



Climate Resilient Livestock & Production System

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Karnal-132001 (Haryana), INDIA



Climate Resilient Livestock & Production System

National Training

(November 18 to December 1, 2013)

of

Division of Dairy Cattle Physiology

National Dairy Research Institute (Deemed University)

Indian Council of Agriculture Research

Karnal-132001 (Haryana), INDIA

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Published by: **Dr. A. K. Srivastava**

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The views expressed in the article including the contents are sole responsibility of the respective authors. The editors bear no responsibility with regards to source, and authenticity of the contents.

Printed by: Intech Printers & Publishers

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FOREWORD

Climate change has become an important area of concern for India to ensure food and nutritional security for growing population. The impacts of climate change are global, but countries like India are more vulnerable in view of the high population depending on agriculture. In India, significant negative impacts have been implied with medium-term (2010-2039) climate change, predicted to reduce yields by 4.5 to 9 percent, depending on the magnitude and distribution of warming. Since agriculture makes up roughly 16 percent of India's GDP, a 4.5 to 9% negative impact on production implies a cost of climate change to be roughly up to 1.5 percent of GDP per year. The Government of India has accorded high priority on research and development to cope with climate change in agriculture sector. The Prime Minister's National Action Plan on climate change has identified Agriculture as one of the eight national missions. With this background, the ICAR has launched a major Project entitled, National Initiative on Climate Resilient Agriculture (NICRA) during 2010-11 for the XI Plan with the objectives to enhance the resilience of Indian agriculture covering crops, livestock and fisheries to climatic variability and climate change through development and application of improved production and risk management technologies. Hence, this training programme on "**Climate Resilient livestock and Production system**" is designed to address some of these burning issues and aims at strengthening our understanding on challenges and opportunities associated with climate change impacts and Climate Resilient Livestock Production system. It is expected to immensely help the researchers and teachers to re-orient and fine tune their research and teaching priorities especially in livestock production system. In this context, the compilation of lectures and practical exercises to be covered during this course shall prove highly informative and useful for the participants of the training in their research and teaching.

I congratulate the dedicated faculty of the National Training Programme for conceptualization, development and organization of this very important training. I also congratulate and welcome all the delegates, who are expected to join this training programme not only for knowledge gain but also for knowledge sharing as well. I convey my best wishes for a grand success for this National Training Programme.

(A.K. Srivastava)

Director cum Vice-Chancellor

PREFACE

There are number of reasons to explore the inter-relationship between climate change and the livestock sector. Firstly, the livestock sector has been blamed for contributing more to global climate change than the automobile industry (FAO 2006). This sector is booming due to a increasing global demand, which is closely linked to both economic growth and urbanization: two factors which are in turn linked to climate change. Thirdly, livestock play a critical role in the livelihoods of many of the world's poorest people, acting as a source of both credit and savings in rural areas, providing food and cash income for the urban as well as the rural poor. Fourthly, livestock systems are credited with providing environmental services, including promoting soil health (total biomass) and thereby helping to capture atmospheric carbon and mitigate climate change. Therefore, there is an urgent need to identify the resilient breeds of livestock to fulfill the demand of milk and milk products for ever growing human population in changing scenario of climate change/ global warming.

National Agricultural Innovation Project (NAIP) funded by World Bank under the Umbrella of Indian Council of Agricultural Research (ICAR), New Delhi initiated the activities for capacity building to undertake basic and strategic research in the frontier areas of agriculture and allied sciences to meet challenges in technology development in the immediate and predictable future. NAIP sanctioned the National Training on "**Climate Resilient livestock and Production system**" in the frontier area of climate change and mitigation strategies. This training is aims to address some of the dimensions of climate resilient livestock and Production system. Therefore, it is an interdisciplinary approach for addressing the trends in climate change, unique characteristics of resilient breeds of livestock, water requirement and water footprints, green house emission and carbon sequestration, vulnerability and likely impacts, methane mitigation etc. The compilation of lectures in the form of book is being brought on this occasion is a collective efforts of the resource persons and publication committee of this national training programme. It would provide useful literature on climate change and production system in India especially on cattle and buffaloes.

We express gratitude to Dr. A.K. Srivastava, Director & Vice Chancellor, NDRI, Karnal for guidance and support for conducting this National Training Programme. Financial support received from NAIP, ICAR, New Delhi for conducting the training programme is gratefully acknowledged. We are grateful to all the speakers who took lot of pain in preparing the lectures and come forward for delivering the talk in time and interacted with the participants. We are specially thankful to the guest speakers who spared the time for delivering the lectures in time.

The support of whole faculty and other staff is gratefully acknowledged. The support and help of Dr. A.K. Singh, Ms. Syma Ashraf, Ms. Lovely Sharma, Ms. Beenam, Ms. Jyoti Dewedi, Ms. Renuka, Ms. Rajani and Mr. Yogendra for smooth conduct of training is gratefully acknowledged.

Last but not the least, we are grateful to the participants who reached at NDRI, Karnal well in time after travelling a long distances and remained away from their family during the period of the training. Thanks are also due to their respective organizations for sparing them to attend the training programme. We are thankful to one and all associated whose names are not mentioned for providing help during this training programme.

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Course Director

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Course Co-Directors

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Indian Livestock Production has Resilience for Climate Change

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In India livestock rearing is a part of mixed farming combined with crop production. Large livestock like zebu cattle are primarily kept for draught purpose and contribute to energy needs of farms. However, some cattle breeds and buffaloes were maintained for milk production, and draught power. Farmers involved in crop-livestock mixed farming maintain 2-3 large animals (cattle and buffaloes). Small farmers and landless particularly low-income groups for supplementing income raise small ruminants, thus more than 80% of small ruminants population is raised by low-income. The pig production is based mostly on scavenging and economically weak section of population rears them. However, the poultry production is mostly in backyard, but in recent years, it has grown and has assumed a status of industry. Therefore, multiple livestock production system of India is not only sustainable but also environment friendly. The mixed crop-livestock integration further avoids human and livestock competition for food and feed ingredients.

1.0 Resilience in Indian livestock:

a) Morphological and physiological adaptation:

Livestock breeds of India have well adapted to soil, plant and climatic conditions that prevail in different agroclimatic zones. Most of the breeds have been named on the basis of their habitat or location to which they have well adapted. One or more specific regions have used these breeds for their improvement.

Zebu breeds have small size and low body weight with small barrel shaped body and slender legs. They have a hump and a dewlap. The head is held high in most zebu breeds. Since most of these breeds have been developed for draught purpose long legs with articulate joints provide ample capacity to run and swiftly move even under moist soils. The balanced fore and hind body quarters help them in propelling body and moving

forward with loads at moderate speeds. Balanced body is mainly due to small size and low volume of internal organs. Small sized rumen, reticulum, omasum and abomasums do not distend down belly of these Zebu draught breeds contrary to heavy bodied Taurine breeds.

The Zebu non-descript cattle of India due to their genotype environment interaction have adapted to thermally stressful conditions by reducing metabolic rate, heart rate and high sweating capacity. In the process of adaptation, characters favorable for low metabolism have been selected. High milk production requires high metabolic activity and more energy expenditure therefore, in tropical conditions livestock species capable of withstanding high heat loads with low metabolism have been preferred. Effect of temperature and humidity on cattle, buffaloes, sheep and goats has been investigated with emphasis on their thermal stability and adaptability. Metabolism of livestock species is affected by ambient temperature rise and humidity levels. The magnitude of response depends upon species, breed and physical environment factors.

The rectal temperature has been considered as an index of thermal balance of animal. The increase in body temperature of animals may be to an extent of 1-2 °C during normal diurnal variations and 4-5 °C during thermal exposure/work in heat. Increase in body temperature over a hot day has been considered a test of adaptability and gross deviation has been attributed to poor adaptation (Rhoad, 1944). Various other physiological processes like respiratory rate and body temperature have also been used in scoring heat tolerance/adaptability (Benezra, 1954).

The necessity of heat loss to maintain thermal balance particularly during hot humid conditions force thermal distressed animals (Taurine breeds crossbreds and buffaloes) to employ open mouth panting with tongue protruded out to complement heat elimination process. Studies on Sahiwal and Sahiwal X Holstein cattle have revealed that during hot dry summers and under direct sun exposure, Sahiwal cattle are able to withstand extra environmental heat loads due to their capacity to increase skin evaporative losses. However, Sahiwal-Holstein crossbreds, in spite of their ability to increase pulmonary and skin evaporative losses, are not able to dissipate extra heat of environmental or work and raise their body temperature too much higher levels. The

Crossbreds exhibit distress symptoms like Open mouth panting, tongue protrusion, profuse salivation and restlessness. The ability of Sahiwal cattle to increase evaporative cooling at higher temperatures without increasing their respiratory frequency much is an important factor in establishing the heat tolerance superiority of the Sahiwal compared with the heat tolerance of Sahiwal-Holstein crossbreds (Aggarwal and Upadhyay, 1997).

Zebu cattle breeds of India have special adaptive mechanisms to deal with extra heat loads of tropical climate. The mechanisms that facilitate easy transfer of heat from body without much loss of moisture are unique in Zebu and other tropical livestock species in addition to mechanisms that conserve energy for body maintenance at high temperatures. Adaptive mechanisms to deal with heat gain and loss are coat colour, length of hair coat, skin pigmentation, number of sweat glands and their secretion. The small body size with low energy requirement for maintenance and capacity to use poor quality feeds and fodders make them superior to many breeds of livestock in efficiency of feed conversion. The water recycling and economy in these animals is much more than give them higher capacity to dehydrate and withstand higher thermal stress. Body appendages and higher body surface area per unit of weight help them in heat dissipation. Some of the Zebu breeds (Tharparkar, Nagori and Sahiwal) well adapted to hot dry desert conditions are able to reduce their metabolic requirements to minimum and conserve energy for diversion to production (milk and /or work) without extra energy expenditure. These mechanisms are rarely found in livestock species located in other areas.

b) Adaptations to the vegetation and environment:

The major feed resources for livestock in India are grasses, agriculture crop residues, cultivated fodders, and leafy fodder from trees. The quantity of fodder obtained through grazing varies in different agroclimatic zones and on the extent of the forest areas. The crop residues of cereal and pulse crops constitute major source of roughages and fodder for livestock. The crop residues are able to meet minimum maintenance need of Indian livestock and but require supplementation with greens and/or concentrate for maintenance and production. There is a deficit of dry, green fodder and concentrates for high producing livestock feeding and more than 20% livestock population do not get enough dry matter for maintenance. Deficit of green fodder, dry fodder and concentrate

in almost all the regions of the country lead to under-feeding of animals that result in to poor growth, meat, milk, fiber and egg production.

Different agroclimatic zones in India have typically adapted livestock breeds of cattle, buffaloes, sheep, goats etc. There is very high variability in feed and fodder supply in these agroclimatic conditions as a result a fluctuation in growth and productivity is observed. Zebu cattle are superior to European cattle in their capacity to digest food because of differences in the rates of fermentation in the rumen. The zebu had higher fermentation rates and could utilize the low protein coarse feeds and fodders that are available under tropical conditions.

Indian livestock species are resistant to certain diseases and less susceptible to others. Cattle and buffaloes are resistant to many parasitic and viral diseases that affect Taurus cattle. Skin thickness of animals and capacity to invade through by many parasites make them more resistant to vector born diseases. Buffalo skin surface attain high temperature during direct solar radiation during summer and rainy season. Long wallowing hours of buffaloes, to alleviate thermal loads, in water and mud kill many ectoparasites. Tropically adapted breeds of goat and sheep also do not suffer from many viral and bacterial diseases. Zebu breeds are tolerant to foot rot and mastitis than European breeds or their crosses. Similarly, zebras have genetic immunity for protozoan diseases like trypanosomiasis, anaplasmosis, piroplasmiosis etc.

India is characterized by dry land / rainfed land, which constitutes about 70 per cent of the arable land. These areas face great instability in crop production due to erratic and inadequate rainfall. In the face of uncertainties in the crop yields, livestock production has been found to provide economic stability to farmers. The dry land / rainfed land can sustain livestock better than crop production. In the arid region, it has been observed that drought occurrence has less effect on dairy production. During a drought year, crop production falls to less than 10 per cent of the production of a favorable year, but the production of milk is still over 50 per cent of that of a good year. Dairy farming by its very nature, therefore, offers a scope for remunerative employment in arid districts of Rajasthan, Gujarat and Haryana. Dairy farming has been recognized as an instrument of drought proofing (Patel, 1997). Arid regions of India are the home tracts

of the best dual purpose/dairy breeds of the country, namely Red Sindhi, Sahiwal, Gir, Tharparkar, Rathi, Kankarej and Haryana, which are well known for their milk production potential and efficiency.

2.0 Efficiency of production system and climate change:

The production efficiency of Indian livestock production in totality is comparable as the contribution of Indian livestock is not only in terms of milk but is also for animal power and majority of farms in India are still dependent on animal power. The resource poor farmers under stressful climatic conditions and difficult terrains use animals for farm energy. People, animals and the natural environment have become particularly vulnerable to the impacts of climate change. The livestock biodiversity and its efficiency are threatened by globalization and climate change but proper management of livestock biodiversity can help in reducing the impacts of climate change on their use and efficiency. Human activities that lead to the degradation of the environment, such as deforestation and overgrazing, can exacerbate the consequences of climate change. In rural and urban areas, people with meager resources, lower income, are forced to live in exposed and marginal areas like floodplains, exposed hillsides, arid or semiarid lands that put them at risk to the negative impacts of climate change. Even a minor variability or change in climate can affect their lives and livelihoods.

3.0 Vulnerability and impact of climate change:

The temperature and humidity above threshold level of tolerance affect all livestock species. Temperature higher than 25°C and relative humidity more than 50% has negative impact on animal productivity. Different livestock species and breeds have different tolerance level for temperature and humidity. Temperature Humidity Index (THI) has been used to relate animal stress and develop algorithms with decline in productivity of milk at different temperature ranges all over world including India (Bohmanova, *et al.*, 2007 Ravagnolo *et al.*, 2008; Upadhyay *et al.*, 2007; 2008). Animals are comfortable at THI between 65 and 72, under mild stress from 72 to 78 and under severe stress above 80. THI map of India has also been developed at NDRI. Analysis of THI in relation to habitat of cattle breeds indicate that indigenous or non-descript animals, due to their

better adaptive capacity and ability to cop up with feed scarcity / harsh environmental conditions, predominantly are distributed in high THI zones.

a) Impact on milk production:

The potential direct effects of possible climate change and global warming on milk production of Indigenous, crossbred cattle and buffaloes were evaluated using widely known global circulation model UKMO to represent possible scenarios of future climate (Ruosteenoja *et al.*, 2003). The recent studies on impact of climate change on Indian livestock under tropical conditions indicate that production of livestock is greatly impacted by temperature variations and rise in temperature.

A small change in THI as a result of rise in temperature due to climate change is not likely to cause much effect on physiological functions as animals have enough capacity to adapt. However, both milk production and reproductive functions of cattle and buffaloes will be adversely affected by projected temperature rise of 2-6 °C over existing temperatures for time slice 2070-2099. The negative impact of temperature rise on total milk production for India has been estimated about 1.6 million tones in 2020 and more than 15 million tones in 2050. The partitioning of milk production impact indicated that high producing crossbred cows and buffaloes will be affected more, accounting 0.4 million and 0.89 million respective annual decline in 2020. Global warming will also negatively impact productivity of indigenous cows and productivity loss will be about 0.33 million tones milk in 2020. The Northern India is likely to experience more negative impact of climate change on milk production of both cattle and buffaloes due to rise in temperature during 2040-2069 and 2070-2099.

A sudden changes in temperature, either a rise in T max during summer i.e. heat wave or a fall in T min during winter i.e. cold wave; cause a decline in milk yield. Both increase in T max ($>4^{\circ}\text{C}$ above normal) during summer and decline in T min ($>3^{\circ}\text{C}$ than normal) during winter negatively impact milk production of crossbred cattle and buffaloes. The decline in yield varies from 10- 30% in first lactation and 5-20% in second and third lactation. The extent of decline in milk yield occurs less at mid lactation stage than either late or early stage. The negative impact of cold wave or heat wave on milk yield of buffaloes are not only observed on next day of extreme event but also on the

subsequent day(s), thereby indicating that heat and cold waves cause short to long term cumulative effect on milk yield and production in cattle and buffaloes. The return to normal milk yield takes 2-5 days normally, however a variable response may also be observed in individual animal depending on stage of lactation. The decline in milk yield and return to normal after an extreme event was also influenced by subsequent day(s) T max and T min. Therefore, global warming due to climate change with increased number of stressful days (THI more than 80) and increase in frequency of warm days will impact yield and production of cattle and buffaloes. A rise in temperature due to global warming will impact high producing animals and they need to be cooled artificially either by sprinklers or by increasing wind velocity or air movement to sustain milk production. High producing crossbred cows vulnerable to thermal stress and unable to maintain thermal balance through skin and pulmonary evaporation requires protection from direct solar radiation during summer with cooling provisions. Increase in incidence of stress days with high heat loads will necessitate higher water use for animal intake and maintenance.

b) Impact on animal growth and reproduction:

The rise in temperature due to global warming will negatively impact growth and time to attain puberty of livestock species. The negative impact of THI rise on animals growing at higher rates (500g/day or more) will be more than slow growing (300-400g/day) cattle. The crossbreds have been observed to be more sensitive to rise in THI than either Zebu cattle or buffaloes. The temperature changes are also likely to affect the normal reproductive rhythm of animals. The reproductive cycles of seasonally breeding domestic animals are closely linked to the rhythmicity of the seasons; climatic changes can cause desynchronization of such events due to responses of pineal-hypothalamo-hypophyseal-gonadal axis that may lead to interdependent pairs of hormonal events therefore a change in temperature with changes in photoperiodicity could lead to reproductive malfunctioning.

