of a collagenous region and a C-type lectin (CTL) domain; typically they form oligomeric receptors. MBL binds specifically to terminal mannose residues, which are abundant on the surface of many microorganisms, and associates with MBL-associated serine proteases (MASP). MASP1 and MASP2 are activated by MBL and initiate the lectin pathway of complement by cleaving C2 and C4 proteins. Several cell surface receptors expressed on macrophages function as pattern recognition receptors that mediate phagocytosis of microorganisms. Macrophage mannose receptor (MMR) is a member of the C-type lectin family and it interacts with a variety of gram-positive and gram-negative bacteria and fungal pathogens. The main function of the MMR is thought to be phagocytosis
of microbial pathogens, and their delivery into the lysosomal compartment where they are destroyed by lysosomal enzymes. Macrophage scavenger receptor (MSR) is another phagocytic pattern recognition receptor expressed on macrophages and has an unusually broad specificity to a variety of polyanionic ligands, including double-stranded RNA (dsRNA), LPS, and LTA. MSR protects against endotoxic shock by scavenging LPS. Another member, MARCO, is a macrophage receptor that binds to bacterial cell walls and LPS, and mediates phagocytosis of bacterial pathogens. Toll receptors and the associated signaling pathways represent the most ancient host defense mechanism found in insects, plants, and mammals. Studies of the fruit fly have shown that the Toll family is one of the most crucial signaling receptors in innate immunity. Among PRRs, TLRs are expressed mainly on antigen-presenting cells such as DCs and macrophages, as well as on B cells.

**Intracellular Recognition Systems**

Viruses and some bacterial pathogens can gain access to the intracellular compartments, such as the cytosol. Several pattern recognition receptors are expressed in the cytosol where they detect these intracellular pathogens and induce responses that block their replication. The protein kinase PKR is activated upon binding to dsRNA, which is produced during viral infection. Activated PKR results in a block of viral and cellular protein synthesis. In addition, PKR leads to the induction of the antiviral type-I IFN genes. PKR also inhibits viral spread by inducing apoptosis in infected cells. Another group of proteins likely involved in intracellular pattern recognition is the family of NOD proteins. NOD proteins contain an N-terminal CARD domain, a nucleotide binding domain (NBD), and a C-terminal leucine-rich repeat (LRR) region. The full range of ligands recognized by NOD proteins is currently unknown, but both NOD1 and NOD2 are reported to activate NF-κB response to LPS, presumably through binding to their LRR regions.

Although the TLR family detects PAMPs either on the cell surface or the lumen of intracellular vesicles such as endosomes or lysosomes, recent studies have shown the existence of a cytosolic detection system for intracellular PAMPs. These cytosolic PRRs include retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). RLRs belong to the RNA helicases family that specifically detects RNA species derived from viruses in the cytoplasm and coordinate anti-viral programs via type I IFN induction. NLRs constitute a large family of intracellular PPRRs, several of which—such as NOD1, NOD2 and NALP3 (NACHT [neuronal apoptosis inhibitory protein (NAIP), CIITA, HET-E and TP-1], LRR [leucine-rich repeat] and PYD [pyrin domain] domains-containing protein 3)—are well characterized. NOD1 and NOD2 recognize intracellular bacterial cell products, and NALP3 responds to multiple stimuli to form a multi-protein complex termed the NALP3 inflammasome, which promotes the release of the IL-1 family of cytokines. Furthermore, intracellular double-stranded DNA (dsDNA) released by DNA viruses or bacteria function as PAMPs that induce type I IFN through unidentified pathways. In addition to PAMPs, innate immunity has the potential to respond to endogenous molecules that are released by host cells as a result of necrosis, pathogen infection, damage, injury and certain pathological conditions, which are directly or indirectly recognized by TLRs, NLRs, RLRs or as-yet-undefined sensors. The recognition of endogenous molecules by PRRs is tightly linked to the pathogenesis of autoimmune and inflammatory diseases.

**Toll-like Receptors**

TLRs are type I transmembrane proteins (i.e. the N-terminal is outside the membrane) composed of three major domains and characterized by LRRs in the ectodomain, which
mediate the recognition of their respective PAMPs; there is also a transmembrane domain and an intracellular domain that is homologous to that of the IL-1R and is known as the Toll/IL-1R (TIR) domain, which is required for initiating downstream signaling pathways. So far, the mammalian TLR family comprises more than 12 members. The TLR family members can be conveniently divided into two subpopulations with regard to their cellular localization. On the one hand, TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 are expressed exclusively on the cell surface and recognize microbial membrane components such as lipids, lipoproteins and proteins. On the other hand, TLR3, TLR7, TLR8 and TLR9 are localized in intracellular vesicles such as the endosome or lysosome and the endoplasmic reticulum (ER) and predominantly recognize microbial nucleic acid species.

**Pathogen Recognition by TLR**

**Bacteria**

Lipopolysaccharide is a strong immunostimulant cell wall component of Gram-negative bacteria and is composed of lipid A (endotoxin), core oligosaccharide, and O-antigen. TLR4 is essential for recognition of LPS which recognizes lipid A of LPS. For LPS recognition, a complex formation of TLR4, MD2, and CD14 on various cells, such as macrophages and dendritic cells, is necessary. LPS is associated with an accessory protein, LPS-binding protein (LBP) in serum, which converts oligomeric micelles of LPS to monomers for delivery to CD14, which is a glycosyl phosphatidylinositol (GPI)-anchored, high-affinity membrane protein. CD14 concentrates LPS for binding to the TLR4/MD2 complex.

TLR2 recognizes various bacterial components, such as lipoproteins/lipopeptides and peptidoglycans from Gram-positive and Gram-negative bacteria, and lipoteichoic acid from Gram-positive bacteria.

TLR1 and TLR6 are structural relatives of TLR2. TLR2 and TLR1 or TLR6 form a heterodimer that is involved in the discrimination of subtle changes in the lipid portion of lipoproteins.

Bacterial flagellin is a structural protein that forms the major portion of flagella that contribute to virulence through chemotaxis, adhesion to, and invasion of host surfaces. TLR5 is responsible for the recognition of flagellin. Unlike other TLRs, TLR5 is not expressed on conventional dendritic cells or macrophages in mice. TLR5 is expressed on the basolateral surface, but not the apical side of intestinal epithelial cells, suggesting that flagellin is detected when bacteria invade across the epithelium.

Bacterial DNA is a potent stimulator of the host immune response. This immune stimulation is mediated by unmethylated CpG motifs. In vertebrates, the frequency of CpG motifs is severely reduced and the cytosine residues of CpG motifs are highly methylated, which leads to abrogation of the immunostimulatory activity. CpG DNA recognition is mediated by TLR9.