Hot dry summers with limited access to water affect buffaloes heat expressions particularly from March to June, when animals have relatively non-functional gonads with less number of sperms in semen of males and poor expression of heat in females

mainly due to higher thermal heat loads that animals are unable to dissipate. Non-availability of water or limited access for drinking and wallowing affects buffaloes during summer. Buffalo heifers have a greater sensitivity to high temperature than other livestock. Therefore, climate change with a rise in temperature and change in precipitation will not only alter reproductive rhythmicity but also affect maturity and reproductive functioning of vulnerable livestock species like buffalo.

c) Impact on physiological responses and functions:

The sensitivity of livestock to increasing ambient temperatures under open ambient conditions and in climatic chamber have been evaluated by exposing Zebu, crossbred cattle and Murrah buffaloes to warm/hot ambient temperature (26-40°C) and low/cool temperatures (6-16°C). Physiological responses, surface temperature and sweating rate increased at high temperatures. Body heat storage based on weighted skin and rectal temperature change indicated that body heat loads of crossbreds and buffaloes increased beyond their capacity to tolerate heat particularly on days, when THI exceeded 80 during summer and hot-humid conditions. The study also revealed that Zebu animals under hot dry/ hot humid conditions have better heat tolerance than crossbreds or buffaloes. The sensitivity of buffaloes to temperature rise above 35°C was observed to be higher than either Zebu or crossbreds.

Since a rise in ambient temperature increases rate of animal energy expenditure/oxygen consumption therefore maintenance of thermal balance during climate change scenario during summer or hot-humid conditions will impact animal functions and productivity negatively. The crossbred cattle and buffaloes are likely to suffer more than zebu cattle at high temperatures and increased heat stress days mainly due to differences in heat dissipation mechanisms and higher water requirements. Increased heat loads, particularly in water scarce situations under climate change scenario, with more frequent warm days with higher thermal load; will impact physiological functions adversely.

d) Animal diseases:

Temperature rise due to global warming is likely to cause an increase in animal diseases that are spread by insects and vectors. Elevated 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 than now. Some of the viral diseases (PPR or RP like 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.

Summary:

The Indian breeds of livestock have resilience for climate change due to their capacity to withstand high thermal stress, feed and water scarcity, diseases and parasite load. The zebu breeds perform across multi dimensions of use. The tropical livestock are more resilient due to their genotype and capacity to interact with environment. Indian livestock have adaptive mechanisms to deal with high heat loads and water scarcity observed in tropical climate. The mechanisms are unique in Zebu and other tropical livestock species that help conserve energy for body maintenance at high temperatures. Adaptive mechanisms to deal with heat gain and loss are coat colour, length of hair coat, skin pigmentation, number of sweat glands and their secretion. The small body size, low energy requirement for maintenance and capacity to use poor quality feeds and fodders make them superior to many breeds of cattle and other livestock in efficiency of feed conversion. The water recycling, capacity to dehydrate and water economy in animals is much more than other animals. Body appendages and higher body surface area per unit of weight help them in heat dissipation and cope with higher heat loads.

Temperature rise due to climate change is likely to impact livestock production and livestock health. The temperature rise will cause a change in composition of species, breeds (quantity, quality) and their mix at farm level due to availability of resources. Adapted species of livestock (e.g. like Tharparker cattle, Murrah buffalo, Jamunapari goats etc) will be able to sustain and single purpose superior breeds with higher productivity will be impacted more. Higher temperatures and prolonged period of stress

may alter diseases and pest challenges. Temperature rise may also cause a shift in habitat of livestock species (yak and mithun). Inadequate resources and infrastructure will put stress on livestock and livestock production system with further substantial increase in stressful days due to climate change. India is likely to face a major water crisis that will severely impact both crop and livestock production system. The biodiversity in crop and livestock with traditional farming knowledge make them resilient and can help cope and adaptation to climate variability and change.

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Impact of Temperature and Climate Variability on Milk Productivity of Bovines

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Dairying is an important enterprise for many countries of the world and is especially important source of income generation for rural families. With increase in population the demand for milk increases (Hammami *et al.*, 2009; Tollens *et al.*, 2004). Local breeds of tropics and subtropics are well adapted to the prevailing environmental stresses but have very low milk production, whereas, on the other hand, the specialized temperate dairy breeds, despite of having their high genetic potential for high milk yield in the intensive systems in which they were selected, cannot survive the harsh tropical conditions, to the point of risk of their survival (Vaccaro, 1990). The expression of the inherit genetics merit varies from one environmental condition to another conditions and is greatly influenced by non-genetic factors (Javed *et al.*, 2002; Das *et al.*, 2003). The performances of temperate genotype in tropical conditions are 30-40% lower than that of the countries of their origin. Environment and genetic (G x E) interaction plays an important role in the expression of the full genetic worth (Misztal *et al.*, 2002; Rivas, 2006; Scholtz *et al.*, 2010). Seasonal heat stress effect on milk production, fertility and reproduction of dairy cattle is worldwide and exert heavy economic loses to the dairy industry (Wolfenson *et al.*, 2000). In lactating Holstein cattle, the comfortable temperature is between the range of 4- 24°C (Hahn, 1981). Heat stress effects on the cattle can be observed above 24°C, and milk yield markedly decreases above 27°C (Jhonson, 1976). Whereas, in most of the tropical and subtropical areas the ambient temperature rises even above 40°C that is obviously suboptimal for temperate breeds cattle to maintain their health and production performance.

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, snowstorms 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 in 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.

Table1. Number of cold waves recorded in different states of India in different years

State	Years and number of extreme events				
	1901-10	1911-67	1968-77	1978-99	1901-99
West Bengal	2	14	3	28	47
Bihar	7	27	8	67	109
Uttar Pradesh	21	51	8	47	127
Rajasthan	11	124	7	53	195
Gujarat, Saurashtra & Kutch	2	85	6	6	99
Punjab	3	34	4	19	60
Himachal Pradesh	-	-	4	18	22
Jammu & Kashmir	1	189	6	15	211

Maharashtra	-	60	4	18	82
Madhya Pradesh	9	88	7	12	116
Orissa	4	5	-	-	9
Andhra Pradesh	2	-	-	-	2
Assam	1	1	-	-	2
Haryana, Delhi & Chandigarh	-	-	4	15	19
Tamil Nadu	-	-	-	-	-
Karnataka	-	10	-	-	10
Telangana	-	5	1	-	6
Rayalaseema	-	3	-	-	3

Source: De *et al*; 2005

Table2. Number of heat waves recorded in different states of India in different years

State	Years and number of extreme events				
	1901-10	1911-67	1968-77	1978-99	1901-99
West Bengal		76	9	28	113
Bihar		105	6	23	134
Uttar Pradesh		27	3	42	72
Rajasthan		43	1	7	51
Gujarat, Saurashtra & Kutch	-	2	-	-	2
Punjab	-	1	-	-	1
Himachal Pradesh	-	-	-	-	-
Jammu & Kashmir	26	5	35	66	
Maharashtra	82	4	13	99	
Madhya Pradesh	32	4	15	51	
Orissa	25	8	18	51	
Andhra Pradesh	21	-	3	24	
Assam	-	4	19	23	
Haryana, Delhi & Chandigarh	-	1	2	3	
Tamil Nadu	5	-	2	7	
Karnataka	-	-	-	-	
Telangana	-	-	-	-	
Rayalaseema	31	2	28	61	

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 evapo-transpiration 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 draught affected state of Gujarat, 18 million cattle out of 34 million were reported to have died 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 draught 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.

Impact of temperature humidity index (THI) and global warming:

Temperature humidity index (THI) is a very useful tool to measure heat tolerance of bovine species. Preez *et al.* (1990) and Johnson (1976) reported that milk production is not affected by heat stress when mean THI values are up to 72. Whereas the milk production and feed intake begin to decline when THI reaches 72 and continue to decline sharply at a THI value exceeds 76 (Molee *et al.*, 2011). Bouraoui *et al.* (2011) reported a reduction (21%) in milk production and DMI (9.6%) when the THI value increased from 68 to 78, milk yield decreased by 0.41 kg /cow/day for each point increase in the THI values above 69. Holter *et al.* (1997) also reported a significant negative correlation between THI and DMI for dairy cattle. All these studies indicated that in tropics and subtropics it is not only the production that is adversely affected but also the survival and adaptability of crossbred and buffalo is at risk. West *et al.* (2003) reported that in the absence of protective measures, global warming could create conditions that not only impair productivity of cattle but can also increase mortality of cattle.

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

There is normally a decrease in milk production for cattle/ buffaloes under heat stress (Singh and Upadhyay, 2008; 2009). This decrease can be either transitory or long term depending on the length and severity of heat stress. The decrease in milk production can range from 10 to >25% (Upadhyay *et al.*, 2009a). If heat stress lowers milk production in early lactation of dairy animals, potential milk production for the lactation will decreased. The negative impact of temperature rise due to global warming on total milk production for India has been estimated about 1.6 million tones in 2020 and more than 15 million tons in 2050. The partitioning of negative impact indicated that high producing crossbred cows and buffaloes will be affected more than Indigenous cattle, accounting 0.4 million and 0.89 million tons (milk) respective annual decline in 2020. The Northern India is likely to experience greater impact of global warming on milk production of both cattle and buffaloes during 2040-2069 and 2070-2099. The decline in milk production will be higher in crossbreds (0.63%) followed by buffalo (0.5%) and indigenous cattle (0.4%) (Upadhyay *et al.*, 2009).

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} ($>4^{\circ}$) than normal during summer and decline in T_{min} ($>3^{\circ}c$) 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 an 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).

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 microenvironment 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 agro climatic 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 α-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) .
- ❖ Selection of heat tolerant animals within breed for future breeding programmes.

Conclusions:

Under tropical and subtropical zones, there is a need to select animal not only for the production genetic merit but also for resistance to environmental and physiological stresses, and for that, it is suggested to model the genetic components to the environmental stress factors for selection. It is assumed that in the near future global warming effect would be more disastrous in the tropics. To avoid this mishap, considering an integrated approach by using improved cooling capability, efficient nutrition formulation and assisted techniques such as proxy indicators for adaptability, quantitative trait loci (QTL), gene chip and marker assisted selection are supposed to enhance the production combined with adaptability of temperate and buffaloes under hot environmental conditions.

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Thermal Stress Depression in Production and Reproduction of Farm Animals

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Growth and reproduction in farm animals are complex, highly integrated processes involving interactions between nutrients and a host of different hormones and their receptors. More than hundred years ago, the Burgman-Allen rule pointed out that there is a tendency for animals living in the cooler regions of the world to have a greater body size and shorter extremities than those in the warmer regions. Animals in the warm regions appear to be lean, elongated, while those in the cold, even when they are littermates of the same body weight, have a round appearance with short limbs and snout. There are both direct and indirect effects of the thermal environment on growth and reproduction. The direct effects are due to air temperature, air movement, solar radiation, humidity and precipitation. Indirectly the thermal environment affects the supply of food through its effects on the growth and quality of plants.

The new born animals:

Relative to body size, heat production in the mammals is high in the period following birth and declines progressively as the animal grows and thermal insulation increases. The susceptibility of the new born to cold gives way to the susceptibility of the mature animals to high temperature. This trend is associated with the thermo-neutral zone moving down the environmental temperature scale, an effect that is much more pronounced in animals that develop thick coats or layers of subcutaneous fat than it is in man. Observations on pigs, lambs, calf and human infant demonstrate the need to consider the animal's critical temperature when estimating the effects of the thermal and environment on survival, growth and production.

The newly born pig lacks thermal insulation of an effective coat and has a critical temperature as high as 34°C. The newly born pig is only about 1% of the mature adult size and it grows rapidly to double its birth weight during the first week. During the same period its body fat content increases from the very low value of about 1-2% at birth to 10%, so that its thermal insulation increases appreciably. Metabolic rate of new born lambs rise soon after birth, associated with the intake of milk and the type of coat. The lamb's thermal insulation is

considerably higher than that of pig. Calves are relatively larger animals with considerable cold resistance; neither the growth rate nor the metabolic heat production of the veal calves is affected by air temperature in the range of 5-20°C. Although beef and dairy calves receive less food, produce less heat and are less cold tolerant than veal calves, the critical temperature is still as low as 8-10°C.

Reproduction:

The patterns of reproductive behavior of farm animals vary with the variation in climatic conditions. Pronounced variations observed in the signs of estrus, rates of conception and frequency of calving are generally attributed to climatic factors. During the four months from February to May, not more than 10% calving take place. Summer sub fertility/ infertility may be due to many reproductive disorders such as i) anoestrus ii) weak or silent estrus iii) anovulatory estrus iv) early embryonic mortality. Estrus in buffaloes can occur all the year round in wild conditions. However in water buffalo the average number of services per conception is 1.46 and the breeding efficiency is 85.80%. Study of slaughter house genitalia of Egyptian buffaloes showed that the percentage of functional ovaries were 99.32, 93.49, 85.22 and 92.29 in winter, spring, summer and autumn respectively. Ovaries and corpora lutea were lightest in summer and graffian follicles were largest in summer and autumn. Persistent follicles 12.55% and smooth ovaries 5.1% have been reported during high temperature. Luktuке reported that 86.04% of the estrus commenced during the morning hours, 9.30% during afternoon and 4.66% during night hours. Duration of heat was 15.6 ± 1.19 hr in animals which came in heat during summer as against 18.8 ± 1.06 hr in winter. Conception rate is highest during the cool season (October to January) and lowest during hot and dry season (May to July). Winter months were most favorable for conception. Mehta reported only 20% conception rate during summer. The number of insemination per conception in winter was minimum (3.1) and maximum (4.5) in summer. Deficiency of green fodder and deteriorating semen quality and neglect on the part of owner may be responsible for lower conception during summer. It was observed that high conception occurred in buffaloes when both the environmental temperature and relative humidity were less. It was seen that the effect of air temperature on conception when RH was held constant was negative. As the air temperature increases the conception decreases and *vice versa*.

Various managerial practices like providing shelter, fan cooling or sprinkling of water have been tried, but these approaches have not met with full success. So, physiological approaches could be thought of as a possible solution to overcome summer infertility.

Different phases of reproductive cycle are regulated by intricate sequential events and interactions between hormone releasing factors from hypothalamus, gonadotropins from pituitary and sex steroids from ovary. Lack of integration of synchronization and hormonal imbalances at any phase of sequence will result in reproductive failure.

Follicular Dynamics:

Follicular dynamics can be summarized as the continuous process of growth and regression of a group of antral follicles, one of which develops to attain pre-ovulation stage. Follicles are formed when primary oocytes are enveloped by a single layer of flattened granulose cells during pre-natal period. Hence, the primordial follicle is a simple structure consisting of primordial oocytes surrounded by a single layer of flattened granulose cells demarcated from the stroma by the non-cellular basal lamina. This primordial follicle reserve formed during fetal life or soon after birth remains in the resting stage until they are stimulated. Follicular growth pattern proceeds through integrated stages of follicular recruitment, selection and dominances. A group of primordial follicles are recruited periodically to grow and pass through a phase of growth till either ovulate or degenerate. Follicular growth is believed to commence in a centrifugal pattern within the ovary.

On examination of cattle ovaries from slaughter house, it was observed that the number of follicles >5mm in diameter were not evenly distributed throughout the estrous cycle but appropriately exposed to two growth waves. The term follicular wave was proposed for this phenomenon. Later on with the advent of ultra-sonography technique to monitor follicle development on daily basis, several workers reported that follicle grow in a wave pattern (Adams *et al.*, 1994; Savio *et al.*, 1988). The follicular wave begins with the recruitment of a cohort of follicles from which a single follicle continues to grow while the other follicles undergo atresia (Wolfenson *et al.*, 1997). Pre-ovulatory FSH release appears to be critical for the recruitment of follicles and the variability in the responsiveness to gonadotropic stimulation among follicles probably determines which follicle continues to grow (selection) from the recruited pool. Selected follicles exert its dominance through inhibition of recruitment of additional follicles for the next wave and the dominant follicle appears to remain active until approximately days 10-11 of estrous cycle (Ginther *et al.*, 1995). Under normal circumstances, the first dominant follicle (DF) regresses and initiation of the second follicular wave results in growth of the second active DF in the ovary. Maturation of the second DF of estrous cycle is often associated with regression of corpus luteum and, therefore, this follicle is ovulated after luteolysis.

A different number of follicular waves can occur in each estrous cycle. Variation in number of follicular waves per cycle is detected; in cattle 2 or 3 wave pattern (Ginther *et al.*, 1995; Bartlewski *et al.*, 2000), goat 2-5 waves (Menchaka and Rubians, 2002; Meidan *et al.*, 2003), buffalo 2 or 3 wave (Baruselli *et al.*, 1997, Lohan *et al.*, 2003). Buffaloes have estrous cycle with one wave (3.3%), two waves (63.3%) or three waves (33.3%) of follicular growth (Baruselli *et al.*, 1997), with the first wave beginning on day 1, the second around day 9-11 while the third wave appeared on day 17 of the estrous cycle. It is unclear whether two or more follicular waves reflect environmental conditions, a casual occurrence or specific characteristics of an individual.

Regulators of follicular dynamics:

The ovarian follicle is a balanced physiological unit whose structure, function depends on extra-cellular factors and complex system of intra-follicular relationship. Follicular development, differentiation and growth are controlled by intra-ovarian, intra-follicular and extra-ovarian endocrine and growth factors (Gurya, 1997). Follicular growth is regulated under the dynamic interaction between FSH and LH produced by pituitary. The pituitary gonadotropins, ovarian steroids and non-steroidal factors interact to regulate the initiation of meiosis in fetal ovary, recruitment of primordial follicles and selection of ovulatory or atretic follicles (Revahand and Butler, 1996). Cyclic growth and atresia of follicles occur during the mid luteal phase which characterized by high levels of progesterone in plasma and the concomitant alteration in gonadotropin production. Studies indicated that alterations in secretion of FSH and LH are related to follicle growth and recruitment of antral follicles in cattle. There is a minimal threshold of FSH concentration below which recruitment cannot proceed. This threshold appears to be variable between animals. A rise in the levels of both the gonadotropin (FSH & LH) prior to ovulation appears to be related to the formation of a large pool of small antral follicles during the post-ovulatory and early luteal periods. It is well established that gonadotropins are needed for the development of dominant follicles and production of estradiol -17- β from these follicles (Driancourt *et al.*, 1993). Mean LH concentration, its frequency and amplitude were significantly higher during the follicular phase than during the luteal phase of the cycle.

All the follicles of a cohort develop in the same endocrine environment, however, the dominant follicle survives and the neighboring sub-dominant follicle dies by apoptosis. It seems possible that individual follicles respond to their endocrine environments, thereby, controlling degeneration or continued growth. Prior to selection, there is a surge of

circulating FSH that stimulates follicular recruitment (Evans *et al.*, 1997) and LH appears to be minimally involved in the control of recruitment. Within 1 to 2 days the FSH levels drop significantly, which is key mechanism in selection. This decline was coincidental with dominant follicle selection, it was believed that the dominant follicle was selected because it contained more FSH receptors and could compete more effectively for gonadotropins than other subordinate follicles (Mihm *et al.*, 1997). Follicles produce intra-follicular growth factors that contribute to the success of folliculogenesis (Webb *et al.*, 1999) and the later stages of follicle development are reliant upon the gonadotropic hormones LH and FSH. There is general agreement that LH is the key hormone involved in the final growth of the dominant follicle while other follicles in the cohort complete atresia. In contrast, estradiol production does not appear to be pre-requisite for follicular dominance. FSH induces aromatase activity within granulose cells resulting in follicles to gain the ability to produce estradiol. It also stimulates the production of inhibin and follistatin. Low serum FSH levels maintained by inhibin and estradiol production in dominant follicle, are believed to be the indirect cause of atresia in the sub-ordinate follicles.

Dominant follicles are exquisitely sensitive to LH, thus it is no surprise that change in the pattern of pulsatile LH secretion may alter their fate. Hence, inducing wave turnover reduction in dominant follicle could be induced by doubling concentration of circulating progesterone, which results in a 50 % reduction in LH frequency. Dominant follicle finally either ovulates (during final wave) or loses its dominance and declines due to the feedback inhibition by elevated oestradiol levels which permits FSH surge and the recruitment of new cohort of follicles. If regression of CL occurs coincident with dominance phase, the dominant follicle ovulates in response to a pre-ovulatory gonadotropin surge. If luteal regression does not coincide with the dominance phase, the dominant follicle undergoes atresia, probably in response to rising titers of intra-ovarian factors involved with atresia.

Heat stress and follicular dynamics:

Heat stress can be defined as the sum of forces external to a homoeothermic animal that acts to displace body temperature from the resting stage. The increase in body temperature caused by heat stress has direct adverse consequences on cellular function. The follicle destined to ovulate emerges as an antral follicle 41.5 days before ovulation. Therefore, heat stress during the period of follicular growth has the potential to compromise the oocyte, either because of direct actions of elevated temperature on the oocyte or because of alterations in follicular function that could compromise oocyte quality. Some investigators employed

ultrasonography to examine follicular growth. Badinga *et al.* (1993) observed that acute heat stress reduced the size of the first wave dominant follicle by day 8 of the estrous cycle and the follicle contained less follicular fluid than that of non-heat stressed cow. Wilson *et al.* (1998) also observed reduction in follicular size in heat stressed cattle and related it to decreased steroidogenesis within the theca cells, granulose cells or both. The possible mediators of heat stress (HS) response were reduced variability of granulose cells or more specific changes in steroidogenic enzyme aromatase activity in granulose cells (Wolfenson *et al.*, 1997).

Badinga *et al* (1994) observed follicular growth in lactating Holstein cows and reported that first follicular wave was characterized by development of a single layer follicle, which was consistently larger in April as compare to June, August and November. The first wave dominant follicle in April was associated with an earlier regression of the largest follicle and a sharper decrease in medium size follicles by day 9 of the estrous cycle. In contrast, the growth of the first wave dominant follicle was slower and largest sub-dominant follicle was more persistent in August than in April, June and November. This resulted in increase in number of medium size follicles between day 4 to 12 of the estrous cycle in August than in April, June and November. Wolfsdorf *et al.* (1997) observed no difference in follicular diameter and follicular fluid of cows during summer, autumn and winter season, but the winter heat stressed cows had low follicular fluid volume than winter control cows.

Wolfenson *et al* (1995) reported decrease number of small follicles to heat stressed cows. Perhaps the population of small follicles is particularly sensitive to physiological changes that occur during heat stress. Approximately 40 days are required for bovine follicles to grow through the antral stage (0.13 to 8.56 mm). A direct effect of heat stress on small follicles may explain why the effects of heat stress on fertility are observed in the late summer and fall. Developing small follicles that are damaged by heat stress during summer may ovulate an infertile oocyte or develop sub-functional corpus luteum.

Wilson *et al* (1998) studied follicular growth from day 11 to 21 of the estrous cycle and reported that heifers subjected to heat stress tended to have smaller second wave dominant follicles than heifers in thermo-neutral environments. The second largest follicle increased in diameter till day 15 in both groups. The size of the follicle decreased rapidly between days 15 to 21 in heifers kept under thermo-neutral environment, however, in heat stressed heifers the follicle size decreased between days 15 to day 17 of the estrous cycle and remained unchanged thereafter. Heat stress compromised ovarian follicular dynamics

(Badinga *et al.*, 1993) and the ability of the dominant ovarian follicle to exert dominance (Wilson *et al.*, 1998). Heat stress induced depression of dominance of the largest follicle of first wave found to be associated with 2-3 days earlier emergence of the second wave dominant follicle / pre-ovulatory follicle (Wolfenson *et al.*, 1995) and the authors suggested that this earlier emergence of pre-ovulatory follicle may result in ovulation of older follicles. The loss of follicular dominance may be related to reduced plasma concentrations of estradiol - 17β and inhibin (Wolfenson *et al.*, 1995) and increased plasma concentration of FSH (Roth *et al.*, 2000). The duration of dominance of pre-ovulatory follicle was found to be negatively co-related with fertility of cattle (Mihm *et al.*, 1997).

Goeloglu *et al.* (2001) reported reduced follicular dominance during a follicular cycle in heat stressed dairy cows. The acute decrease in follicular dominance was reflected by a transit increase in number of class 3 follicles of days 7 and 8 of the follicular cycle that occurred immediately after the period of heat exposure for an 8 day period. The heat stressed induced decrease in follicular dominance as reflected by the acute increase in the number of class 3 follicles, was not reflected in class 2 follicles on day 4 of the follicular cycle. Accordingly, Wolfenson *et al.* (1995) observed an increase in the number of large follicles in heat stressed cows during days 6-10 of the estrous cycle. Collectively, these results and those of Wolfenson *et al.* (1995) and Badinga *et al.* (1994) indicate less follicular dominance in terms of higher environmental temperature. Perhaps this is due to a heat stress induced decrease in inhibin secretion as suggested by Wolfenson *et al.* (1995).

Elevated body temperature may also directly affect follicular function. Culture of theca cells at 40.5°C reduced androsteindione production from cultured theca cells but generally had no effect on estradiol- 17β production from cultured granulose cells (Wolfsdorf *et al.*, 1997). It is not clear whether effects of heat stress on follicular development are sufficient to alter subsequent fertility. Al Katnani *et al.* (1998) reported that cows experiencing air temperature $> 20^{\circ}\text{C}$ on day 10 before breeding had lower 90-d non-return rate than those cows experiencing air temperature $> 20^{\circ}\text{C}$. Torres *et al.* (2008) demonstrated that in Gir cows (*Bos indicus*) heat stress exerted a delayed effect on reproductive functions, manifested by an increased incidence of large follicles, more follicular co-dominance and reduction in estrous cycle length, progesterone concentration and oocyte developmental capacity. Heat stress did not affect follicular recruitment, since the population of > 3 mm follicles and number of cumulus oophores complexes (COCs) not affected. However, heat stress increased the number of large follicles and number of follicles > 9 mm characterized as

follicular co-dominance. Heat stressed induced co-dominance which may compromise oocyte quality has been reported in cow (Sartori *et al.*, 2004) and goats (Vikash *et al.*, 2007).

Conclusion

Heat stress resulted in decreased growth rate, average daily gain and feed intake, oestrus frequency, oestrus duration, calving rate and increased in inseminations per conception. Heat stress inhibited follicular growth and follicular dominance, decrease proestrus rise in estradiol-17 β and induce smaller size of the second wave dominant follicle and longer luteal phase, decrease biosynthetic capacity of theca cells but has less effect on granulose cells. These changes can account for the well documented low breeding efficiency during warm months in sub-tropical environments.

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Thermal Stress Mitigation Strategies for Maximizing Production in Cattle and Buffaloes

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Thermal stress occurs when the ambient temperature lies above thermoneutral zone. Dairy animals have been found to be negatively impacted with increased temperature and humidity. Environmental variables which influence the ambient temperature significantly are- dry bulb temperature, wet bulb temperature, wind velocity and intensity of solar radiation and radiations from surrounding structures in the animal byre. High producing animals are more vulnerable to heat and the problem of thermal stress becomes more acute as the production level increases (Armstrong, 1994). Thermal stress in cow and buffaloes may impair health, decrease in milk yield and time to achieve peak milk production delayed and decreased feed intake. Transition cows are particularly susceptible to infectious diseases and metabolic disorders. Research reports that prepartum cooling consistently decreases rectal temperature, lowers respiration rate, and increases calf birth weight. Cooled cows showed greater lactation persistency compared to non-cooled control cows. Thermal stress during dry period can also affect endocrine responses that may increase abortions and shorten gestation length. In addition, it may also decrease thyroid hormones and placental estrogen levels, while increasing blood non-esterified fatty acid concentrations. The endocrine system is more sensitive to moderate thermal stress during the dry period than during lactation. Prepartum thermal stress affects growth of maternal tissues, influences mammary function after calving, decreases calf birth weight by as much as 10 percent, reduces immunoglobulin content, and lowers nutrient (fat, protein, and lactose) concentration in colostrum. Summer depression of production causes significant economic loss in the dairy industry. Cooling of cows and buffaloes in hot climates is essential to maintain cow health, calf birth weights, and milk production in early lactation.

Development of information system on the animal responses to temperature humidity index (THI) under different agro-ecological zones and allele mining for biotic and abiotic stress are few options, which could determine the type of livestock suitable and adaptable in different agro-ecological conditions. Molecular markers like heat shock proteins (HSPs) to assess the animal response to heat stress are important to study adaptive strategies.

Heat Shock Proteins:

At the cellular level, eukaryotic cells respond to stresses by the production of a specific set of proteins called heat shock proteins or stress proteins, which help the cell to survive thermal stress. Animals have been shown to acquire thermo-tolerance by exposure to a conditioning body heat dose and hyperthermia of whole animals induces the synthesis of stress proteins.

Allele Mining for Biotic Stress:

One of the strategies for combating the impending climate change is to develop a livestock population having high frequency of better adaptive genes for stress and heat tolerance. There are a number of genes that are involved in the thermoregulation and homeostatic mechanism of mammals. There is a need to identify the animals that can tolerate a high THI and identify the candidate genes. Once the candidates are identified markers can be developed that can be included in the breeding programme for adaptive tolerance. Molecular mechanism of animal behavior (stress, reproduction, lactation, feeding, shelter seeking etc.) with advanced tools of nanotechnology and on-field study by telemetry facility, will definitely produce valuable output of research to augment the production and sustainability.

Different methods for reducing thermal stress in dairy animals are:

1. Provision of shade and water:

The animals should be provided with shade and fresh water *ad lib* especially adjacent to the feeding area. Shade can be natural (trees) or artificial in order to cut down direct solar radiation. Direct sunlight adds a tremendous heat load to the cow, but heat energy that is reflected from areas exposed to the sun such as concrete floors, barn walls and other exposed surfaces add to cow's heat load. Shading reduces black globe environmental temperature (a measure of temperature and radiant energy) and lowers the rectal temperature and respiration rate of cows. Painting galvanized steel with white paint reduces the surface temperature. Shades of 2 to 3 meters in height are more suitable for warm, humid climates than the 4 to 5 meter height for dry climates. Regulating air velocity around the animals facilitates heat dissipation as long as the ambient temperature is below body temperature. Dairy cows need approximately 3-4 liters of water per liter milk produced.

The floor area should be larger than the roof area so that the animal can move to avoid direct sun (Bucklin *et al.*, 1991). The minimum height of the roof should be 3.6 m (4.3 m if wider than 12.2 m) for sufficient air movement in the centre of the shade (Buffington *et al.*, 1983). Metal roofs should be painted white on the upper side of the roof and painted black on the underside. The shade decreases the radiation from the sun and thereby the net heat load on the animal, but the humidity and temperature of the air remain the same.

2. Ceiling fans:

In holding areas and free stall shelters the fans should be installed longitudinally, spaced not more than 10 times their blade diameter. They should be located vertically and just high enough so that they are out of reach of the cattle and do not interfere with alley scraping or bedding operations (Shearer *et al.*, 1991). If air is flowing over the animal with a velocity between two and three meters per second, it increases convective heat loss during stressful conditions.

3. Evaporative cooling:

When environmental temperature exceeds cow body temperature, evaporative cooling of the air may be necessary. Air conditioning would be the most effective by reducing air temperature and relative humidity. However, due to energy cost and system maintenance issues, it is not considered as a practical solution on commercial dairies. Low-pressure sprinkler systems, high-pressure misting systems and evaporative cooling pads produce cooling by evaporating water. Evaporation converts liquid water to water vapor. It takes energy to evaporate water and as water evaporates the energy source is cooled. The low-pressure sprinkler system gets the energy from a cow's skin. The high-pressure misting system and evaporative cooling pads get the energy from air. All of these evaporative cooling systems add moisture to the air and raise the relative humidity. Good ventilation air exchange is important to bring in drier air and avoid excessive relative humidity levels. High-pressure systems operate at water pressures 200 psi or higher. Higher water pressures can produce smaller droplets. Avoid letting water pressures decline. At low water pressures, droplet sizes increase so that they do not evaporate before reaching the ground where they can wet bedding or feed. High-pressure system nozzles can be attached to fans that direct the mist or they can be located in the inlet side of a cross-ventilated barn or above head locks. High-pressure systems require clean water and in-line filters to minimize plugging of

the high-pressure nozzles. Low-pressure systems operate at water pressures between 20 and 40 pounds per square inch (psi) and use nozzles that have flow rates near 0.5 gallons per minute.

4. Cooling ponds:

Animals in pond loses heat primarily by conduction, since the animal surface is in contact with water but during 5-10 minutes after exiting the pond, a small amount of heat is lost by evaporative cooling (Shearer *et al.*, 1999). The man-made pond has a continuously inflow of water with an overflow at the end of the pool. Buffaloes, because of their black skin and far less number of sweat glands than cows, especially like to wallow in water for alleviation of heat stress (Chauhan, 1998; Anjali and Singh, 2008). Cooling ponds should be located close to a water supply, as fresh water needs to enter the ponds constantly. Cooling ponds should not be too far from the milking parlor as long walks in the sun increase heat stress.

5. Passive solar cooling:

In temperate and hot climates, solar heat infiltrating the building has typically been the most costly thing to mitigate (i.e. air conditioning). However, effective passive solar cooling design can eliminate much of this conventional operating cost with proper building design. Passive cooling includes –overhangs for south-facing windows, few windows on the west, shade trees, thermal mass and cross ventilation. Preventing excess solar heat from entering the building envelope can help in reduction of heat load on the animal.

6. Night grazing:

When the cattle were allowed out into a pasture at night, the physiological responses (rectal temperature, respiration rate and pulse rate) decreased immediately as a result of the reduction in radiant heat during night from the surroundings and the rise in heat loss from the cattle.