**Fungi**

TLRs have been implicated in the recognition of the fungal pathogens such as *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis carinii*. Several components located in the cell wall or cell surface of fungi have been identified as potential ligands. Yeast zymosan, derived from *Saccharomyces cerevisiae*, activates TLR2/TLR6 heterodimers, whereas mannan, derived from *S. cerevisiae* and *C. albicans*, are detected by TLR4. Phospholipomannan, present on the cell surface of *C. albicans*, is also recognized by TLR2, while TLR4 mainly interacts with lucuronoxylomannan, the major capsular polysaccharide of *C. neoformans*. 
Protozoa

Several studies have shown that glycosylphosphatidylinositol (GPI) anchors from protozoan parasites activate cells of both lymphoid and myeloid lineages. GPI moieties are abundantly expressed by many protozoan parasites and function as anchors to the surface of eukaryotic cells. GPI anchors consist of a glycan core and a lipid component. TLRs sense GPI anchors of protozoa. TLR9, a receptor for unmethylated bacterial CpG DNA motifs, is also important for resistance to protozoan parasite infections. DNA from the protozoan parasites activate macrophages and DCs, leading to the induction of inflammatory responses.

Virus

TLR4 recognizes not only bacterial components but also viral envelope proteins. The fusion (F) protein from respiratory syncytial virus (RSV) is sensed by TLR4. The envelope protein of mouse mammary tumor virus (MMTV) directly activates B cells via TLR4. TLR2 has also been reported to be involved in the recognition of envelope proteins of measles virus, human cytomegalovirus, and HSV-1. Double-stranded (ds) RNA is generated during viral replication. TLR3 is involved in the recognition of a synthetic analog of dsRNA, polyinosine-deoxycytidylic acid (poly I:C), a potent inducer of type I interferons (IFNs). CpG DNA motifs are also found in genomes of DNA viruses. Mouse pDCs produce IFN-α by recognizing CpG DNA via TLR9. TLR7 and TLR8 are structurally highly conserved proteins. They are predicted to recognize a guanosine- or uridine-rich single-stranded RNA (ssRNA) from viruses such as human immunodeficiency virus (HIV), vesicular stomatitis virus (VSV), and influenza virus. Although ssRNA is abundant in hosts, host-derived ssRNA is not usually detected by TLR7 or TLR8. As TLR7 and TLR8 are expressed in the endosome, host-derived ssRNA is not delivered to the endosome and so is not recognized by TLR7 and TLR8. Besides TLR7 and TLR8, TLR3 and TLR9 are exclusively expressed in endosomal compartments not on cell surfaces. After phagocytes internalize viruses or virus-infected apoptotic cells, viral nucleic acids are released in phagolysosomes and are recognized by TLRs.

RIG-I-like receptors

Once viruses enter the cytoplasm and generate dsRNA during the course of replication, infected host cells can sense them and, thus, activate intrinsic anti-viral signaling pathways. This sensing occurs in the cytoplasm of both immune and non-immune cells and is independent of the TLRs that can detect the RNA species present within endosome. In this regard, the RLR family, which has three members—RIG-I, melanoma differentiation associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2)—was reported to recognize viral RNA in the cytoplasm. RIG-I, a prototypical member of the RLR family, contains tandem caspase recruitment domain (CARD)-like regions at its N-terminus that function as an interaction domain with other CARD-containing proteins. RIG-I also has a C-terminal repressor domain (RD), which binds to RNA. In resting cells, RIG-I is inactive as a monomer, but virus infection and RNA binding trigger conformational changes to facilitate self-association, which promotes CARD interaction with downstream signaling molecules. The roles of RLRs in the detection of RNA viruses have been elucidated through analyses of mice deficient for each respective RLR. RIG-I is essential for the recognition of various ssRNA viruses, which include paramyxoviruses, influenza A virus, VSV and Japanese encephalitis virus.

NOD-like receptors

The NLR family detects the presence of PAMPs and endogenous molecules in the
cytosol. NLRs consist of three domains characterized by an N-terminal protein interaction domain, a central nucleotide-binding domain and a C-terminal LRR. Members of the NLR family are categorized into at least five subfamilies distinguished by their N-terminal structures. These include NLRA (which contain an acidic transactivation domain), NLRB (contain a baculovirus inhibitor of apoptosis protein repeat [BIR]), NLRC (contain a CARD), NLRP (contain a Pyrin domain) and NLRX (contain an unknown domain). So far, at least 23 human and 34 murine NLR genes have been identified, although the physiological function of most NLRs is poorly understood. Certain NLRs respond to many PAMPs and lead to the release of the IL-1 family of inflammatory cytokines including IL-1β, IL-18 and IL-33 through the formation of the ‘inflammasome’, which involves caspase-1. Caspase-1 mediates the processing of the pro-form of these cytokines into mature forms, which results in the secretion of bioactive cytokines. On the basis of the NLR protein involved, inflammasomes are grouped into three main types—the NALP3 inflammasome (also known as the NLRP3 inflammasome), the NALP1 (NLRP1) inflammasome and the IPAF (NLRC4) inflammasome.

**Conclusion**

Innate immune recognition is very complex, as it has to protect the host against a highly diverse microbial world. But it seems to be in essence much simpler than the adaptive immune response, which operates by recognizing fine details of pathogenic microorganisms. The discovery of the transmembrane TLRs and cytosolic sensing systems such as RLRs, NLRs has revealed that the innate immune system possesses multiple recognition mechanisms in different cellular compartments and in different cell types. Understanding of the complexity of PRRs, with respect to the coordinated control of both innate and adaptive immune responses, is thus required for future development of therapeutic drugs that effectively or qualitatively control immune-associated diseases, including infectious diseases, inflammatory diseases, allergies, autoimmune diseases and cancer.

**Further reading**


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Introduction

To achieve optimal production and productivity, the trend in poultry production has undergone sea-change over last few years as apparently improved feeding patterns, genetic selection, management practices etc. have been adopted.

Several long-term selection studies using a variety of small animals have clearly shown that over-selection for a single trait may have adverse or unexpected effects on other traits (Lerner, 1954; Dobzhansky, 1970; Wright, 1978). Belyaev (1979) and Belyaev and Borodin (1982) demonstrated the effects of over-selection for a single trait in long-term selection experiments in foxes. Selecting foxes for a single behavioral trait (tameness) caused unexpected changes in coat color, breeding cycles, hormonal profiles, and subsequent changes in body traits.

Today broilers are reared to attain the desired marketable weight for dressing at even as low as 28-30 days and it is made possible through genetic manipulation which imparts genetic stress. During recent years, there is growing concern over the welfare activities on the issue of stress. In near future, the most serious animal welfare problems may be caused by over-selection for production traits such as rapid growth, leanness, and high yield.

“Stress is the nonspecific response of the body to any demand”, and stressor can be defined as “an agent that produces stress at any time”. Thus, stress represents the reaction of the animal (biological response) to stimuli that disturbs its normal physiological equilibrium or homeostasis (Selye, 1976). We attempt, to briefly have an overview, of major stressors, neuroendocrine-immunological interactions and possible mitigation measures.

Physiological mechanism of stress and impact of stress on the Immune Response:

Exposure of birds to stress, particularly in commercial production, is an inevitable event in poultry husbandry and when the threshold level of stress is crossed, it results in distress to birds. Thereafter, the birds show stress syndromes, which were earlier classified into three stages (Mohan J, undated) viz. short-term regulation of stress: neurogenic (sympatho-adrenal) system, long-term regulation of stress: endocrine system and exhaustion stage.