7. Feeding during summer:

A proper emphasis should be given to feeding of animals during summer so that they eat to appetite and produce more milk. Generally, there is a chronic shortage of green fodders in months of May-June and the animals are fed dry materials, like straws and stalks (Kadbi) of Bajra, sorghum and maize. They have poor nutritive value, lower the productive capacity, increase internal heat of fermentation and reduce fertility in animals. In order to obtain maximum milk yields a concentrate mixture containing 16% DCP and 70% TDN along with palatable, digestible and laxative fodders should be provided to the animals. Multi-cut fodders like guinea

grass, napier, bajra hybrid can be maintained for milk production throughout summer. Mixing of cowpeas to the above fodders benefit additionally to overcome the deficiency of protein. Conserve surplus oats as silage in March and Berseem as hay so that they can be used during scarcity period of green fodders for cheap milk production. As a homoeothermic measure animals tries to get rid of excess heat or cut down heat production in the body by reducing feed intake and its utilization. Feeding schedule should be shifted to early morning or late evening during summer. Also, soak the concentrate in equal amount water for 20-30 minutes, which helps in better utilization of nutrients and reduces dustiness in concentrates. Mix smaller meals and feed more often during the day to keep feed fresher while encouraging intake and rumen stability. Heat stressed cows don't like eating but they require more energy from their diet to maintain body temperature. At the same time, nutrient availability is limited as heat stress redirects blood flow to the skin and away from the stomach, mammary glands and uterus. Therefore, feeding high quality forages with better taste and higher digestibility is very important during the summer. Highly palatable forages encourage intake, and higher digestibility means more relative feed value (energy) with less metabolic heat generated in breaking it down. Digestive aids like yeast cultures help break down fiber in the rumen, reduces the metabolic heat produced and helps stabilize rumen microbial populations.

High temperature, moisture and humidity are the basic needs for growth of moulds and other undesirable organisms, which produce mycotoxins and other substances that are toxic to the animals. So, store the feeds and feed ingredients in dry, cool and ventilated go downs. After harvesting, the green fodder should not be left in a heap for longer periods to avoid initiation of anaerobic fermentation and hence to spoilage of fodders. Do not feed moldy feedstuffs/fodders to animals.

8. Thermal stress and antioxidants:

Heat stress is associated with reduced activity by antioxidants in the blood plasma. Vitamin E (α -tocopherol), a strong reducing agent that can give electrons to lipids undergoing peroxidation, is a major antioxidant present in plasma membranes. The treatment of cows with antioxidants to improve fertility in summer has given inconsistent results.

Effects of antioxidants on reproductive function may be more pronounced during heat stress because of the increased metabolic rates associated with cellular hyperthermia. Elevated

temperature increases liver peroxidation (Ando *et al.*, 1997) and activity of enzymes involved in free radical production such as xanthine oxidase. Exposure of dairy cows to heat stress decreased total antioxidant activity in blood. Like most cells, pre-implantation embryos can produce free radicals (Yang *et al.*, 1998). Multiple vitamin E and selenium injections failed to improve reproductive function or milk yield in lactating Holstein cows. In this respect, results are in agreement with others in which injection or feeding of supplemental vitamin E, selenium, or vitamin E and selenium failed to affect reproductive function (Kappel *et al.*, 1984) or milk yield (Baldi *et al.*, 2000). In contrast, other studies have reported a beneficial effect of supplemental vitamin E or vitamin E and selenium on fertility (Arechiga *et al.*, 1998b; Baldi *et al.*, 2000) and milk yield. Much of the variation in results among studies may be due to differences in dietary content of vitamin E and selenium, as well as timing, dosage, and method of administration of supplemental vitamin E and selenium. Other antioxidant treatments were without effect on fertility of heat-stressed dairy cows including injection of 3000 IU of vitamin E at the time of AI and multiple injections of β-carotene (Arechiga *et al.*, 1998b). Feeding supplemental β-carotene to heat-stressed cows had only slight effects on reproductive function (Arechiga *et al.*, 1998a). Studies indicate that oxygen consumption of lactating cows actually decreases during heat stress, probably because feed consumption and milk yield is reduced, and such an effect would decrease free-radical production.

9. Effect of evaporative cooling on animal health and production

9.1 Immunity: Cows that were actively cooled during the dry period had lower circulating prolactin compared with heat stressed cows, and the lymphocyte expression of prolactin receptor was increased (do Amaral *et al.*, 2010). Cooled cows had greater lymphocyte proliferation than heat stressed cows indicating improved immune status. Cooling also increased the capacity for neutrophils to destroy bacteria through the process of oxidative burst generation (do Amaral *et al.*, 2011). Thus, cooling dry cows improved the ability to resist infection in early lactation. With regard to acquired immune function, cow's ability to generate antibodies to a specific non-self protein, i.e. ovalbumin was studied (do Amaral *et al.*, 2011). Cows were injected with ovalbumin early in the dry period and then received boosters of ovalbumin through the dry period and into lactation. Cooled cows had greater ovalbumin IgG responses relative to heat stressed cows, but that effect was evident only during the dry period indicating that the two arms of the immune system act differently in response to environmental factors. That is, the acquired immune

function appears to be influenced during the dry period, whereas the innate system is affected during the initial stages of the next lactation. In both cases, however, improvements in immune status would result from cooling of dry cows.

9.2 Milk production: Based on studies, there is compelling evidence that cooling dry cows improves milk yield in the next lactation. In addition, those cows that are cooled are better able to navigate the metabolic challenges of early lactation, and have improved immune status at a time of significant risk for disease. The approach to cooling is the same as that for lactating cows but should be implemented as early in the dry period as possible to maximize the benefits to the cow as the cow transitions into lactation. Milk yield is a function of the number of mammary cells and the relative metabolic activity of those cells. Although an increase in either cell number or activity can increase yield, an increase in cell number would be expected to cause persistent increments whereas an effect on metabolism may more transient.

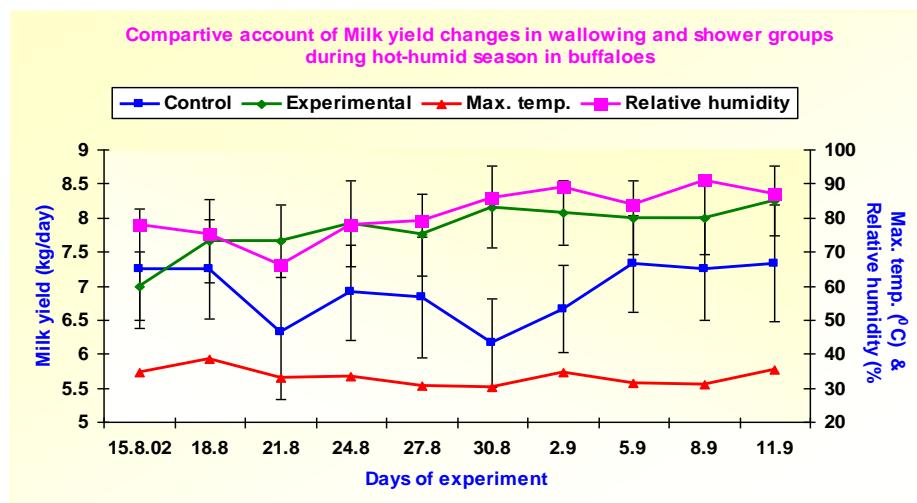


Figure1: Milk yield in buffaloes kept under water showers and allowed for wallowing

Using a serial mammary biopsy technique, mammary samples from cows during the dry period and into early lactation were collected, cows were cooled or heat stressed when dry (Tao *et al.*, 2011). Relative to heat stressed cows, an increase in the proliferation of mammary cells in cooled cows during the transition was observed, which indicates greater capacity for milk synthesis. Another factor that influences total mammary cell number is the rate of loss of cells, or apoptosis. There was no difference in the rate of apoptosis between heat stressed and cooled cows. Therefore, cooled cows have a greater net gain in secretory capacity during the dry period, and that is expressed as higher milk yield in lactation. In a study, while comparing two different

cooling systems, one group of buffaloes in early lactation were kept under water showers and other group was allowed to wallow in a water pond. It was observed that wallowing resulted in significantly higher milk yield as compared to the group kept under water showers (Figure 1; Anjali and Singh, 2008).

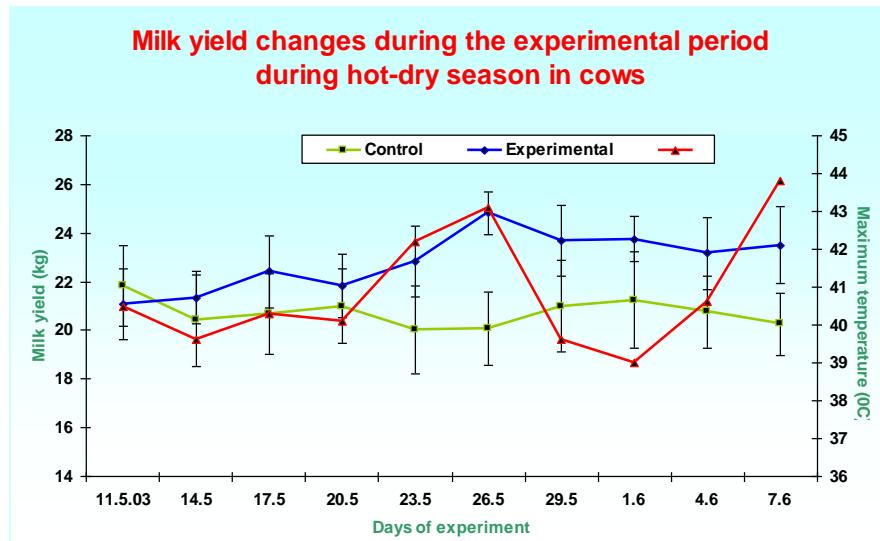


Figure 2: Milk yield in cows kept with and without mist and fan cooling system

In another experiment in early lactating cows, one group of cows was kept without providing any cooling and the other group of cows was provided with mist and fan cooling system. Cooling of cows during hot dry season significantly increased milk yield (Figure 2; Anjali, 2004).

Conclusions:

The development of a strategy for the prevention of production diseases in dairy cattle relies upon the use of optimal nutrition and management. Thermal stress generally increases the production of free radicals, leading to oxidative stress. In dairy cows, oxidative stress has a negative impact on immune and reproductive functions, increased chances of mastitis and higher somatic cells counts in milk, increased embryo mortality, post-partum retained placenta, and early calving, with consequences on the calves live weight, mortality and health. The existence of numerous antioxidants that can function at both the hydrophilic (cytosol and extracellular fluids) and lipophilic (membrane lipids) phases is essential to maintain tissue integrity during oxygen metabolism. Other antioxidant nutrients also may influence immunity independent of their antioxidant roles. Cows need to consume a certain quantity of biologically available

minerals and vitamins to maintain optimal status. When cows are below optimal status, supplementation of a biologically available form of the nutrient elicits a positive response. Evaporative cooling systems improve the environment for lactating dairy cows and buffaloes in arid climates and the reduced air temperature results from the removal of heat energy required to evaporate water. The fan cum mist cooling system is very effective during the hot-dry climate when temperature may rise as high as 46°C but, effectiveness of evaporative systems in climates with high relative humidity is limited. Sprinkler and fan cooling systems generate a large volume of waste water which must be processed. Mechanical cooling or air conditioning is generally considered too expensive in animal housing.

Selection of Livestock Breeds for Greater Tolerance to Heat Stress

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The best method of reducing the impact of the stressors to improve productivity and animal welfare is to breed cattle that are productive in their presence, without the need for managerial interventions.

Dr Heather Burrow

The livestock in the tropics adapt well with the heat stress than the breeds native to tropical climate. The breeds that are more tolerant to heat stress are likely to sweat and drink more in hot weather than the other breeds (Espinoza *et al.*, 2009). Livestock genetic erosion is major criticized aspect in the present era of crossbreeding to improve the genetic merit of the tropical cattle. The alternate approach that's being suggested is to go for the selective breeding. Low reproductive rates and high calf mortality reduce the intensity of selection. The other major constraints that limit the selective breeding in the Indian tropical cattle are the long generation interval, late sexual maturity and the long calving intervals in most tropical breeds. The extensive milk recording schemes which support dairy cattle breeding programmes in many temperate countries are almost non-existent in the tropics. In this situation, the most realistic approach to improvement the Indian cattle breeds can be through selection and may be started with a single nucleus herd (or a group of cooperating herds) (Syrstad and Ruane, 1998).

Global warming has risen the surface temperature about 0.7°C since the early 20th century, about 0.5°C of this increase is since 1978. Climate change is one of the most potentially serious environmental problems confronting the global community. High environmental temperature exerts a negative influence on the performance of livestock population (Liu *et al.*, 2011). By 2100, the temperature will be about $1.4 - 5.8^{\circ}\text{C}$ over 1990 levels (IPCC, 2007).

The taurine group is adapted to temperate environments and the zebu cattle are adapted to tropical environments. The tropical environment is characterized by stress factors; notable among them is the high temperature which can lead to heat stress and thus affecting the

performance of the animals. Livestock resources when maintained under suitable conditions bring about maximum utilization of the natural resources and there is a close correlation between an animal's physiological response and productivity. Environmental temperature is most important in determining which type of animal can be maintained in a particular region (Finch, 1986). The productive process of an animal is disturbed when it moves away from its ambient body temperature. Even small upward shifts in core temperature have profound effects on tissues and neuro-endocrine functions, which in turn reduce fertility, growth, lactation, and ability to work (Finch, 1986).

Turner and Schleger (1960) indicated that a sleek coat may have a greater significance as an indicator of metabolic efficiency or of a capacity to react favorably to stress. Mader *et al.* (2002) demonstrated that coat color also had a large impact on the heat tolerance of cattle. Presently researchers started to look at the existence of a gene(s) associated with heat tolerance in slick haired *Bos taurus* cattle. A method to estimate genetic merit for thermotolerance was developed by Hayes *et al.* (2009) in Australia and the heat stress index may be used to select bulls for thermotolerance.

Dikmen *et al.* (2012) estimated the heritability of rectal temperature under heat stress conditions and the values ranged from 0.13 to 0.17. This value is relatively low compared to a trait like milk yield, where heritability is ~0.30 (Pritchard *et al.*, 2013), but it is high enough to allow selection for rectal temperature. Hammond *et al.* (1996) investigated rectal temperature as an index of heat tolerance in Senepol, Brahman, Angus, Hereford, and crossbred Senepol cattle under summer conditions in subtropical Florida and found that Angus females always had the highest RT and Senepol cows the lowest. Rectal temperature (RT) and respiration rate (RR) are the most sensitive indices of heat tolerance among the physiological reactions studied (Verma *et al.*, 2000). Change in rectal temperature has been considered an index of heat storage in large animals and other mammals. Under the heat ameliorating measures, reduction in rectal temperature (about 0.4°C has been reported in Murrah buffaloes (Rahangdale *et al.*, 2010) under hot summer season.

Skin temperature have been reported to fluctuate much more in buffaloes (0.6°C) than in tropical cattle under increased ambient temperature. The greater sensitivity of the buffaloes to hot conditions especially when exposed to direct solar radiation could be due to the dark body,

lesser density of sweat glands and the thick epidermis which reduce the capacity of cutaneous evaporation. Moreover, the water excretion rate through urine in buffaloes is higher than in cattle and that buffaloes have a greater dependence on external water because of the evolutionary adaptation of buffaloes to wet environments (Koga *et al.*, 2004). Diurnal variation in skin temperature of different body parts in response to heat stress in male buffalo calves was reported by Das *et al.* (1999) and at middle neck region, it has been reported to vary between $29.66 \pm 0.17^\circ\text{C}$ at 06.30 hrs and $35.75 \pm 0.38^\circ\text{C}$ at 18.30 hrs. Saravanakumar and Thiagarajan (1991) characterized the sweat gland and skin qualities of epidermis and total thickness and also compared the heat tolerance ability in Murrah, Surti and non-descript buffaloes and found that Murrah was least heat tolerant compared to Surti and non-descript.

Seath and Miller (1946) observed that increased respiration rate is the first reaction when animals were exposed to environmental temperature above the thermo-neutral zone. Increase in respiratory frequency may be used as an index of discomfort in large animals (Gaalas, 1945). Bhatnagar and Choudhary (1960) found that the combination of relative humidity and air temperature caused variation in body temperature and respiration rate of animals, whereas the relative humidity caused variation in pulse rate. Das *et al.* (1999) reported that the respiration rate in buffaloes found to vary diurnally 11.25 ± 0.38 at 06.30 hrs and 19.00 ± 1.48 at 18.30 hrs. At higher temperature e.g. 41/ 31°C (DB/WB), the respiration rate rises rapidly to about three to four times the normal values (Chaiyabutr *et al.*, 1987).

Exposure of animals to thermal stress leads to the elevation of pulse rate with concomitant decline in feed intake and production (Kabuga, 2005). Increase in pulse rate of Murrah buffalo heifers from 39 ± 1.00 to 70 ± 4.00 beats/min after exposure at 45°C for 4 hours in a climatic chamber has been reported by Patir and Upadhyay (2007) indicating that exposure was stressful. Reduction in pulse rate to normal due to heat ameliorating measures has been reported in lactating Murrah buffaloes (Aggarwal and Singh, 2008).

India is currently losing nearly two per cent of the total milk production, amounting to a whopping over Rs 2,661 crore due to rise in heat stress among cattle and buffaloes because of the global warming (Upadhyay, 2010). Majority of places in India observe THI > 75 and more than 85% places in India experience moderate to high heat stress during April, May and June (NDRI Vision 2030, 2011).