Post ganglionic and adrenal medullary tissue controls the rapid response to the animal i.e. fight or flight reaction which lasts only a short time (Cannon, 1929) characterized by increased rates secretion of the catecholamine from the adrenal medulla. These catecholamines prepare the bird for "Fight or Flight" reaction and commanding a rapid release of glucose in blood. This leads to depletion of liver glycogen, increases peripheral vasomotor activity, alters the ventilation rate and increased neural sensitivity (Selye, 1950; Siegel. 1980). Catecholamines also stimulate the activity of hepatic adenyl cyclase, the enzyme required for the production of cAMP (Robinson et. al., 1971) which regulates the number of physiological processes and directly increases the formation of antibody (Braun et al. 1971).

A bird, like other vertebrates, depends upon its hypothalamo-pituitary-adrenal (HPA) axis to respond to a stressor on long term. A reciprocal regulation exists between the central nervous and immune systems through which the CNS signals the immune system via hormonal and neuronal
pathways and the immune system signals the CNS through cytokines. The primary hormonal pathway by which the CNS regulates the immune system is the hypothalamic-pituitary-adrenal axis, through the hormones of the neuroendocrine stress response. The sympathetic nervous system regulates the function of the immune system primarily via adrenergic neurotransmitters released through neuronal routes. Neuroendocrine regulation of immune function is essential for survival during stress or infection and to modulate immune responses in inflammatory disease (Webster et al. 2002). It has been known for at least 4 decades that corticotrophin-releasing hormone (CRH) and arginine vasotocin (AVT) are the 2 major neuropeptides responsible for the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary of birds (Salem et al., 1970a,b; Carsia et al., 1986). The pituitary hormone ACTH stimulates the release of the stress hormone corticosterone, a glucocorticoid, from the adrenal gland.

Glucocorticoids are the main effector end point of this neuroendocrine system and, through the glucocorticoid receptor, have multiple effects on immune cells and molecules. This review focuses on the regulation of the immune response via the neuroendocrine system. Particular details are presented on the effects of interruptions of this regulatory loop at multiple levels in predisposition and expression of immune diseases and on mechanisms of glucocorticoid effects on immune cells and molecules. Physiological regulation of the immune system by glucocorticoid is only one part of an extensive regulatory network between the central nervous system (CNS), neuroendocrine system, and immune system. This network of connections through nerve pathways, hormonal cascades, and cellular interactions allows the CNS to regulate the immune system locally at sites of inflammation, regionally in immune organs, and systemically through hormonal routes.

Other neuropeptides like Vasoactive Intestinal Peptide (VIP), substance P and nor-epinephrin also play a vital role. The endocrine mechanism of stress regulation is triggered with the stimulation of hypothalamus and release of ACTH from anterior pituitary, which causes the increase of adrenal cortical steroid secretions. Continuous stimulation to adrenal cortex leads to chronically high levels of corticosteroid hormone. This hormone is responsible for the formation of glucose from body's reserve of carbohydrates, lipid and proteins. Corticosteroids contribute many of the disease associated with long-term stress, such as, cardiovascular and gastointestinal disease, hypercholesterolemia, metabolic rearrangements and antibody suppression. (Siegel, 1985). Other hormones like Glucagon from α cells of the pancreas (Freeman, 1980) and Thyroid are also involved in stress regulation.

Finally, on the eventuality of the bird not recovering from the stressor and the availability of body reserves and hormones from the adrenal gland are inadequate, a third or exhaustion phase leads to fatigue of the homeostatic mechanisms and death (Brake, 1985; Freeman,1987; Maxwell,1993).
As stated above, modulation of the immune response by the central nervous system (CNS) is mediated by a complex network that operates multi-directionally between the nervous, endocrine and immune systems. The hypothalamic–pituitary–adrenal (HPA) and the sympathetic–adrenal medullar (SAM) axes constitute the main pathways through which the immune response can be modulated: (a) Most lymphoid tissues receive direct sympathetic innervations, both of the blood vessels passing through the tissues, and directly to the lymphocytes themselves. 
(b) The nervous system directly or indirectly controls the output of various hormones, in particular, corticosteroids, growth hormones, thyroxin and adrenaline particularly. It has been shown that lymphocytes, monocytes or macrophages, and granulocytes express receptors for many neuroendocrine products like cortisol and catecholamines, which can affect cellular trafficking, proliferation, cytokine secretion, antibody production and cytolytic activity. Inter-relation between the nervous, endocrine and immune systems is still examined.

**Major factors inducing stress**

The critical inputs in poultry production are nutrition, health, management, genetic, which, if mishandled create stress. Many physical factors such as social, air, temperature, humidity, light, water, dust, noise and wastes etc. keep disturbing the micro-environment of poultry and make its living conditions harsh and difficult from time to time. These stressful conditions individually and collectively exhibit their impact on the physiological performance, food intake, growth, production efficiencies, well-being and livability of the birds and quality of products obtained from them.

A typical broiler 4-5 decades back was reared for 55-56 days, which until a few years back was done at an age of 45 days taking around seven to ten days lesser to get to slaughter weight per age. This was possible due to a huge leap in management practices that was applied to a typical broiler flock, in 17-18% less time. Now, we can appreciate the whopping management sensitivity with an even further reduction of the marketable age with desired weight to 28-30 days. Thus, the management period is condensed and, while the birds' performance is improved in terms of health, liveability, welfare and growth, the sensitivity to inputs increases manifold. During this rearing period typically 3.5kg of feed (containing approximately 660g of protein and 10,500Kcal of energy) is used and of the six liters of water, only around 25-30% goes towards meat production; the rest is lost through panting, in faeces and evaporation. In a 100,000-bird shed, that adds up to a "huge amount of water", and has necessitated the development of more efficient ventilation systems that can micro-manage temperature and humidity (Thomson A. 2013). It is extremely challenging over time, the expectations we have from the birds to take the feed, utilize and metabolize the energy and the protein, to live and thrive, and to grow. The first seven days are 20% of a bird’s life, and crucial to effective development. He points to a number of studies that show poor seven-day weight translates to poor finishing weight. That is why we pay so much attention to get chicks started, gut development, development of appetite and stress management. The management involved in optimizing intestinal performance is increasingly becoming important. In particular, the development of the surface area in the first seven days makes the real difference to digestibility and efficient feed conversion. The villi
increases surface area by up to 15 times, the microvilli by about another 30 and their development can be hampered by a number of factors, including stress, feed distribution and intestinal bacteria. Management of villi and microvilli, as well as careful control of the bacterial environment, is becoming a yardstick for good performance.

It is very difficult to categorize stressors separately as entire management, feeding and health care is inter-related. Still the following major points are enumerated for the sake of understanding along with mitigation measures:

**Stress induced immunosuppression:**

Most environmental factors causing immunosuppression relate to mismanagement such as inadequate water or food supply, ammonia in the houses, temperature stress, social interactions within a flock (social stress), etc. Social stress, is a result of overcrowding, non-uniform growth of the flock or less number of feeding/watering equipments than required etc.. It is one of the ignored stressor that affects production efficiency and physiological responses. It may occur in layers housed in multiple birdcages and in broilers housed in intensively confined floor pens. Productivity rate generally declines as population size increases and space allowance per bird decreases. Social and environmental stresses are common in the life of birds and influence the immune system and susceptibility to diseases. Changes in immunity are thought to be dominated primarily by hormonal play, although direct neural modulation and nutritional changes may also be involved. The diet itself is also a major determinant of the type and/or magnitude of immune response. Although nutrient deficiencies receive the most attention, there are many nutrients that modulate immunity even at levels between the dietary requirement and toxic levels. In fact, many nutrients that are not normally considered to be either required or toxic are immunomodulatory.

**Temperature, light, noise, gas/ ventilation Stressors:**

Potential stressors include temperature, light (e.g. ultraviolet light), air quality (e.g. ammonia, ozone), infectious agents, environmental contaminants (e.g. mycotoxins, pesticides) and nutrients. Exposure of birds to extreme temperatures can impact immune responses and the effect of heat or cold stress is generally immunosuppressive (reviewed by Dohms and Metz, 1991). The mechanism by which heat stress modulates immunity is in part due to the extent of induction of heat shock proteins in lymphocytes, heterophils and macrophages (Dietert et al., 1994), while cold stress suppresses plasma corticosterone levels and enhances thyroid hormone levels (Hangalapura et al., 2004). In general, all studies show an immunosuppression effect of heat stress on broilers and laying hens. For instance, under environmental stressful conditions, as the bird’s body attempts to maintain its thermal homeostasis, increased levels of reactive oxygen species (ROS) occur. As a consequence, the body enters a stage of oxidative stress, and starts producing and releasing heat shock proteins (HSP) to try and protect itself from the deleterious cellular effects of ROS. Higher concentrations of HSP70 were found in broilers and laying hens exposed to heat stress. Heat stress is one of the most challenging environmental conditions affecting commercial poultry. Compared to other species of domestic animals, broiler chickens are more sensitive to high ambient temperatures. They have no sweat glands, a rapid metabolism, and high body temperature. As living organism in the world, chickens have their protective measures against environmental disadvantages. Having no sweat glands, birds dissipate heat via the respiratory system during heat stressing course. A group of highly conserved proteins known as HSPs are rapidly synthesized and these proteins are essential for organisms living at the edge of their thermal range. One of the most important functions of HSPs is to protect organisms from the toxic effects of heating and play important roles in protein assembly and disassembly, protein folding and unfolding, protein translocation and the refolding of damaged proteins. Of the many expressed HSPs, those with a molecular weight of approximately 70 kDa appear to be most closely associated with heat tolerance (Yu and Bao, 2008). In summers in tropical countries like India, foggers, sprinkling the roof etc. will help cooling the houses.
Improper lighting of poultry houses is another reason inhibiting egg production and growth (Nagra SS., Undated). The most common physiological effect of light on growing pullets is the effect of day length on sexual maturity. Sexual maturity is delayed in pullets grown under decreasing day length. Practically, the pullets should be grown under decreasing or constant photoperiod length. In addition to day length, light intensity is also to be paid attention to. High intensity of light in brooder house may result in poor survival of chicks due to the pecking during first few days of their life. Many alternate intermittent lighting programs have been investigated found to be more promising for better production efficiencies and welfare. Electronic coloured light lamps, the use of which not only saves on electricity consumption but also improves welfare and production efficiencies. Rodenburg et al (2004) reported that yellow sodium lighting and green/blue lighting reduced breast smearing while green/blue lighting positively affected footpad lesions and gait score in broilers. Under red illumination turkeys and chickens had shown inferior growth, more activity and aggressive behavior than blue or green light as per some studies. Red light was sexually more stimulatory and blue light had calming effects.

Water intake is correlated with feed intake. Any decrease in water consumption due to failure in the water supply or lack of watering space lowers the consumption of feed to varying extent depending on the age, type of chickens, season etc. Regular supply of clean, sanitary and fresh water must, therefore, be maintained. Water temperature particularly in summer months is important. Any contamination of water will influence the performance. Enclosed watering systems is believed to improve feed conversion as the water remains protected from carriers of bacterial contaminants such as dust, litter, feed and fecal materials. Open type waterers remain exposed to such contaminants. It would require more effort to clean and disinfect the watering system on regular basis, but it certainly leads to better feed efficiency. Automated watering systems now-a-days helps address these issues.

It has always been recommended that the poultry rearing facilities should be located away from the noisy areas such as main roads and heavy mechanical industries. In small farms where the land area is a limiting factor, alleviating the noise stress by this means may not be a practical approach. Under such cases, the installation of resonators or exterior application of sound baffles should be considered.

The bird’s main response to the social stress interactions of many birds housed in a cage is an increase in the circulating level of corticosterone released from the adrenal gland as a protective mechanism. Studies found that the laying hens maintained at 0.094 m²/bird density had elevated levels of corticosterone than those maintained at 0.373 m²/bird. In addition, egg production, per cent livability and average body weight were depressed for those birds maintained in floor pens compared to those kept in cages. Thus the number of hens kept per cage and the type of rearing environment - cage or floor pens- affect the bird’s productivity and physiological response to social stressors. Similarly, broiler chicks provided 2000 cm² floor space showed less agonistic behavior than those provided 1000 cm². It is, therefore, important in commercial operations not to overcrowd pens or cages and to provide sufficient number of feeding and watering equipments. The physical environment can affect the stress response and thus the immune response. For example, chickens raised on slatted floors have elevated heterophil:lymphocyte ratios and duration of tonic immobility, and reduced antibody titers and Cutaneous Basophil Hypersensitivity (CBH) responses as compared with birds in a litter-based environment In laying hens, providing access to perches and the ability to engage in natural behaviors decreases heterophil:lymphocyte ratios (Campo et al., 2005).

Air quality is determined by the level of suspended dust particles and toxic gases. Excessive level of suspended dust particles is a result primarily of too dry litter, particularly in dry-hot season. These dust particles damage the lung surface and increase the susceptibility of birds to diseases. On the other hand, high concentration of toxic gases such as ammonia, methane, hydrogen sulphide and carbon dioxide is a result of excessive fermentation of litter due to high moisture content. These gases are lethal at high concentration. Ammonia is the major gas which causes health and production losses.
Continuous high concentration of ammonia reduces the activity of cilia of respiratory trait. A wide range of ammonia concentration is associated with building design and management practices. Chemicals like zeolites are used to neutralize ammonia and some act by inhibiting microbial growth reducing uric acid decomposition. If a cautionary approach is taken to promote welfare, performance and environmental impact, then the permissible concentration should be lowered to 10 ppm (Nagra SS., Undated). It is, therefore, necessary to use optimum management measures that can help in the elimination of toxic gases from the house while retaining a certain amount of moisture in the litter to avoid dust problem. This would require controlled air flow in the poultry house through proper ventilation and exhaust system, adequate feeding and optimum manure management.

The geographical environment in which a bird lives also affects immune responses, thus the same species may have different responses depending on geographical location. It is also conjectured that birds living in tropical environments have increased parasite loads, and thus mount lower corticosterone responses in order to maintain adequate immune defences (Martin et al., 2005). Alternately, birds living in temperate environments may mount corticosterone responses when challenged and when other resources (like nutritional resources) are available to allow this response to occur.