Genes and thermo-tolerance:

Nearly 50 genes not traditionally considered to be HSPs have been found to undergo changes in expression in response to heat stress (Sonja *et al.*, 2002). Unraveling molecular changes associated with seasonal acclimation will offer new insights into selecting domestic animals for thermal tolerance. Selection within breeds for thermoregulatory characteristics would be a sound strategy for increasing productivity in the tropics (Finch *et al.*, 1986).

Genetic polymorphism studies would facilitate to identify genes which control part of the variability of the phenotypic traits. Second approach focuses on the analysis of the genetic polymorphism by the candidate genes and its association with the desired trait.

Global warming has a great impact on productivity of livestock, especially on buffaloes due to their sensitivity to temperature changes. Moreover, Murrah buffaloes are the commonly preferred and high milk producing animal. The authors have undertaken the present investigation to study the effect of thermo-regulatory genes (ATP1A1, ATP1B2 and Osteopontin genes) on the physiological parameters in adult Murrah buffaloes. Physiological parameters were recorded *viz.* respiratory rate, pulse rate, skin temperature and rectal temperature. Genomic DNA was isolated using standard procedure of proteinase-K digestion (Sambrook *et al.*, 1989). The ATP1A1, ATP1B2 and Osteopontin genes were amplified by the primers already reported by Liu *et al.* (2011), Wang *et al.* (2010) and Tantia *et al.* (2008) respectively. The DNA sequence data were analyzed using Chromas (*Ver. 1.45*, <http://www.chromas.html>). Multiple sequence alignments for identification of SNPs were performed and were selected for further genotyping by using high-throughput MALDI-TOF mass spectrometry.

The significant effect of genotypes on physiological functions was analyzed using the following model:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where,

Y_{ij} =Physiological parameter of jth animal belonging to ith genotype

μ = Overall mean,

P_i = Effect of i^{th} genotype ($i = 1$ to --)

e_{ij} = Random error associated with Y_{ij} observation, and assumed to be NID ($0, \sigma_e^2$)

Statistical analysis was carried out using PROCGLM of SAS version 9.3 to find out the association between the polymorphic SNPs of ATP1A1 gene.

Nine SNPs were identified in the three genes taken in the study. All the nine SNPs were genotyped using high-throughput MALDI-TOF mass spectrometry. The SNPs at position 19333 (G/T) of ATP1A1 gene, 2775 (T/G) of ATP1B2 gene and 2387 (C/T) of OPN gene have been found to have significant association with physiological parameters (RR & PR) may be used as markers in the breeding programs.

GENE / PARAMETERS	ATP1A1 (g.19333 G>T)	ATP1B2 (g.1457 A>G)	ATP1B2 (g.2775 T>G)	OPN (g.2387 C>T)
RECTAL TEMPERATURE	NS	NS	NS	NS
RESPIRATION RATE	S (P<0.05)	NS	S (P<0.01)	S (P<0.05)
PULSE RATE	S (P<0.01)	NS	NS	NS
SKIN TEMPERATURE	NS	NS	NS	NS

The DNA based Sequenom assay developed in the study may be used for screening the buffalo populations for the presence or absence of alleles for thermotolerance. More SNPs need to be identified using either conventional method or whole genome sequencing and association with physiological parameters should be explored. The data generated can be included in the SNP chip development for screening the herd.

With the advent of genotyping and sequencing technologies in agriculture, genome-wide exploration of the genetic basis for the differences in tropical adaptation has only just become possible. Variation in rectal temperature has been used to identify genetic markers that predict thermotolerance on the Illumina Bovine SNP50 BeadChip (Dikmen, Cole, Null and Hansen, unpublished). The Bovine SNP50 BeadChip is a genetic tool to estimate an animal's inheritance of specific mutations at 50,000 places on its chromosomes and make it possible to identify genetic markers that predict thermotolerance. Selection for these markers could lead to an increase in thermotolerance without having adverse effects on milk yield. Selection for heat

tolerance is possible by study of variation within and between the breeds to reduce the negative effects of heat stress on performance traits of livestock.

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Role of Animal Skin in Thermoregulation

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Introduction:

The impact of weather affecting physiological adaptations and behavior are on the immediate vicinity in animals. Global climate change requires diversity of livestock breeds that withstand greater extremes in temperature and rainfall (Seo and Mendelsohn, 2007; Hoffmann, 2010). Species level assessment showed that 31% of cattle breeds are currently at risk and already extinct (FAO, 2009). However, there is evidence of genetic differences within ruminants with respect to heat tolerance and this may provide clues or tools to select productive and thermotolerant subjects (Bernabucci *et al.*, 2010). Livestock genetic diversity can provide insurance against changes in production circumstances, new diseases, or changes in market demands. The increasing concern with the thermal comfort of animals is justifiable for countries occupying in both tropical zones and temperate zones (Nardone *et al.*, 2010). The situation is worrying in the developing world where rapid changes in production systems are leading to the replacement of breeds or crossbreeding. India is currently losing nearly 2 % of the total milk production due to rise in heat stress among cattle and buffaloes because of the global warming (Upadhyay, 2010). In India more than 85 % places experience moderate to high heat stress (THI>72) during April, May and June (NDRI Vision 2030, 2011). There is therefore an urgent need to document the diversity of our livestock genetic resources and design strategies for their sustainable conservation.

Animal adaptation is a broad term used to describe the ability of animals to adjust to environmental conditions or to infer genetic modifications that make animals more suitable for existence under specific environmental conditions. Integumentary system of animal has multiple roles in homeostasis, including thermoregulation, sensory reception, biochemical synthesis, absorption and protects the entire body from thermal damage, mechanical damage, chemical damage, ultraviolet radiation etc. Heat and cold receptors are located in the skin to perceive thermal changes in environment. When the body temperature rises, the hypothalamus sends a

nerve signal to the sweat-producing skin glands, causing them to release the sweat for cooling the body. The hypothalamus also helps dilation of the peripheral blood vessels in the skin to allow more blood flow to help heat convection away from the skin surface. When body temperature falls, the sweat glands constrict and sweat production decreases. *Zebu* cattle are widely recognized as adaptable to tropical and subtropical environments that are restrictive to *exotic* and *crossbred* cattle. We supposed that there are some differences in the physiological role of skin in different breeds of animals. Therefore, an important understanding on physiological role of skin will help in modulating heat tolerance and improve functions under different heat load conditions.

Structure and function of the skin:

The skin, sometimes known as the integumentary system is, in fact, the largest organ of the body and protects animals from external influences. It has a complex structure, being composed of many different tissues. The integument of an animal is in direct contact with its environment. It affords mechanical protection of the soft tissues and also acts as a barrier against infection and physiological stresses encountered in its habitat. It performs many functions that are important in maintaining homeostasis in the body. Probably the most important of these functions is the control of body temperature. The skin also protects the body from physical damage and bacterial invasion. The skin has an array of sense organs, which sense the external environment. Skin serves as a barrier, a temperature regulator, it has a prominent role in immune regulation, and it functions as a cushion for external mechanical loads (Ebling *et al.*, 1992). Skin properties of an animal may change in relation to the environment (Hildebrand, 1988). The skin is one of the first systems affected when an animal becomes sick so it is important for anyone working with animals to have a sound knowledge of the structure and functioning of the skin so they can quickly recognize signs of disease.

The skin consists of mainly the layers: the epidermis, dermis and hypodermis. Epidermis and dermis separated by a basal lamina mainly consist of collagen VII (Haake *et al.*, 2001; Palson and Bhatia, 2004). The third part of the skin, the hypodermis is crucial in controlling the temperature and nutrient storage, as well as secreting important hormones such as leptin (Frank *et al.*, 2000).

Epidermis:

The epidermis is the avascular outer layer of skin and constitutes about 95% of keratinocytes that produce a tough protein called keratin, which function as a barrier to protect from harmful substances and also prevent water and other essential substance from escaping the body. The epidermis is 50-150 μm thick, depending on the part of the body and skin type (Lewis *et al.*, 1994). It consists of a multilayered sheet of keratin synthesizing cells, called keratinocytes. In between those cells melanocytes, Langerhans cells and Merker cells are present. The keratinocytes are distributed in layers in order of increasing differentiation starting from the basement membrane zone, located immediately on top of the dermis (Humbert and Agache, 2004; Lewis *et al.*, 1994; Mitchell *et al.*, 1999). New skin cells that help to replace the older cells, are produced at the basal membrane and push the older cells towards the surface (Fairley, 2001). The keratinocytes that get older and migrate to the skin's surface changes their shape from square-shaped to flat, and become engorged with keratin and eventually die, losing all of their internal structures. The other epidermal cells are melanocytes, which function to produce melanin for the purpose of ultraviolet protection; Langerhans cells, in the skin serve an immune function, and Merkel cells serve as mechanoreceptors. The normal epidermis also contains appendages such as hair follicles, sebaceous and sweat glands.

Dermis:

The dermis or the “true skin” is thicker than the epidermis and lies immediately underneath the epidermis (Ebling *et al.*, 1992; Humbert and Agache, 2004). The dermis is composed of collagen rich connective tissue that contains fibroblast cells that produce collagen and elastin, which are responsible for the pliability and strength of skin (Humbert and Agache, 2004). The mechanical properties of the dermis are primarily determined by the supporting extracellular matrix. The main components of this matrix are proteins such as collagen, elastin, fibronectin, and proteoglycans (Mitchell *et al.*, 1999; Prydz and Dalen, 2007). The dermis is structurally organized into the uppermost papillary and the innermost reticular dermis (Lewis *et al.*, 1994). The sub-papillary plexus, a horizontal plane of vessels, constitutes the boundary between the papillary and reticular regions, and sends capillaries toward the epidermis, which furnish nutrients to the epidermis (Marieb, 1997). The papillary dermis is a narrow zone immediately under the basement membrane zone of the epidermis. Directly underneath the papillary dermis

the reticular dermis is situated. This part contains most of the dermal collagen, organized into coarse bundles, cross-linked with one another and with elastic fibers (Daamen *et al.*, 2007; Tzaphlidou, 2007). The majority of cells found in the dermis are the fibroblasts (Ebling *et al.*, 1992; Lewis *et al.*, 1994). Fibroblasts synthesize the extracellular matrix proteins such as collagen, elastin and proteoglycans. Characteristic for these cells is that, if their activity is stimulated, for instance by heat, their endoplasmatic reticula and ribosomes become well developed and they start to synthesize heat shock proteins (Snoeckx *et al.*, 2007). Compared to the reticular dermis, fibroblasts are more abundant in the papillary dermis and have higher proliferative and metabolic activity at this location. The reticular dermis is more fibrous and mainly composed of collagen. The connective tissue of the dermis gives the skin its pliability, elasticity, tensile strength and protection from mechanical injury.

Hypodermis:

The hypodermis lies below the dermis. There is an abrupt transition from the reticular dermis to the hypodermis, or subcutaneous tissue, rich in adipose tissue. However, structurally and functionally the two regions are integrated through nerve and vascular networks, as well as epidermal appendages. The adipocytes are organized into lobules separated by septa of connective tissue that contain nerves, vessels and lymphatics. The subcutaneous tissue insulates the body, provides for energy, and allows for mobility of the skin on underlying structures (Palson and Bhatia, 2004).

Dermal fibroblasts:

Dermal fibroblasts are the most common cells of dermis that synthesizes and maintains the extracellular matrix of skin. Fibroblasts secrete the precursors of all the components of the extracellular matrix, primarily the ground substance and a variety of fibers. The composition of the dermal extracellular matrix determines the physical properties of the skin. The dermal fibroblast population is not as homogenous as previously thought. Sorrel and Caplan (2004) describe differences between papillary fibroblasts, which reside in the superficial dermis, and reticular fibroblasts, which reside in the deeper dermis. Active or young fibroblasts are recognized by their numerous rough endoplasmic reticulum, a feature of protein secreting cells (Kierszenbaum, 2002; Midwood *et al.*, 2004). The capacity of the skin fibroblasts to grow,

remodel the matrix, secrete and degrade proteins, and produce numerous growth factors or cytokines is crucial for the regulation of the tissue structure and its cellular microenvironment.

The main function of fibroblasts is to preserve the structural integrity of connective tissue through constantly secreting precursors of the extracellular matrix. Dermal fibroblasts are an essential component of skin; they not only produce and organize the extracellular matrix of the dermis but they also communicate with each other and other type of cells. Fibroblasts also engage in paracrine and autocrine interactions in skin (Kondo, 2000; Werner and Smola, 2001). Dermal fibroblast cultures provide a powerful tool for investigating the molecular mechanisms that regulate skin behavior (Kondo, 2000; Werner and Smola, 2001).

Animal-Environment interactions:

The components of thermal environment include air temperature, relative humidity, radiation, and wind speed. Living objects interact with these environmental conditions in complex ways.

Physiological responses observed- when animals are exposed to thermal stress include body core temperature, skin temperature, sweating rate, respiration rate, feed intake and, production performance. The immunity and animal behavior also changes under thermal stress (Hillman, 2009). Animal to relieve heat stress utilize various behavioral and autonomic thermoregulatory mechanisms. A heat stressed animal may seek shade, change its orientation to the sun, and increase water intake (Blackshaw and Blackshaw, 1994).

Skin temperature is dynamic, which is affected by physical, environmental, physiological factors and hair coat's optical properties. One of the physiological factors that influence skin temperature is evaporative cooling. This occurs when sensible heat on the skin surface is converted to latent heat. Evaporative cooling is affected by wind velocity, relative humidity, and thermal and solar radiation. Other factors that affect the efficacy of evaporative cooling from the skin surface are hair coat physical and optical characteristics. Hair coat density and thickness, hair length and color, and skin color are also factors that influence evaporative cooling. For example, black, hair and skin enhance solar absorption and thus increase heat load on the skin surface. Other properties such as hair coat density obstruct evaporation of water from the skin surface by trapping a thin film of water at the skin-hair coat interface. Gebremedhin *et al.* (2008, 2010) reported variation in sweating rates of different breeds of high producing dairy and feedlot heifers in their natural habitats. There is also considerable variation in sweating rates between cows of the same breed (Gebremedhin *et al.*, 2010), which is attributed to genetic variation in thermo regulation.

Role of integumentary system in homeostasis:

The integumentary system has multiple roles in homeostasis, including thermoregulation, sensory reception, biochemical synthesis, absorption and protects the entire body from thermal damage, mechanical damage, chemical damage, ultraviolet radiation etc. Heat and cold receptors are located in the skin to perceive thermal changes in environment. When the body temperature rises, the hypothalamus sends a nerve signal to the sweat-producing skin glands, causing them to release the sweat for cooling the body. The hypothalamus also helps dilation of the peripheral blood vessels in the skin to allow more blood flow to help heat convection away from the skin surface. When body temperature falls, the sweat glands constrict and sweat production decreases.

A continuous fall in body temperature induces thermogenesis by shivering. The skin also helps in excretion and acts as a mini-excretory system for urea, salts and water. The skin also synthesizes vitamin D and manufactures several proteins important for immunity. The tiny sensory receptor like touch, pressure, temperature and pain receptors provides a great deal of external environment information (Marieb, 1997).

Environmental adaptation in *Zebu* cattle and buffalo:

Properties of the skin help in achieving the thermo-tolerance in *zebu* cattle. The tissue resistance to heat flow from the body core to the skin was lower for Brahman cattle than for Shorthorn cattle at high air temperatures (Finch, 1985). The physiological basis for this difference has not been identified. In *Bos indicus*, high density of arteriovenous anastomoses have, lower resistance to flow than vascular passages involving capillary networks, facilitate increased blood flow to the skin during heat stress (Hales *et al.*, 1978). Many *Bos indicus* breeds have smooth and shiny hair coats, which reduce heat exchange *via* radiation (Finch, 1984; Hutchinson and Brown, 1969). In cattle, the hair clipping reduced the magnitude of hyperthermia in response to heat stress (O'Bannon *et al.*, 1955). While *zebu* cattle have reduced resistance of heat flow from the body core to the skin than European breeds, the converse is true when considering flow of heat from the skin to the surrounding air. The vascularity and degree of insulation of the skin and quality of the hair coat (hair and skin coat color, thickness and hair density) also contribute to the effectiveness of heat loss in cattle (Gebremedhin *et al.*, 2008; 2010). The actual rate of heat loss *via* sweating depends not only upon the extrusion of water at the skin surface but also upon the evaporation of that water. It has been observed that evaporative heat loss rates were less affected by humidity for Indicus cows than for Holstein and Brown Swiss cows.

Bonsma (1973) expanded *Zebu* attributes to include coat color, pigmentation, conformation, genetic adaptation to the source of nutrition (forages) and resistance to pests and disease. The implications are that *Zebu* cattle are adaptable to poorer quality forages and soils of low pH and are resistant to ectoparasites and diseases transmitted by ectoparasites. *Zebu* cattle of hot climates have acquired genes that protect cells from the deleterious actions of high temperature (Hansen, 2004). The *zebu* genotype has been utilized for improving cattle for beef and dairy production in hot climates with limited success. Therefore, identification of the genes conferring cellular thermo-tolerance offers the possibility of transferring these genes to heat-

sensitive breeds to improve reproduction and other physiological systems compromised by hyperthermia.