In addition, the presence of fungal toxins in feed is an environmental stressor. Most of these stressors enhance the production of corticosterone. Selection for high versus low corticosterone concentration in blood plasma can influence the degree of stress induced immunosuppression. It has been recognized for a long time that social stress can exacerbate disease. Stress can reduce T cell responses to mitogens, NK activity, IL-2 production, and expression of IL-2R on lymphocytes. Reduction in stress can have a reverse effect. Stress may induce susceptibility to coccidiosis, mycoplasmosis, salmonellosis infection and also mycotoxiosis due to moldy feed. MDV-exposed chickens were kept in a socially stressful environment by moving every day one chicken from one cage into another cage. These chickens developed a higher incidence of tumors than the MDV-infected chickens kept in a low stress environment. The effects of social stress on MD were especially enhanced in birds selected for high plasma corticosterone concentrations. Inoculation with chemicals blocking 11-β hydroxylase, which mediates the conversion of deoxycorticosterone to corticosterone in the adrenal glands, reduced the impact of social stress on MD and other diseases (Gross, 1972).

Dohms and Metz (1991) suggested that bacterial infections (e.g. Escherichia coli) may cause stress-type lesions in the bursa similar to corticosterone-induced lymphoid depletion. Aflatoxin is the best known fungal stressor for poultry but fumonisins and ochratoxins have also been implicated as immunotoxicants in chickens and turkeys. Bondy and Pestka (2000) suggested that the effects are more immunomodulatory than immunosuppressive. Ingestion of aflatoxins can result in decreased antibody responses to T cell-dependent and -independent antigens, decreased macrophage functions, and decreased cell-mediated immune (CMI) responses, but these effects are dose-dependent.

Psychological stress has been shown to elevate the level of IL-6 and Acute Phase Proteins(APP). It is not known how stress induces the acute phase response, but activation of the hypothalamic–pituitary–adrenal axis may trigger systemic or local cytokine production by stress signals, thereby stimulating the production of APP.

Unavoidable stressors: without environmentally controlled houses, the birds are subjected to extreme weather, rapid growth (production stress), vaccination, debeaking, medication etc. are other fallouts of an intensive system.
Wrapping-up: Future Stress Management Challenges:

Production stress, welfare requirements, food safety requirements and inevitable climate change are all putting newer stressors on the poultry production system. Poultry flocks are particularly vulnerable to climate change because birds can only tolerate narrow temperature ranges. Industrialized poultry sector needs to consider making adaptations now to help reduce cost, risk and concern in future. Most of large firms and integrators are having environmentally controlled establishments for their breeding stocks. Unorganized poultry sector is still relying mostly on indigenous varieties, which adapt to changes better than exotic varieties. The molecular research into the disease refractoriness and major histocompatibility complex will also help reveal possible avenues for mitigating climate change risks.

Heat stress during the growth period of broilers has been associated with undesirable meat characteristics and quality loss. Further, transportation of broilers from farms to processing facilities under high temperature conditions have also been shown to cause meat quality losses. In laying hens, heat stress has been shown to negatively affect egg production and quality. Food safety is increasingly being considered an important and indispensable. Colonization of birds by food borne pathogens, such as *Salmonella* and *Campylobacter*, and their subsequent dissemination along the human food chain are a major public health and economic concern in poultry and egg production. In fact, consumption and handling of undercooked poultry products constitutes one of the most commonly implicated sources of food borne illness. There is increasing evidence to demonstrate that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms.

Intervention strategies to deal with heat stress conditions are fairly easy to implement if the stressor is identified timely and provided sufficient space and resources are there. Environmental management (such as facilities design, ventilation, sprinkling, shading, etc.), nutritional manipulation (i.e., diet formulation according to the metabolic condition of the birds), inclusion of feed additives in the diet (e.g., antioxidants, vitamins, minerals, probiotics, prebiotics, essential oils etc.) and water supplementation with electrolytes are commonly followed. Lately, two innovative approaches viz. early-life conditioning (i.e., perinatal heat acclimation) and genetic selection of breeds with increased capacity of coping with heat stress conditions (i.e., increased heat tolerance) are promising (Lara and Rostagno, 2013).

Farm animal welfare issues is related to an apparent conflict of interest, as some management practices that increase farm profitability may negatively impact welfare (like increased stocking density, beak trimming, toe clipping). Leg disorders due to sudden weight gains and ascites etc. are blamed to be related to production stress. In Europe, inspite of the industry’s concerns, the minimum welfare standards imposed by McDonald’s upon their food suppliers and by many other food industries has pushed the issue forward more quickly and strongly than any government action. In addition, international pressure to ensure animal welfare is increasing, particularly from the Developed countries, where concern has been expressed, for example, about the impact of feed restriction, litter quality, ammonia, catching and transportation etc. on the welfare of breeding and now slowly the commercial stock. These concerns have led the EU to request that animal welfare issues be included in future international trade negotiations.

Proposed further research in precision feeding along with development of melioration techniques for incriminating and toxic substances in poultry feed and products is proposed along with database creation on gut metagenome of poultry species and identification of active clones for application as prebiotics, developing stocks with better immunocompetency and tropical adaptability, and climate resilient poultry production systems using Thermal Humidity Index and modulation of Heat Shock Proteins (HSPs) along with exploring epigenesis under extreme climatic and production stress.
It is also proposed to revisit the management practices to make them more eco-friendly, have a low carbon footprint, and to address welfare concerns of both poultry and also of workers by reducing pollution etc. Value-addition, food safety, quality assurance areas will be given due emphasis by paying attention to crucial areas like developing areas of Salmonella and Campylobacter serotypes of zoonotic importance using real-time Polymerase Chain Reaction (rt-PCR) techniques, developing thermal inactivation model to decontaminate by assessing thermal death time and studying thermotolerance gene expression profiling e.g. in S. typhimurium isolates from broilers, assessing efficacy of Generally Recognized As Safe (GRAS) antimicrobials, develop database for residues of chemical contaminants in poultry feed and products, thus helping in developing quality standard. All the above steps will ensure a better stress management in Poultry in near future.

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There is a need to re-energize and re-vitalize Veterinary education for better tomorrow. Past achievements have been laudable. Over the times, progressive, study growth and developments have infused confidence in us to take up any challenging and daunting tasks for effectively participating in national/ global developmental programs. Looking back from the modern era in historical developments of veterinary education, this profession made its beginning from a mono-professional approach, has now reached to stage of multi-dimensional profession with inbuilt components of societal growth, public health awareness, food and nutritional security, poverty alleviation programs, business and market trades under global order, bio-safety & bio-terrorism etc., besides, medical health care and management of livestock, wild animal and birds. The livestock has been and shall continue to be the centre of focus of rural uplifting and contributing substantially to provide sustainability to the agro dependant livelihood.

Soon after independence, veterinary education became an integral component of Agricultural system. Indian Council of Agricultural Research (ICAR) as a central apex body and the State Agricultural Universities (SAU’s) becoming mother body, assumed the role of regulatory and governance forces for managing the affairs of agricultural education that include veterinary education as well. All faculties falling under the umbrella of agricultural system were made to move on a common path for governance, academic management, basic infrastructural lay out and human resource needs as per the laid down procedures on the US pattern of land grant system as adopted by the ICAR.

A landmark decision was taken by the GOI by enacting Indian Veterinary Council Act, 1884. (IVC, Act). This Act empowered the Veterinary Council of India (VCI) to regulate veterinary practice in all the States and UT to which the Act extends. The Act also has empowered the VCI to lay down the Regulations for Minimum Standards of Veterinary Education (MSVE). Creation of VCI has galvanized the educational institutions in the country by bringing greater amount of uniformity in academic regulations including admission and examinations, course distributions and course contents throughout in the country.

The State Government of Tamil Nadu in 1989 took a bold decision by creating an independent Veterinary and Animal Sciences University by carving it out from the exiting Tamil Nadu Agricultural University. This decision of the State was considered deviation from normal by Central organizations. However, with liberal support from the State Government and dedicated efforts by the faculty and management bodies, the University demonstrated positive gains. Initially other States adopted a very cautious approach and only two more Universities at West Bengal and Maharashtra were established in text 10 years. Nine more Universities have been established in the first decade of 21st century. It is expected that next decade will witness double the numbers of these universities. In such situation 75% of the Veterinary colleges will fall in the fold of Veterinary Universities. The much needed autonomy, this profession was striving for is on the cards. There is now every reason for the organizational net work to re-vitalize the system to bridge the gaps and translate needed reforms.

As of now Veterinary Universities are moving in a narrow spectrum. These Universities have to UNIVERALISE EDUCATION AND TRAINING IN LIVESTOCK SECTOR WHILE ENCOMPASSING ON THE DIVERSIFIED BOUNDRIES OF THIS SECTOR. Programs should be technological driven, tuned to fulfill Industrial needs, besides, the graduates to be thoroughly equipped with latest and innovative skills. Every university should develop unique character though which it must have a national and global recognition with a stamp of excellence. Universities of course are duty bond to introduce programs and translate the national regulatory standards in the University.
The VCI is the authorized body to lay and update the educational standards. The Council has to ensure the efficacy of the regulations in the country and suggest amendments when ever and where ever needed. The vital changes that have taken place within the professional system and revolutionary advancements occurring nationally and world over in every sphere, calls for revamping basic veterinary education to keep it relevant to the present time and ever changing needs in future. 

The primary responsibility of a veterinarian is medical health care and management of animals. This aspect should not be lost sight while making structural changed to the basic degree course(s). The existing program is already over loaded. A proper balance is required to be struck while affecting vital transformations. The total situation has to be assessed from multiple angles .One of the following broad approach (any other alternative) could be critically examined to bring changes.

- Should there be drastic modifications in the existing course curriculum and academic regulations over the degree program in vogue with some addition and deletion as has been the practice in the past? 

  OR

- Making room for diversification within the same degree course after imparting core competence of knowledge and skills for initial period of 3 years or so.

  OR

- Should there be separate degree courses in broad disciplines of Veterinary and Livestock sector?

  OR

- Would it be feasible to integrate courses from different degree courses/ faculties or even outside the university jurisdiction?

However, a very cautious approach is required while introducing new degree course(s). Its market feasibility is required to be assessed on long term perspective. We have had unpleasant experience of starting B.Sc in Animal Sciences degree course which did not yield desired result and the program had to be abundant. Pulse of the industry and other end users is required to be assessed before starting new programs.

The author has proposed a model for introducing diversification in the same degree course by providing option to the student to pick up courses on Professional Focus and area of application during the currency of his degree pursuits in his presentation on ‘Policy issues in national education: Delivery and HRD Sustainability’ at the National annual Convention and a seminar of the National Academy of Veterinary Sciences at Mathura on 2-3 Nov, 2012. See below.

Policy Changes (proposed) for Educational needs of tomorrow.

The profession has grown in multidimensional spheres. Presently the country needs different breeds of professionals. Further, under the fast changing world order, challenges are compounding and veterinarians shall have to shoulder multifarious responsibilities. Already there is very wide gap between demand and supply and the basic work force being generated in this country have unidirectional stereotype orientation. We cannot afford to waste any more time to go in a big way in expanding our base in quantitative and qualitative terms. Policy formulation, planning and execution processes should go simultaneously. Institutes and the Universities today have mainly limited themselves in water jacketed regulatory frame work. These organizations should become truly autonomous in their actions and not merely in words.

There is a dire need to restructure our degree program so that we are able to produce veterinarians who could be equipped to cater to the responsibilities in different professional arenas.

In this direction a very rough sketch is being presented for restructuring veterinary degree course.

- Degree program should be restructured by incorporating greater flexibility and wide variability. Instead of a single degree course in vogue, program should necessary have inbuilt mechanisms to impart basic knowledge and skills in veterinary and animal science, besides, allowing graduates to be equipped in certain identified areas for acquisition of specific skills (professional focus) At the initial stage three areas are proposed to be identified (which can
be further expanded in due course of time) such as (i) veterinary health (ii) production sciences (iii) Livestock product processing.(technology driven).

The degree course is divided in three phases viz.

a. **Compulsory core courses** for 3 years
b. One year program on **professional focus** (60% courses from main area-professional focus and 40% from rest two areas.

c. Last year should be earmarked for adjusting courses on **area of application** (one semester) and internship or skill empowerment (based upon professional focus and its application (one semester)

**Area of application** could be

**Health group:** Pet (companion) animals, large animal, equine, wild and zoo animals, orthopedic, diagnostics, public health & zoonic diseases, pharmaceuticals, indigenous medicine

**Production group:** Species specific specialization, nutrition, breeding & genetics, production physiologist, feeding technologies, LS economics marketing, social sciences

**LS Product technology:** Meat technology, Milk technology, quality control, wool, fur and fiber technology, leather, skin and hide tech.

- The VCI should give course objective and outline while it should be left to the universities to develop course contents. Further, Universities should be free to add 25-30% courses especially in areas of professional focus and in area of application based upon its strength.
- Special grants may be given for each college to develop facilities and expertise in identified areas of professional focus and area of application
- Nomenclature of the degree may appropriately change.

**PG Education**

PG education presently is regulated through ICAR-SAUI/SVU system. The VCI had made a mammoth exercise to draft PG regulations and widely circulated for suggestions and comments more than a decade back. However, this exercise has lost its momentum and now there is a complete silence over this issue by the VCI. It will be in professional interest in there is proper synchronization of higher education in totality. Revamping of degree course will add value only if it is linked with PG programs as well.

**Education through private sector**

The most disheartening factor that has created dis-harmony and imbalances in the veterinary education system is the participation of veterinary education through private sector. The private sector has made its entry in this country in early 2000. It is unfortunate that over a span of more than 10 years nothing appears sound and stable. Government is yet to come out with a definite policy framework. Under such situations sometime there are compulsions to extrapolate certain provisions of the Act or regulations which leave rooms open for legal scrutiny. Implementation of these with a questionable force of law makes its feasibility and implementation a daunting task. Government ought to have taken effective steps to put a hold to the sliding trend, instead of giving leverage to adopt retaliatory approach. The existing scenario is questioning the efficacy of system management by the authorities.