When the body's heat production is stable, the blood flowing into the dermis is regulated depending on changes in ambient temperature. The purpose of this regulation is to ensure that the difference in temperature between the skin's surface and the environment remains constant, thus regulating heat loss and maintaining an almost constant body temperature. The extent of heat lost from the skin is dependent on the amount of blood flow to the dermis. During heat stress, blood flow to the dermis also increases, which, in turn, increases the heat lost from the skin to the same rate as the excess heat production and *vice versa* during cold stress.

The study of animal skin demonstrates the peculiar adaptation of skin and organs to environment. Turner and Schleger (1960) indicated that a sleek coat may have a greater significance as an indicator of metabolic efficiency or of a capacity to react favourably to stress. Mader *et al.* (2002) demonstrated that coat color also had a large impact on the heat tolerance of cattle.

The greater sensitivity of the buffaloes to hot conditions especially when exposed to direct solar radiation could be due to the dark body, lesser density of sweat glands and the thick epidermis, which reduce the capacity of cutaneous evaporation (Koga *et al.*, 2004). Diurnal variation in skin temperature of different body parts in response to heat stress in male buffalo calves was reported by Das *et al.* (1999) and at middle neck region, it has been reported to vary between $29.66 \pm 0.17^{\circ}\text{C}$ at 0630 hrs and $35.75 \pm 0.38^{\circ}\text{C}$ at 1830 hrs. Saravanakumar and Thiagarajan (1992) characterized the sweat gland and skin qualities of epidermis and total thickness and also compared the heat tolerance ability in Murrah, Surti and non-descript buffaloes and found that Murrah was least heat tolerant compared to Surti and non-descript.

Our research on dermal fibroblast of cattle and buffalo:

Although dermal fibroblast cells constitute a major portion of skin, the information on fibroblasts and its response to heat stress, particularly on the molecular behavior of dermal fibroblast in livestock is not available. To understand the dermal fibroblasts responses upon heat-stress, a study was conducted in our laboratory to establish dermal fibroblast cell culture of cattle (Tharparkar, Sahiwal, Karan-Fries cows) and Murrah buffalo and assess the effect of heat stress on the gene expression, apoptosis, biochemical and enzymatic characteristics. Results indicating

that there are species and breed differences in cellular stress markers (ROS, NO, Cytotoxicity and IL-6), Antioxidants (SOD, Catalase, GPx, GR and Glutathione). These differences are important for certain aspects of species and breed differences in resistance to thermal stress. The results also showed that heat stress affect expression of a significant numbers of genes (HSP70, MMPs, iNOS, Caspase and Bcl-2 family) of diverse biological functions and apoptotic responses to thermal stress. Tharparkar and Sahiwal showing high basal levels of HSP70, Low level of inducible MMPs and iNOS which indicate more thermo-tolerance at given temperature than Karan-Fries and Murrah buffaloes. The lower expressions of Caspase genes (Caspase-3 & 7), less reduction in Bcl-2/Bax gene ratio, lower activities of Caspase enzymes (Caspase-3, 7, 8 & 9) and lesser number of apoptotic nuclei in Tharparkar and Sahiwal than Karan-Fries and Murrah buffaloes indicate that Tharparkar and Sahiwal are more thermotolerant as compared to Karan-Fries and Murrah buffaloes.

Conclusions:

From the results obtained during the present study, it may be concluded that there are species and breed differences in cellular stress markers, antioxidants, significant numbers of genes (HSP70, MMPs, iNOS, Caspase and Bcl-2 family) of diverse biological functions and apoptotic responses to thermal stress. The present research pin pointing the local role of dermal fibroblast cells in thermoregulation. Present research also revealed that skin of Tharparkar and Sahiwal are highly adapted to thermal stress than Karan-Fries and Murrah buffaloes. Comparative study of the heat stress to dermal fibroblast of different cattle breeds and species can lead to the identification of unique stress defence mechanisms. Therefore, further detailed studies are required to understand their functional role in livestock during heat stress.

Acknowledgements:

The authors express sincere thanks to the Director, NDRI, Karnal for providing necessary facilities for research. The financial assistance for the research work was provided from National Initiative on Climate Resilient Agriculture (NICRA) project of Indian Council of Agricultural Research (ICAR), New Delhi. The authors acknowledge the able technical help provided by J. Choudhary, S. Panda, V. Anand, and A.P. Singh.

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Role of Small Ruminants for Livelihood Security under Changing Climate

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Introduction:

Livestock sector plays an important role in the rural economy at national level and this sector is emerging as a driving force in agriculture by contributing towards the income and employment of rural households. The livestock share to the agricultural GDP has increased from 14 percent (1980-81) to 23% (1997-98) and further increased to 32% in 2001-02. Sheep and goats represent second and fourth most numerous livestock species in the world. Small ruminants in the country contribute 4.8 and 3.4% of the world meat and milk production. Small ruminant sector plays an important role in the rural economy of the country as more than 70% of the landless, marginal and small farmers are involved in its farming and the population growth has been substantial compared to large ruminants. The sheep population in the country has been gradually increasing at the rate of >1% annually and would be around 92 million in 2030 from 71.6 million in 2007. Similarly, the goat population growth rate of India varies from 0.94 to 5.13% with an average of 3.55% during 1951-2002, which may reach to around 170 million at the rate near 1% growth (Prasad, 2011).

Significance of small ruminants in Indian economy:

In small ruminant sector the shepherd communities mainly depended on sheep rearing for a source of livelihood and income generation, this community is generally economically poor, uneducated, socially backward and migratory. This sheep sector ensure them self-employment and family labour under extensive system and act as cushion at the time of drought and famine. Sheep flocks act as movable assets of high liquidity and a source of household nutritional food security. Sheep husbandry contributes 48.5 kg wool, 263 m kg mutton, 56.3 m kg skin and 190 m kg manure (GOI, 2010). In national economy, it contributes Rs 3518.4 crores through meat, Rs 683.8 crores through offal, Rs 231.8 crores through skin, Rs 9.77 crores through blood, Rs 226 crores from wool, Rs 682.1 crores through manure, Rs 82.1 crores through increment in stock totaling to Rs 5434 crores. Sheep husbandry provides export earning of Rs 250 crores from meat and animal casing and processed meat. Woolen industry provides employment to about 2.6 million people and earns more than Rs 5000 crores of foreign exchange through exports. Mutton is an important

component in sheep husbandry, it was 125 m kg in 1980-81, 230 m kg in 2001-02 which will be around 356 m kg by 2030 and during the same period mutton requirement in the country would be 579.5 m kg indicating a deficit of 37.2%.

The goat population over the past few decades has increased 197.7% (from 47.2 m in 1951-52 to 140.5 m in 2007). Combining the annual growth rate with mean slaughter rate around 41 % and mortality rate of about 15%, goat have shown the potential population growth of above 58% per year. According to Compound Annual Growth Rate (CAGR) of sheep and goat during (1992-2003) in the country, shows that their population has been increase at a fast rate in some of the districts (Kumar *et. al.*, 2010). This is a main factor that makes goat as most desired species of animal for meat production in the country. The goats and their products contribute Rs 17661.7 crores annually to the national economy and sheep contributes to 5434 crores. Thus, the annual contribution per animal works out to Rs 1407 for goat and Rs 845 per sheep. Among different products, meat accounts for largest share i.e. 50% of the total value of goat products and 65% of the total value of sheep products. Indian Council of Medical Research (ICMR) recommends 30g of meat as against the present availability 15g/head/day. There is wide gap between the requirement and the availability as the present production is meeting only 50% of requirement. Demand for the livestock based food is higher and would like to increase in comparison to food grains. The quantity of wool production varied widely in different regions of the country. As per recent estimates, 11.42 m kg of fine wool is produced in the north temperate region, 22.77 m kg of ideal carpet wool in the north western region, 11.55 m kg of very coarse /hairy and coloured fleece in southern region and 2.08 m kg of hairy types and unsuitable for woollen textile is produced in eastern region.

In India the average wool per sheep is very low (0.7 kg) against the world average of 2.70 kg. The country's wool requirement would not match with the current production trend and thus the gap of raw wool (220 m kg by 2030) will have to be met through imports or by increasing production. The challenges for research and development is to initiate integrated approaches of breeding, health, feeding management and marketing in cost effective manner for enhancing wool and meat.

Economics of small ruminant rearing:

In comparison to large ruminant the initial investment for small unit of sheep/goats are modest with quick returns due to fast multiplication. Studies on this issue have shown that

sheep and goats are economical in arid and semi-arid regions. It was reported that a goat in Jodhpur (Rajasthan) provided net income of Rs 250 per year/goat (Ghosh and Khan, 1980). Pasha (1991) reported a net income of Rs 114 per small ruminant per annum. Acharya and Singh (1992) highlighted the crucial role of goats in livelihood security of resource poor rural households. Deoghare and Rekib (1996) found that the farmers could earn a net income of Rs 331 per goat/annum and Rs 74 per sheep/annum. Kumar and Deoghare (2002) estimated actual cost of rearing which was Rs 395 per annum with net returns of Rs 1126 per goat /annum in Mathura district. Shinde *et al.* (2003) have shown goat rearing was also profitable on organized farms. The income generated from goat rearing was estimated at Rs 1.29 in first year and Rs 1.36 in the second year per rupee of investment. All above studies indicate that rearing of small ruminant is quite remunerative with higher profitability in goats compared to sheep.

Although the goat and sheep both are well adapted to a variety of agro-ecological region there is marked variation in their density among different states. Among them, goat are more widely distributed (Rath, 1992). The density of goats per sq km was highest in West Bengal (212) followed by Tamil Nadu and Rajasthan. Whereas the density of sheep was highest in Andhra Pradesh with 78 sheep/km sq followed by Tamil Nadu, Karnataka and Rajasthan.

In a study Kumar and Pant (2003) demonstrates that average size of holding and percent net irrigates area were negatively associated with density of goats validating the general perception that goats are associated with marginal and small farmers and provide livelihood to the people in rain fed regions. However, the associates between goat density and area under pasture/waste lands were positive, which indicates the role of common property resources (CPRs) in small nutrient production system. The CPRs comprise of barren and uncultivable lands, cultivable wastes, permanent pastures and other grazing land and land under miscellaneous trees, crops and other fallow land. Despite of critical role of CPRs in livelihood security of rural people, the area under CPRs are continuously declining (Jodha, 1986). The sheep and goats are blamed for this deterioration but the reality is that poverty and demographic pressures on the land drive environmental degradation through deforestation, overgrazing, overstocking and indiscriminate exploitation of fragile marginal ecosystem. Further, the increase in occurrence of drought and famine frequency has worst the situation in availability of good quality forage biomass. Moreover, in the wake of climate scenario the extremes of hot and cold climatic conditions directly affect the health and productivity of

small ruminants under extensive system of rearing. Considering the above mentioned opportunity and challenges in small ruminant rearing, there is need to assess the effect of extremes of climate on animal productivity and way out to resolve the problem of environmental stress through shelter management.

Effect of environment stress on animal physiology and productivity:

(1) Animal physiology:

A major part of arid and semi-arid region is characterized by harsh environmental conditions, poor quality of water and forage, higher variation in air temperature etc. Small ruminants of this region are mostly maintained on extensive grazing on pasture/rangelands, where these animals were not provided any kind of shelter during the grazing/browsing, but in extremes of summer and winter animals seek shelter and physical protection. Extremes in the environmental conditions can have enormous effects on physiology and productivity of farm animals (Singh, *et al.*, 2001 and Singh and Upadhyay, 2009). Patel *et al.* (2009) analyzed the stressful and comfortable period based on weekly Temperature Humidity Index (THI) values for Central Farm of CAZRI, Jodhpur, the stressful period for animals starts from May to Ist week of October, where THI ranged from 79 to 83. Moreover, the comfortable period was found to start from IInd week of October to March, where the values were found equal to or below 74. The cold stress period was also observed in this region from IInd week of December to IIIrd week of February during night and early morning hours, especially during the time when there were cold waves. The average THI values of morning and evening were 61.62 and 74.75 for winter; 75.58 and 83.81 for summer dry and 77.59 and 83.70 for summer wet seasons, respectively. A long period of summer, monsoon and post monsoon are stressful seasons in arid and semi-arid regions. Animals should be protected from direct solar radiation during the hottest hours of the day to ameliorate the effect of heat stress (Shinde, *et. al.*, 2002).

Adaptability of small ruminants for water restriction: The small ruminants of desert were observed for unusual ability to maintain circulation even when faced with considerable haemo concentration. When water was reduced to below 75 % of normal daily requirement, there was a steady decline in the body water stores. On an average, there was an 18 % loss in body weight after remaining without water for 3 days during winter and 25 % during summer. Interestingly, the digestibility of crude fibres was found to be increased in water-

restricted sheep while nitrogen balance was not to be affected due to water stress. The rate of passage of feed is slow in water-restricted sheep in comparison to normally hydrated animals.

The goat breeds of Beetal and Black Bengal were divided into 2 groups, in one group water was not withheld, whereas it was withheld for 36 h in other group before exposure to thermal stress ($43\pm0.5^{\circ}\text{C}$, RH 55%). Increase in all the three body responses was seen under thermal stress. Maximum respiration rates were observed when goats attained rectal temperature between 40.3°C and 40.5°C . The rectal temperature level at which maximum respiratory frequency was observed was lower when the animals were under combined stress of thermal exposure and water restriction. Significant differences were observed between breeds and groups in pulse and respiration rates (Kaushish *et al.*, 1987).

Responses of sheep to solar exposure: Hogget of native Nali, Chokla and their crosses with Rambouillet and Soviet Merino were maintained in shade or sun during summer. The respiration rate, rectal temperature, skin temperature and fleece temperature 1 and 2 cm above the skin were higher in the solar exposed group as compared to the animals maintained in shed. In general, the rectal temperature of the crossbred animals was lower than the native; lower in Merino x Nali and highest in Nali. Animals maintained under hot sun experienced greater stress as compared to the animals under shade. It also appeared that in crossbreds the thermolytic mechanism was activated at a lower ambient temperature resulting in their higher respiration rate and lower rectal temperature (Kaushish *et al.*, 1989). The physiological response *viz.* respiration rate and heart rate were lower in sheep provided shade and nutritionally restricted or fed ad lib in comparison to those, which were exposed in sun (Hooda and Naqvi, 1990).

Genetic variation in heat stress adaptation: The sheep is globally distributed as per the different climatic conditions. About 42 breeds of sheep are reared in almost all climates of the country ranging from cold climate to hot and dry climate of north western part and hot humid climate of eastern part. Climate plays an important role in nature of produce from sheep. Prolific sheep breeds in hot and humid coastal region of West Bengal and Odisha, excellent carpet wool breeds in hot and dry western part of Rajasthan, fine wool breeds in cold and dry temperate climate, and mutton breeds in hot and humid plains of southern states evolved through the process of adaptation. Open fleece in hot and humid region facilitates the dissipation of body heat while close fleece in temperate region helps in conservation of body heat. Similarly, prolificacy traits in coastal sheep breeds are associated with higher temperature coupled with humidity. The adaptation of sheep breeds to different

location/climates depends upon temperature, humidity, vegetation and wool cover and resistance to various diseases. Sheep breeds can tolerate wide range of climate and convert poor quality forage into wool and animal protein. These characters favour their rearing under extensive system among rural people in harsh climate (Karim and Shinde, 2007). Farmers prefer sheep breeds which can withstand thermal and nutritional stresses and able to walk long distance during migration. Magra breed found in and around Bikaner district produce excellent carpet wool with fibre diameter of 32-35 μ and lustrous character. Farmers are taking initiative for adopting breeding strategies to cope with changing climate. Kheri sheep, developed by crossing Malpura and Marwari, are hardy, produce carpet type wool, have better walking efficiency and thrive well under migratory system. In changing feed resources, climate and market demand, breeding strategies of farmers for animals are changing for better sustainability and productivity (Shinde and Sejian, 2013). In the recent past, more efforts were made to develop prolific sheep breeds in country for enhancing mutton production. At CSWRI Avikanagar threebreeds cross of Malpura, Garole and Pattanwadi is under progress, which can produce twins/triplet with higher milk yield and higher body weight and can withstand the harsh climate. Therefore, the Sheep rearing is economical even in drought and famine by reducing its flock size and migration and withstands its adverse effect.

(2) Animal Productivity:

Growth performance: The body weight gain in growing age is an important aspect for any domestic animal reared for economic benefit. The climatic stress particularly due to heat during growth period of animal reduced the body weight gain. Higher body weight gain was observed in the 6 month old male kids of Marwari breed, when reared under closed type Improved Animal Shelter during summer months. Kids gained 9.52 kg body weight in Improved Animals Shelter than 7.52 kg in Traditional Animal Shelter during 6 months period of experiment from May to October (Patel *et al.*, 2007). The month of birth of lambs affects the body weights, lambs born in January to February were heaviest at 6 months body weight (30.6+0.93 kg) with decreasing trend in lambs born in subsequent months i.e. March to December time period (Poonia, 2004). Harsh environment conditions of arid zone coupled with low forage availability in grazing area affected the body weights of different goat breeds of semi-arid region. The lowest body weight of adult goats (22.8 kg) was observed in Parbatsari breed followed by Jhakrana and Jamunapari. However, highest body weight (31.0 kg) was maintained in adult Marwari goats, which was native of this region (Patel, *et al.*, 1998). However, the supplementation enhanced the higher body weight gain in Jhakrana

(54%), followed by Jamunapari (53%), Parbatsari (47%) and lowest in Marwari (16.2%). Rohilla and Khem Chand (2004) also observed a significant growth in Marwari kids when they were supplemented with concentrate feeds at daily and alternate day in arid conditions.

Milk production performance: The milk yield of all mammalian species undergoes seasonal variation. The optimum environmental temperature for lactation depends on the species, breed and degree of tolerance to heat or cold. The milk yield of Holstein cattle declines at temperature above 21°C, in case of Brown Swiss and Jersey cattle it declines at about 24 to 27 °C whereas milk yield of Zebu cattle declines only above 34°C. Observations in India on cross-bred cows has shown that the productivity of cross-bred of exotic with indigenous breeds were not seriously affected by the hot dry climate in north western parts.

Shinde *et al.* (1990) observed that milk yield per day decreased with simultaneous increase in ambient temperature and humidity. Dutta *et al.* (1995) reported that temperature and humidity together explained 62.75% of variation in milk yield/cow/day. Thermal stress influenced the milk production in cattle (Singh and Upadhyay, 2009). The Karan Fries showed higher sensitivity for temperature rise. When THI crosses the index value of 72, impacted milk production negatively, the average milk production of Karan Fries and Sahiwal animals at around THI of 72 was 13.4 and 6.6 litre/day. The respective milk production was decreased to around 9 and 5 litre/day in Karan Fries and Sahiwal cows at around THI of 82. The increase in each unit THI impacted in reducing the milk production by 0.43 and 0.16 litre/day/animal, respectively in the range of 72-82 THI. The 305 days lactation yield was influenced by season of calving in Tharparkar cattle, it was higher in winter season calvers (1438.8 litre) than summer season (1274.9 litre) in arid condition (Patel *et al.*, 2000). However, the milk fat content of Tharparkar cattle was found to be lowered in both hot dry (4.30%) and hot humid season (4.64%) in arid zone whereas, higher percentage of fat was observed during Autumn (5.24%) and winter season (5.25%) (Patel *et al.*, 1994). Though the Marwari goat breed of Thar Desert are well adapted against desert vagaries, but the productivity of these animals affected adversely due to extremes of climatic conditions. Marwari goats yielded lower lactation yield during winter when housed in open type house (thatched roof animal shelter) in comparison to closed type pucca animal shelter in arid region. These goats yielded 38 litre more milk in a lactation period of 154 days with higher milk fat 4.77 % (Patel *et. al.*, 2001). In a another study they found 22% more lactation yield in Marwari goats when these were housed in closed type Improved Animal Shelter than open type Tradition Animal Shelter during winter season (Patel *et al.*, 2007), which could be due

to complete to protection of goats against cold weather in closed type Improved Animal Shelter winter season.

Reproductive performance: Climate change plays a critical role in reducing the reproductive efficiency on sheep and goats. In a study of effect of thermal stress on superovulatory response, the occurrence of estrus was late ($30.6+1.2$ h) with shorter estrus period ($31.7+3.6$ h) in a group of ewes exposed to thermal stress in comparison to group of ewes which were maintained under shed (Naqvi, *et al.*, 2004). However, the thermal protected ewes exhibited estrus earlier ($25.5+1.1$ h) and the estrus period was longer ($37.7+1.6$ h). Further, in another study Naqvi *et al.* (1999) concluded that estrous synchronization success percentage was more in winter than in summer season in Kheri sheep. Maurya *et al.* (2005) also observed significant effect of heat stress in sexual behaviour of Malpura ewes. The heat stress affects the spermatogenesis and fertility in rams. Testicular and scrotal measurement and sexual behaviour of rams was also found to be affected by heat stress (Naqvi *et al.*, 2010). The season of calving influenced the reproductive performance in bovine. Lower calving interval period was observed in Tharparkar cows, which calved in winter season (Patel *et al.*, 2000), and Surti buffaloes calved in rainy season had lower service period and calving interval (Patel and Tripathi, 1998).

Climate change and sheep diseases:

Climate changes reduce the natural vegetation in grazing lands leading to metabolic diseases of varying nature. Nutritional problems may be feed and water scarcity, mineral imbalances, poor intake, high-energy loss leading to low body weights, metabolic disturbance, ketosis, secondary metabolic diseases. These situations may cause physiological changes like reduced body fat depots, disturbed reproductive cycles, poor immunity, debility, stunted growth, still birth, abortion, water toxicities (nitrate, nitrite, fluorine etc). Poor availability of green grasses, other than monsoon season increases the deficiency of vitamin A, D, and B₁ minerals (Shinde and Sankhyan, 2007). The increased morbidity and mortality and declined production under climate change leads to economic losses to farmers (Singh *et al.*, 2010). Erratic rainfall leads to emergence of haemorrhagic septicaemia, pneumonia, skin and foot diseases. Early embryonic losses increase in sheep bred during summer due to higher temperature and feed scarcity. Nutritional and thermal stress causes stunted growth and neonatal losses. The availability of safe water is declining rapidly in desert areas it contains fluoride, nitrate and TSS more than tolerance levels which affect health, production and reproduction of animals (Mittal and Ghosh, 1984).

Mitigation strategies for climate change effect:

The probable effect of climate change would be reducing production and reproduction performance of small ruminants and it may also affect their health adversely. Hence, it is imperative to concentrate on reducing the effects of climate change. The mitigation strategies can be grouped in three themes namely genetic, management and nutritional strategies (Shinde and Sejian, 2013). The different strategies available to counteract climate change effect on sheep productivity are as follows.

Genetic intervention: The sheep sector is relatively small in comparison to other livestock species as the certain communities are involved in sheep rearing. However, the promotion of sustainable sheep production will be vital to ensure that the impact of climate change is minimized on the sheep farmers. This will involve rearing of animals, which are more sturdy, heat tolerant, disease resistant, and relatively adaptable to the adverse conditions (Moran *et al.*, 2006). Indigenous breeds as Chokla, Malpura, Marwari, and Magra are well-adapted to hot arid ecology would be better choice in arid and semiarid region of the country. Certain sheep breeds are resistant to diseases and parasites in comparison to others. The resistant line does not require drenching and reduces the problem of drug resistance, drenching cost and drug residues in meat and milk (Swarnkar *et al.*, 2009). This breed may be multiplied and propagated in the region. Identification of thermo tolerance specific genes to makes it possible to select for thermal resistance (Khoei *et al.*, 2004).

Management strategies: The animals should be raised in the optimal thermal zone for welfare point of view. The implementation of the knowledge, for the welfare of animals maintained under extensive management systems, is difficult because of objective limitations to monitor heat stress and economic compulsions in applying measures to ameliorate heat stress (Hansen and Arechiga, 1999). The following recommendations are general rules that can be applied under extensive conditions: (i) provision of shade shelter in areas where typical ambient temperature during summer exceeds the normal (ii) provision of water at reasonable distance between watering spot and grazing area (Hahn *et al.*, 2001, Collier *et al.*, 2003). Shade against direct solar radiation can be provided by either trees like banayan or house made of straw and other locally available materials. Animals kept outside during summer are comfortable under tree shade that protects them from direct sun light during peak hours of the day (Buffington *et al.*, 1983). A properly designed shading structure provides the most adequate protection to the livestock not only in hot summer but also in winter (Armstrong, 1994). For tropical climate condition, loose housing system is considered most

appropriate (Hahn, 1981). The longer side of the animal shelter should have an east-west orientation (Mishra, *et al.*, 2006). This reduces the amount of direct sunlight shining on sidewalls or entering the house (Ugurlu and Uzal, 2010).

Nutritional intervention: In the country, area under fodder cultivation is very low 4% of total geographical area. With the increase in livestock population, the area should increase to 8% so to meet growing demand of fodder for livestock. Since sheep and goats maintain on grazing resources alone in the country, decline in CPR yield and area under climate change is a serious concern. Rehabilitation of grazing lands by perennial grasses resistant to water scarcity in dry areas, development of silvipasture, planting suitable fodder trees, bushes, and grasses for round the year forage supply to animals will ease the fodder scarcity in dry zones (Jakhmola *et al.*, 2005). The cultivation of coarse millets in low and erratic rainfall region and conservation in the form of hay or silage for feeding animals during scarcity is a better option for mitigating fodder scarcity. Supplementary feeding of small ruminants can counteract nutritional stress during lactation and pregnancy. The concept of fodder bank in the form of complete feed block may be strengthened in drought prone areas as mitigating strategy for supplying nutritious diets fodder to animals during scarcity period. Agro-forestry system incorporating grasses, grain crops and tree may be propagated for improving yield per unit of land and ensuring fodder security of animals. Sheep grazing under hot arid and semi-arid environment face extreme fluctuations in the quantity and quality of feed throughout the year. Climate change imparts multiple stresses simultaneously on sheep. There are reports, which suggested that when thermal stress is coupled with nutritional stress, it had severe impact on the productive and reproductive functions in contrast to subjecting to either thermal or nutritional stress separately in sheep (Sejian *et al.*, 2010, Sejian *et al.*, 2011). Hence, developing nutritional strategies, which not only support yield but also address metabolic and physiologic disturbances. These findings are very valuable in improving economy of sheep farms as thermal stress and feed scarcity often occur simultaneously and are the major predisposing factor for the low productivity of sheep under hot arid and semi-arid tropical environment.

Conclusions:

The role of sheep and goats in livelihood of rural people and food security is very well acknowledged, its negative impacts by way of contributing to GHG in the atmosphere raise criticism. In climate change scenario intensity of extremes of hot and cold weather will increased further, which affect the productivity of small ruminants despite of their hardiness

character against harsh climatic conditions. The effort in selecting animals should be primarily planned toward robustness and above all adaptability to heat stress. Animal scientists work with scientists of other disciplines to get solutions to overcome the problems of degradation of pasture lands, non-availability of good quality of forage, thermal stress etc. Research must continue developing new techniques of cooling systems such as thermo-isolation and concentrating on techniques requiring low energy expenditure. Tree shed has been found to be common available shelter to the livestock, with its limitations. Closed type shelter with open space for free-movement of livestock, has been found to be most suitable animal shelter in extremes of climatic conditions.

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Landless Livestock Production System: Perceived Climate Variability and Adaptation Strategies by Pastoral Communities of India

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Abstract

The landless pastoral communities have exclusively been relied on natural resources to sustain their life support systems with conservation of indigenous breeds of animals. The high aberration in climatic events has affected socioecological sustainability. Variable climate and extreme events exuberate vulnerability of landless pastoral communities. This article, based on primary research, demonstrates perceived climatic variability and location specific adaptive strategies of landless three pastoral communities of India. These communities are *Brokpa* from Arunachal Pradesh, *Dewasi* from Rajasthan and *Ganderiya* from Uttar Pradesh. Data pertaining to study was collected using personal interview with open-ended and closed questions. Combination of methods including personal interview, focus group discussions (FGD) and transect walk enabled to collect and comprehend data. Results indicated that these communities are already and most often experiencing climate variability and extreme events. Reduced number of rainy days, expanded summer and preponning or post-poning in seasonal cycles exuberate the vulnerability of pastoral system. Intensity of winter is increased. Variability in climate, loosing local resource-base and low level of adaptive capacities of these communities are responsible factors for poor productivity of animals as the health of animals is deteriorated. The adaptive practices to sustain pastoral system are highly location specific and govern by climate and socioeconomic factors. Still majorities (> 85.0%) of pastoralists follow indigenous practices in grazing and healthcare to rear and manage their animals. Although, 25-30 % pastoral communities use allopathic veterinary medicines in part to treat their animals suffering from various diseases and disorders. Inter and intra level of household strategies during drought, rich social capital (trust, reciprocity on resources and sanctions on violation norms), long distance migration and employing community knowledge in conservation of indigenous breeds have been used in combined risk management strategies by these communities to sustain their livelihood around animals.

Introduction:

“..Pastrolism, for many, is profession destined to die”... (Gupta 1991)

Global climate change is now a reality and is perceived to negatively affect agriculture, livelihoods and socioecological sustainability (Singh, 2013). Small and marginal farmers, tribal communities and nomadic pastoralists are highly vulnerable to climate variability and extreme events (flood, drought, cyclone, etc.). The socioeconomic and climatic factors have different roles and impacts in minimizing vulnerability and enhancing the adaptive capacity of landless pastoral communities (O’Brien *et al.*, 2004). According to Kruska *et al.* (2003), the landless livestock production systems is a subset of the solely livestock systems in which dry matter fed to animals is produced in less quantity on the farm and animals are reared with integrated use of natural resources and migration system. The landless production systems in developing countries, and the pastoral communities who exclusively depend upon natural resources for their livelihoods, face major changes to various socioeconomic and climatic changes (IPCC, 2001). Many regions of the developing world including arid regions of India, are vulnerable to predicted climatic change (IPCC, 2001).

In recent past, the impact of climatic variability has been a major issue of debate among the scholars (Singh and Agrawal, 2011; Singh, 2012; Reid *et al.*, 2013). It is recognized as one of the major problems for the agricultural sustainability including livestock systems. Literature survey and primary experiences with pastoral communities reveled that they have wealth of innovations (Singh, 2012), experiences and location specific strategies to combat climatic variability (Kruska *et al.*, 2003; Singh and Agrawal, 2011; Reid *et al.*, 2013). However, resources of local communities are overlooked by scholars and policy makers while developing plan and strategies for these communities (Reid *et al.*, 2013). Looking at the importance of local knowledge and adaptive practices in combating climatic variability, present study was conducted with three pastoral communities of India with the following objectives: (1) to record perception of landless pastoral communities about climate variability, and (2) to study the adaptation mechanism adapted by these communities.

Research Methodology

Arunachal Pradesh (Ar. P) is a biocultural diversity hot-spot. West Kameng and Tawang districts are unique for the pastoral culture led by the landless *Brokpa* community. The state has alpine, temperate, sub-temperate, tropical and subtropical climates.

Table 1. Attributes of studied pastoral communities of diverse socioecological systems of India

Districts	Climates	Altitudes (in m) of survival	Ecology	Communities	Social systems	Animals reared	Vulnerability level*	Adaptive capacity*
West Kameng district (Arunachal Pradesh)	Sub-temperate to temperate (annual rainfall 170.4 mm and 25°C maximum and 1.14 °C minimum temperature)	1660-4660	Mountainous, highly fragile and risk prone	<i>Brokpaa</i> (<i>Monpa</i>)	Mongoloid, Buddhist and Tibetan culture followers. Totally nature and livestock dependent. Nomadic in nature and follow trans-human system	Yak, sheep	High to highest	Low to lowest
Jodhpur and Pali (Rajasthan)	Arid 300-350 mm annual rainfall, 47.0OC maximum and 3.0 OC minimum temperature; Pali 43°C and 6.0°C	215-300	Sandy soil, saline water, scattered vegetation, fragile and risk prone	<i>Dewasi</i>	Nature and livestock dependent, nomadic and provided some support through government schemes. Follow trans-human system	Sheep, camel, goat	Highest	Low to lowest
Azamgarh (Uttar Pradesh)	Sub-humid (annual average rainfall 1031.0 mm; 43.50 °C maximum and 6.80 °C minimum temperature)	60-70	Indo-gangetic plain, sodic soils, good quality water, moderate to scattered vegetation	<i>Gandaria</i>	Hindu, migrate from one to another socioecological systems, during flood adapt stall feeding. Do herding on large farmers' field to get food and money	Sheep	High to highest	Low to medium

* Vulnerability and adaptive capacity of studied socioecological systems is based on O'Brien *et al.*, 2004.

Ecology is highly fragile with lowest adaptive capacity of people (Table1). Yak, sheep, donkey and horses constitute the major animal species reared for the livelihood. Rajasthan (RS) with its arid climate and highly variable climate is considered one of the largest states for the sheep and cattle population. State is highly vulnerable with low to lowest adaptive capacity (Table 1 O'Brien *et al.*, 2004). Cattles and ruminants form the major chunk in the livelihood security and risk reduction of the state. Eastern Uttar Pradesh (UP) with its sub-humid climate provides habitat for the cattle, sheep and goat. Other than agriculture and livestock rearing, few communities such as *Ganderiya* (landless people) rear sheep and goat as a part of livelihood adaptation strategy. Eastern UP is high to highly vulnerable with low level of adaptive capacity (Table 1, O'Brien *et al.*, 2004).

The present study was conducted with *Brokpa* community of Ar P, *Dewasi* community of RS and *Ganderiya* community of UP. West Kameng from Ar P, Jodhpur and Pali from RS and Azamgarh district from UP were sampled purposively. The selected districts belong to different climates, ecology and vulnerability levels. These districts have different level of adaptive capacity (O'Brien *et al.*, 2004; Singh, 2013) with diverse social and institutional systems. From each district, four villages, thus total 12 villages dominated by studied communities were selected randomly. From each village, 15 pastoralists (a total of 60) aged above 50 years were selected randomly. These respondents were interviewed individually and in focus groups also.

An interview schedule with open-ended and close type of questions was used to collect data on selected objectives of this study. Before employing the interview schedule, it was pilot tested with 3 pastoralists in sampled areas to enhance the reliability of questions and types of response. Focus group discussions (FGD) was adapted to discuss the issues in-depth, while transect walk and moving with pastoralists were chosen as to record the facts relating to types of herding, contingency plan in the pasture, direction and types of movement of pastoral communities. Qualitative exploratory research design was used to conduct this study. Qualitative and quantitative data were analyzed using excel and SPSS package to draw the valid inference.