**Teaching Veterinary Physiology-some comments**

Veterinary Physiology is the central core of Veterinary educational system. However, it has not been due place in the educational program. Physiology is a mother Science and it is not wrong to state that many branches of veterinary and animal sciences are borne out form the womb of Physiology. May it be health management or production related sciences including feeding, breeding and management, or from all aspects of conventional approach to modern science and technologies, molecular and basic sciences etc. are incomplete without the application of Physiology & Physiological sciences.
Physiology presently is seen in isolated as a subject of minor relevance and is offered at the preliminary stage of degree course. The present syllabus is incomplete and isolated. Teaching of Physiology would be of relevance if an integrated approach is adopted in teaching and curricular delivery. It is a sorry state that in the prevailing system- functional aspects (Physiology) are taught prior to teaching structural details (Anatomy). Many such types of ambiguities are required to be addressed in the entire degree program. Further, Physiology teaching is disjointed thereby hampering continuity of teaching since there is no teaching of Physiology in third semester.

There is a need to explore the feasibility of integrated teaching on some aspects so that a student get holistic picture of the subject. Meticulous planning and execution is required to plan for such approach. Few such examples for sequential and co-jointed teaching are as under:

- Structural aspects of blood cells are taught by Anatomy follow by teaching of functional significance of blood. In continuity alteration of blood (Pathology) should be dealt. There after blood diseases and therapeutic aspect should be taken up.
- Anatomy of polygastric system, followed by digestive mechanisms, nutritional aspect, productive potentials, rumen and G.I disorders, their diagnosis, treatment and surgical interventions should be managed sequentially to give a comprehensive view of ruminant system and its management. Same analogy should be followed for mono-gastric animals
- Structural details of reproductive system and associated endocrine glands, physiological regulatory mechanisms their abnormalities, followed by gynecological/ surgical intervention should be taken up together.

It would be prudent if complete transformation to our approach is adopted so that we are able to revolutionize the educational system to make it relevant to the future needs under the changing world order. This can be done by none other than this group of physiologists (SAPI) to take up this gigantic exercise. It is desired that through the wisdom of this body to draw the course contents that fits in the overall umbrella of degree course. In this era of knowledge explosion, students entering this profession are better equipped with skill and knowledge. Our system must be built in keeping in view the strata of knowledge with which students are seeking entry into the profession. It is also equally important that the role of physiology be redefined in the overall ambit of the degree program.

I am keeping this issue open for deliberation in the symposium. I do hope that SAPI will accept the onus to make ground preparation so that same get due weight age as and when the VCI take up the exercise to revise its Regulations. **Mine are only suggestions but must be analyzed context in of its feasibility and relevance to the ever changing needs. Changes definitely are needed and how this can be brought depends upon our collective wisdom.**

**CONCLUDING REMARK:**

Under the changing scenario, metamorphic approach is needed to bring transformation toward making our system more vibrant & productive, especially in producing professionals, who are competent, self-reliant and possess the capabilities to swiftly adjusting to the changing environment. Change of mind set, coupled with coordinated efforts, public-private partnership & critical assessment of input to anticipated output as well as outcome analysis are the key component of the developmental processes. Both Central and State Governments, the scientific organizations, academies and associations, the Universities & Colleges, industrial houses and other stake holder should work in tandem to revamp the system for faster and meaningful developments of this sector.
Wikipedia defines Education in its general sense “is a form of learning in which the knowledge, skills, and habits of a group of people are transferred from one generation to the next through teaching, training, or research”. Education frequently takes place under the guidance of others, but may also be autodidactic. Any experience that has a formative effect on the way one thinks, feels, or acts may be considered educational.

Education has been divided into different stages as preschool, Primary and Secondary etc and further as formal, alternative, special and vocational categories. In general parlance Veterinary education is one of professional degree, which helps one in serving the society through treatment of precious livestock, which is now shifting to augment production and reproduction that augur well with increased economic benefits to society. One qualify to acquire this degree after senior secondary education.

The History of Veterinary education in India goes back to the time of as far back as 3,000 to 4,000 years ago. In the post Vedic period, the teaching of veterinary medicine was included in the curricula of medical schools. There is evidence of the existence of veterinary hospitals and dispensaries under the rule of Chandra Gupta Maurya (300-298 BC). Veterinarians were called salihotriya, after the famous horse medicine authority Salihotra, in ancient times. Salutri as a designation of veterinarians is derived from salihotriya. It may be said that formal veterinary education in India began in 1862 with the establishment of an army veterinary school in Pune. The first civil veterinary school was started in Babugarh (Hapur), in Uttar Pradesh, in 1877. These schools had the limited objective of training Indians to serve as assistants in remount depots and on military farms. Subsequently, the first veterinary college was started at Lahore, now in Pakistan in 1882. The establishment of a Veterinary research laboratory in India was recommended in 1885 and actually took place in 1889 at Pune, which was later shifted to Mukteswar in the Kumaun Hills of Uttar Pradesh. The Bombay Veterinary College was founded in 1886. In year 1928, Royal commission on Agriculture recommended significant increase in employment of Veteriany Surgeons to combat increasing disease outbreaks and suggested developing degree programmes for Veterinary graduates on English model. In 1936 Madras Veterinary college started Bachelor programme and later on Patna, Mathura, Bangalore, Trichur, Hyderabad, Trupati, Ludhiana and Anand started graduate degree for Veterinary professionals. With passage of time many other colleges came into being. The Curriculum adopted by most of the Colleges was based on recommendations of FAO/WHO with some modifications. After Independence Agriculture universities were opened in different states on land grant pattern and the curriculum were again modified time and again as per recommendations of various committees or local requirements.

As my memory goes regarding teaching of Veterinary Physiology, there were 16 credit hours load with four independent courses in trimester system in 1982, when I joined this profession as student. Total credit requirement for degree programme were 221 credit hours with 19 non credit courses. 10 to 15 years later say in 1995 system was shifted to Semester system, total credit hours requirement of degree programme was reduced to 180 Credit hours, but Physiology was still taught in four courses, besides one shared course with 14 credit hours.

In 1993 came the revolutionary change in form of introduction of Minimum standard of Veterinary education regulation (degree programme) introduced by Veterinary Council of India with 188 credit hours requirement for degree programme and 11 credit hours with four courses in veterinary Physiology. Course on adaptation, Growth and Environmental Physiology was introduced, which gave good impetus towards understanding impact of climate change and adapational features in livestock.
Though there were some mismatch in organising systems in different courses, which were later tried to be improved through modified or new Minimum standard of Veterinary education regulation (degree programme) 2008. In these regulations total credit hours requirement for degree programme was reduced to 177 and 10 credit hours with three courses in veterinary Physiology, besides introducing large number of non credit courses in form of study circle, tracking programme and entrepreneurship programme etc.

Though the change in law of nature and in order to survive one should change with time and requirements of society, but at the same time focus should not shift from our basic aim of producing highly trained Veterinary graduates. The present curriculum needs to be examined from this angle. As regards Veterinary Physiology undergraduate teaching I can say is curriculum contents is being squeezed, courses merged with insufficient credit hours. Teaching of VPB – 221 in present set up is not possible in a normal semester. Hence thought should go in for introducing better/newer contents, to be delivered in innovative way, for which post graduate curriculum needs to be designed and developed on these lines.

As regards Postgraduate teaching of Veterinary Physiology as practised in most of the institutes is more towards research centric and emphasis on teaching acumen is not so strong. At present the New and restructured post graduate curriculum & syllabi developed by Indian Council of Agricultural research (ICAR), 2009 is being followed throughout the country and same is again being modified with increased total teaching credit load.

Basic aim of producing a post graduate is to prepare him/her as good teacher/scientist of Veterinary Physiology, who can think innovative in the subject and able to deliver to the stakeholders. Our Masters and Doctorate level curriculum needs to be planned and developed at higher level considering the importance of subject more over its applications.

The subject of Veterinary Physiology should no more be considered as basic subject to be introduced to students of graduate/postgraduate programme, but with development of upcoming topics like climate change, Embryo transfer technology, use of stem cell in reproduction and cell therapy etc has given the subject a new dimension and accordingly the post graduate syllabus needs to be reoriented.

References


New and Restructured post Graduate curriculum & Syllabi. Education Division ICAR 2009.
Teaching veterinary physiology to the students of first year B.V.Sc and A.H. is a challenge for the teacher as it the first subject taught in the first semester. Therefore it is the prime duty of the teacher to make the subject exciting. There are certain strategies that can enhance teaching and learning process which the teacher has to keep in mind.

Planning of Lesson:
A good teacher always plans a lesson. The lesson to be taught should be well organised. The teacher should prepare the material so that valuable instruction time is not lost in disorganized transition from one activity to another. The topic to be covered in a single lecture should be small, as smaller the material presented to the learner at one time, better is the learning process. The importance of the topic to be taught should be discussed while starting the lecture. The first 10 minutes of the lecture are most important as maximum retention takes place at this time. Teaching process should move from easy to difficult, known to unknown, concrete to abstract, general to specific. The aim should be to increase the level of complexity stepwise to enable the student to understand the topic easily. The topic should be completed within the stipulated time. The teacher should quote examples while teaching.

Confidence:
A teacher requires a sound knowledge in his area of specialization, proficiency in language, habit of reading and a good general knowledge and all this makes the teacher confident. A teacher should be well groomed and speak with full confidence, enthusiasm, humour to make the whole process of learning lively. An effective teacher is the one who adapts to the need of the student in terms of task work and friendly relationship. The teacher should be patient as it is through patience you can make the student learn. A teacher should pay attention to the needs of the slow/weak students and at the same time maintain a balance so that the sharp student is not at the disadvantage.

Faculty to whom the lecture is assigned:
Senior teachers should conduct the classes of physiology in the first semester as their experience will help the students in the learning process. Moreover the young staff involved in teaching physiology should not only use the multimedia mode of teaching as it may sometimes create boredom. Moreover, it is the responsibility of the senior professor that there is cooperation and harmony between the staff members of the department. The same harmony will then be maintained by the students and they will start working in a team. The medium of instruction is English in all the veterinary colleges of India. The students admitted to first year in B.V.Sc and A.H. finds difficulty in understanding the subject as he has passed his H.Sc. examination in regional language. Therefore it is the responsibility of the teacher to speak in Hindi or regional language as well as English so that student feels comfortable with the subject. The class should be assigned to the faculty comfortable in two or three languages.

Modes of presentation:
In the era of information technology, the formal education process is no longer about information, and successful teachers have come to realize it. The role of the teacher now is to help learners effectively identify and evaluate information sources. A number of presentation and animations for one topic should be given to the students because the choice of material will be different according to the psychology of the student. The presentation of the material in the imaged
form can be remembered better than the text form. It is said that “I hear and I forget, I see and I remember and I do and I understand.” Models, charts, plastenated specimens, are the visual aids for effective teaching.

**Emphasis to practicals:**

As most of the students have heard theoretical lectures of human physiology at H.Sc. level it will be better if practical related to the theory classes is conducted within the same or the next day. The curriculum designed as per VCI is also based on emphasis to practical along with theory. As per SPCA most of the experiments on frogs have been banned, therefore alternate arrangement for live demonstration on laboratory animals (one or two demonstrations) may be arranged after getting permission from institutional ethics committee. Moreover the facilities of the ILFC may be utilized for physiology practicals such as respiration rate, heart rate, rectal temperature, collection of blood etc. in different species of animals and in different seasons.

**Group Approaches for enhancing learning:**

Teaching is primarily meant for aiding learning process. Teacher assists the learner to acquire desirable knowledge, skills and attitude by providing the right environment and opportunities for the students to achieve the performance through interactive processes. The teacher has to adapt as per the needs of the students and use various strategies for effective teaching and learning. One such strategy may be group approach. Students enjoy learning in a group because of emotional ties. There may be small or large group. Small group may be friendship group and large group may be the whole class. Students understand the concept and principles better in a small group rather than a large group. The small group may range from 4-10 students. Usually when the students are performing practicals of physiology they are usually in group of 3-4 students. In a small group every student receives attention and gets opportunity for interaction. Studies have shown that small group strategies enhance motivation to learn, develop positive attitude, and improve problem solving skills and helps in better understanding of the course content.

**Case based teaching:**

The students may be assigned detailed case study of the clinical cases attended by them. The case study should include history, clinical examination and laboratory findings. The student should be asked to present the diagnosis, etiology, differential diagnosis, treatment and prognosis. Case studies help in analyzing and exchanging ideas to decision making and management orientation. As per the VCI syllabus there is a course on Veterinary laboratory diagnosis and hence students should be given at least 10 cases per semester. Case study is time consuming and requires high level of attention and concentration.

**Reward to students**

The curriculum of veterinary physiology is quiet vast and many students never get around to actually studying the subjects until a few weeks before the examination, they just resort to memorizing the subject rather than understanding it. Self directed learning can be motivated by keeping quiz once in a month and rewarding the students by giving some gifts or acknowledging them in front of other students. Variation is the law of nature for sustainability and that should be adopted in education system in the form of project assignment, educational excursion, group discussion and problem solving in a team. The winning team or the group should be rewarded.

**Feedback**

Regular feedback from the students is very necessary for the improvement of strategy of teaching. Feedbacks in the form of suggestions, individual comments on feedback forms can be obtained from students. A healthy participation from the student side should be encouraged. There should not be any fear of the teacher so that student comes up with the problems and queries.
Student-Teacher relationship

The relationship between the student and teacher should be cordial. A teacher may become a role model for a student for the entire life if he possess some of the good qualities such as good behavior, understanding students feelings, accepting students idea and guiding him in the right direction. Apart from teaching a teacher must also be involved in the extracurricular activities e.g. Physiology quiz conducted at zonal and national level. Conducting quizzes for selection of the students for zonal physiology quiz will help the student to gain in depth knowledge of physiology. The student will also develop the feeling of competition and the teacher who devotes his time for the same will be respected lifelong. Extracurricular activities also decrease the generation gap between the teacher and the student.

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