Fig. 1 Increased *Najala* (cough and cold) in sheep reared by *Ganderiya* community

Results:

Perception about climatic variability:

It is evident from the Table 2 that in general the landless pastoral communities of diverse socioecological systems of Ar P, RS and UP perceived that climate is no more normal. *Gandiriya* community perceives that the intensity of cold has increased resulting in frequent *najala* (cough and cold symptoms) in sheep (Fig. 1). Similarly, the *Brokpa* and *Dewasai* communities reported that the summer duration has extended in the last 40 years. Three studied communities revealed that rainfall has reduced approximately three folds in comparison to the past 40 years. The indigenous indicators (insects, plants, animals, wind pattern, cloud formation, etc.) are being used in predicting the weather pattern. *Dewasi* community use grains of indigenous crops (jowar, bajra, sesame and till) in combination of spiritual acts to predict rainfall pattern. They also experienced that rising population and attack of termites in pasture lands and agricultural crops is a locally recognized indicator of variable climate. *Brokpa* community predicts weather based on the amount and pattern of snowfall at varying altitudes, while women infer weather based on the total duration required for fermentation and preparation of yak based milk product such as *Chhurpi* (wet cheese).

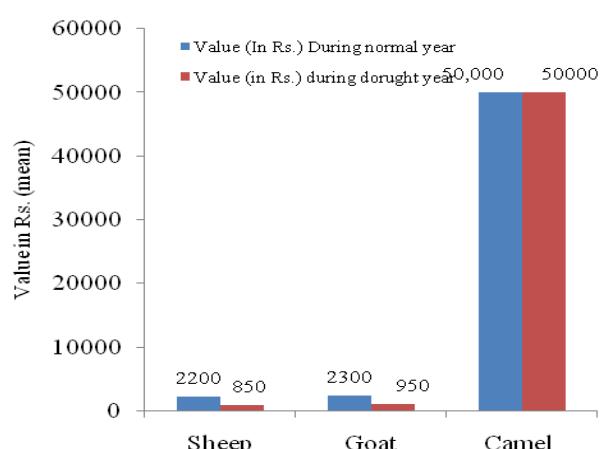
Table 2. Perceived climate variability among pastoral communities of varied socioecological systems of India

Climate variability indicators	Strongly agree			Agree			Undecided			Disagree			Strongly disagree		
	BC	DC	GC	BC	D C	G C	BC	D C	G C	BC	D C	G C	BC	D C	GC
Duration of winter is not decreased (N)	00. 0	00. 0	00. 0	00. 0	00. 0	00. 0	00.0 0	05. 0	10. 0	05.0 0	05. 0	10. 0	95.0 0	85. 0	80. 0
Timing of winter start is postponed	90. 0	85. 0	90. 0	15. 0	15. 0	10. 0	05.0 0	00. 0	00. 0	00.0 0	00. 0	00. 0	00.0 0	00. 0	00. 0
Duration of summer is not increased (N)	00. 0	00. 0	00. 0	00. 0	00. 0	00. 0	00.0 0	05. 0	06. 0	15.0 0	20. 0	12. 0	80.0 0	75. 0	82. 0
Timing of summer start is pre-poned	80. 5	84. 6	78. 0	15. 5	14. 4	18. 0	04.0 0	01. 0	04. 0	00.0 0	00. 0	00. 0	00.0 0	00. 0	00. 0
Duration of rainy season is decreased	95. 0	92. 0	96. 4	05. 0	08. 0	03. 6	00.0 0	00. 0	00. 0	00.0 0	00. 0	00. 0	00.0 0	00. 0	00. 0
Number of rainy days are not decreased (N)	00. 0	00. 0	00. 0	00. 0	02. 0	01. 7	00.0 0	00. 0	00. 0	19.3 0	15. 5	25. 5	80.7 0	82. 5	72. 8
Events of drought	45. 0	65. 0	60. 0	35. 0	25. 0	25. 7	15.0 0	05. 0	10. 0	05.0 0	05. 5	00. 5	00.0 0	00. 0	00. 0

is increased	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Occurrence of flood is normal as was earlier	00.	00.	00.	05.	25.	05.	05.0	35.	05.	30.0	40.	25.	60.0	10.
Extreme events are increased	85.	90.	80.	15.	10.	15.	00.0	00.	05.	00.0	00.	00.	00.	00.
Weather in general has become unpredictable	95.	90.	85.	05.	10.	15.	00.0	00.	00.	00.0	00.	00.	00.	00.
There is no change in general phenomenon of weather (N)	00.	00.	00.	00.	00.	00.	05.0	05.	05.	15.0	05.	10.	80.0	90.
Bio-meteorological indicators are still effective in weather prediction	35.	40.	45.	35.	30.	40.	15.0	20.	10.	15.0	10.	05.	00.0	00.
Pastoral vulnerability did not increase (N)	00.	00.	00.	00.	00.	00.	00.0	00.	00.	25.0	15.	30.	75.0	85.
Climatic variability has increased diseases and health problems in livestock	55.	45.	60.	45.	55.	40.	00.0	00.	00.	00.0	00.	00.	00.0	00.
Climatic variability has threatened pastoralists' livelihood	90.	85.	80.	10.	15.	20.	00.0	00.	00.	00.0	00.	00.	00.0	00.
Neither government nor livestock owners have full-proof adaptive practices to climatic variability	65.	60.	70.	35.	40.	30.	00.0	00.	00.	00.0	00.	00.	00.0	00.

Abbreviations: BC= *Brokpa* community (Ar P); DC= *Dewasi* community (RS); GC= *Ganderia* community (UP); N= Negative sentence to obtain unbiased agreement of pastoralists; Data presented in table are in percentage.

Fig. 2 Economic value of animals during normal rainfall and drought years



Brokpa women are now experiencing that during summer *Chhurpi* gets spoiled and takes less duration, and thus needs to be stored at higher altitudes (4000 meters above mean sea level). Contrary to this, the duration has reduced from 5-6 to 3-4 days in the winter season. The *Ganderia* respondents inferred that there will be an immediate rain if sheep herd is impatient and willing to seat instead of grazing. The majority (85.0%) of respondents reported that climatic aberration has increased and thereby it has impacted the health of livestock. Vulnerability of livestock systems are increased by abrupt changes in climate, and further compounded effect of socioeconomic and cultural factors have increased the risk for these communities. For example, during the normal rainfall year the economic value of sheep would be around Rs. 2200 (for body weight upto 8-9 kg), while during drought it reduces to an average of Rs. 850 per sheep (Fig.

2). This happens due to increased cost of grazing, movement, labour and management. Animals are suffering from frequent infections and stomach disorders and other diseases as perceived by studied communities (Fig. 3). Sheep moving from RS to

Haryana and Punjab suffer from frequent stomach disorders. *Dewasi*

community carry with them tea leaves and dried *kachari* (*Cucumis callosus*) fruits to use them as laxative for sheep, other than using allopathic drugs. *Ganderiya* community use fruits of *bael* (*Aegle marmelos*) and *makten* (a wild tree) to treat *najala/nekda* problem in sheep. Flower buds of *Narar* and wild *beri* (*Ziziphus jujube*) trees are used to make decoction and cure throat infection in sheep. Some *Ganderiya* herders use *mamphal* (fruit like guava) to treat the same problem. Therefore we found that livelihood security of these pastoral communities is at stake due to several factors including abrupt changes in weather and climate.

Adaptation:

Across the socioecological systems, we observed that ‘community held knowledge’ and ‘social capital’ are back bone of adaptive capacity of *Brokpa*, *Dewasi* and *Ganderiya*

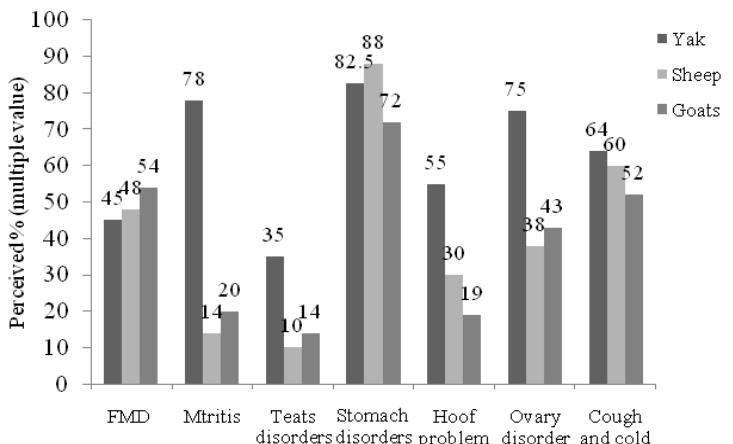


Fig. 3 Impact of climate change on the health of animals

communities. Due to either no land or small size of land holding, these communities have developed collective measures to adapt to changing climatic and socioeconomic scenarios.

Table 3. Adaptive strategies and practices of pastoral communities

Adaptive strategies	Adaptation %*			Adaptation description	Experienced problems
	Ar P	RS	UP		
Increased rotational grazing	89. 0	73. 5	48. 6	Grazing at different altitudes (Ar P) and landscapes (RS and UP).	Recovery period is enlarged, require more energy and labour
Every day and weekly plan of grazing and rearing	12. 5	86. 4	64. 2	Based on the resources, labour, potential of grazing land, nature of herds size and population, <i>Dewasi</i> and <i>Ganderiya</i> make grazing plan every day and week to execute it.	Required more labour, accurate weather information and enlarged migration (upto 30-35 kms per day)
Division of animals	48. 7	56. 8	40. 6	Only the healthy and stronger animals included in the herd for distant migrations.	The cost of rearing and stall feeding for left animals is increased for the animals excluded from herds (RS and UP)
Searching the regions with better amount of rainfall	40. 5	70. 8	62. 8	<i>Dewasi</i> moves to the states like Punjab, Haryana, Gujarat and MP where chances of getting grasses are more. <i>Ganderiya</i> expands their movement to more eastern and western UP. <i>Brokpa</i> expand their movement to Bhutan and Tibet borders.	Time, energy, cost and labour is increased
Diversifying the herd	26. 8	68. 5	48. 6	Yak with sheep (Ar P) and sheep with goat of varying ages (RS and UP).	More integrated plan of grazing, migration and stay is required.
Barter system	85. 5	56. 4	35. 6	<i>Brokpa</i> exchange yak based products with <i>Monpa</i> tribe and of their own community of Bhutan, <i>Dewasi</i> and <i>Ganderiya</i> communities exchange sheep based products with farmers and traders.	Socioeconomic, cultural and political changes now undermine this adaptation
Kinship networks and sharing resources at inter and intra	64. 5	87. 6	74. 8	Over time and space, due to climatic variability people have developed	Changes in sociopolitical systems have

household level					knowledge network to share practices, reciprocate and minimize the risks with diversified enterprises	affected this network
Innovation and community knowledge in developing breeds with high adaptive capacity	28. 6	32. 4	16. 8		<i>Brokpa</i> and <i>Dewasi</i> communities have outstanding knowledge and innovations in conserving and developing climate resilient breeds.	Less interest of young generations affected community knowledge of herd rearing and conservation of indigenous breeds.
Formation of self help groups (SHGs)	16. 4	04. 1	02. 4		SHGs formed by <i>Brokpa</i> women help to share cost collectively, develop yak and sheep based product, reduce drudgery and sale in local market.	In vogue among only few people
Taking the debt from local <i>Baniya</i> and large farmers	28. 4	45. 8	36. 4		The debt helps to purchase food materials and medicines for movement of herds.	Cost of foods and medicines are too high and affect management practices.
Depending on the government schemes	4.5	12. 4	6.4		Getting the wages for family members in MGNREGA scheme and foods through public distribution system	Weakening the dynamics of pastoral system

* Multiple percentages

Even in same villages, there could be three kinds of groups with diverse portfolio of enterprises. There might be one group with its few land, few cattle and few earning members in city areas. The second group might be with its few members engaged in pastoralism while some may be engaged as labourers and in agricultural activities. The third group could be landless people of its income from labour and own few sheep, few goats and engaged as labourers and in crafts. These three groups may have some synergy to assimilate with each and form the risk management strategy together. The risky environments and resource crisis have evolved various social and cultural institutions to sustain pastoral based livelihoods of these communities. For example, all these three communities have evolved *sajha* institution (5-6 households together) to purchase sheep, rear, drive for grazing and trade their sheep products and manage

Various products obtained from reared animals provide assured livelihood base. For example, *Ganderiya* community sale sheep wool @ Rs. 20-25 per kg. They also get an assured money by selling blanket (Rs. 500-1000; size: 6x3 feet) weaved from sheep wool. On

an average, each group of *Ganderiya* (with 5-6 households) herders drive their sheep for rented herding (on 400 sheep, they charge 40 kg wheat per night) in the agricultural fields. These herding are more preferred during March to April in the sugarcane crop. In sandy soils, herding is done before the planting of sugarcane, while in *domat* (clay-sandy) soils even after the planting/sowing, herding can also be done. In one season, about 25-30 herding can be performed. However, landscape and socio-economic changes have affected herding system in farmers' field. As a result, *Ganderiya* community is the sufferer. One member of *Ganderiya* community narrated the situation as:

..”We don't want to continue bhendihara kam (sheep herding) due to high amount of labour required in it, and vulnerability caused by compounded impact of climate and socioeconomic factors”..



Fig. 4 Mr. Mehmud Khan migrating with his sheep herd during May 2013

Other than livelihood security, sheep and goats reared by *Ganderiya* have been playing a pivotal role in sustaining ecological services. For example, the pods of *Babool* (*Acacia nilitica*) fed to sheep and goat, helps in the dispersal of its seeds (after chewing and digestion). Besides this, the germination of different grass seeds is considered to be enhanced this way. The similar success story was narrated by *Dewasi* community of RS about the role of sheep and goat in sustaining plant population of *khejari* (*Prosopis cineraria*) and *Babool* species. Changes in climatic pattern and landscape has affected to a great extent the grazing system, livelihood security of these communities, and thereby reduction in total population of sheep, goat and yak.

Mr. Mehmud Khan (50 yrs) from Ladiya village (Osia, Jodhpur, Fig. 4) revealed that during the drought period in RS, they migrate to Punjab, Haryana and Gujarat states in search of grazing lands. However, in these states too the increasing irrigation percentages have much affected the sustainability of local grasses and pasture systems. As a result, they are forced to reduce the herd size. They sustain their optimum herd size by selling the lambs below the age of 9 months.

Table 4. Indigenous breeds of yak conserved by *Brokpa* community and adapted in fragile ecosystem (altitude ranging from 1660 to 4660 meter above sea level)

S. N.	Parents used in crossing		Produced offspring	
	Female	Male	Female	Male
1	Kot	Yak	Dzomo	Zo
2	Dzomo	Galang	Kot	Kotsan
3	Kot	Galang	Sing Kot	Sing Galang
4	Sing Kot	Galang	Ta Kot	Ta Galang
5	Ta Kot	Galang	Bha Kot	Bha Galang
6	Bha Kot	Galang	Galangmu	Galang (pure male)
7	Galangmu	Yak	Dzomo	Zo
8	Sing Kot	Yak	Sing Dzomo	Sing Zo
9	Dzomo	Yak	Tui Dama	Tui Shang
10	Bha Kot	Yak	Bha Dzomo	Bha Zo
11	Ta Kot	Yak	Ta Dzomo	Ta Zo
12	Galangmu	Yak	Bham Dzomo	Bham Zo
13	Tui Dama	Galang	Tui Dzomo	Tui Zo
14	Tui Dama	Yak	Garmu	Garyak
15	Garmu	Yak	Kyukmu	Kyukyak
16	Kyukmu	Yak	Zyang or Bree	Yak
17	Bree	Yak	Bree	Yak
18	Bree	Galang	Brim Dzomo	Brim Zo
19	Garmu	Galang	Gar Dzomo	Gar Zo
20	Kyukmu	Galang	Kyuk Dzomo	Kyuk Zo
21	Mithun	Dzomo ⁺	Mithak ⁺ (female)	Mithak-male ⁺

⁺Note: This cross was made and result has been obtained after 3 years.

Objective was to get a male breed with high carrying capacity and female with high milk

With sheep, now migration with herds is increased upto 30 to 35 kms per day in search of grasses on road side if climate is too variable and pastoralists are young. They cover 15-20 kms distance in the field for grazing of sheep. While, during normal weather they cover upto 15-20 kms per day. Migration is increased due to less availability of grasses, and changing landscape with increased intensive crop cultivation. The increased migration affects health of animals as experienced by the herders. The herders from RS reported that based on the past climatic uncertainty, they select breed of

sheep with wide and powerful jaw. This criterion is applied to adapt the breed, which can survive even



Fig. 5. Yak driving for grazing at around 3000 meter mean sea level

during the less rainfall or drought when grasses and shrubs are less, and sheep require more energy to graze the grasses from dry lands.

In Ar P, we observed that the *Brokpa* community still conserves about 21 indigenous breeds of yak (Table 4), and some breeds of sheep. These breeds are relatively better adapted to variable climate as compared to the improved ones. The *Brokpa* community has identified different grazing lands at varying altitudes according to the season (Fig. 5) where these yak breeds are reared. During summer, they migrate to the altitude from 3000 m to 6500 m mean sea level. In winter, they come down below 3000 meters. Before migrating to a particular grazing land of a definite altitude, the elders and community chief held meeting to decide the norms and of movement, level of accessing grasses and types of sanctions and fines in order to sustain the grazing land and fulfill their livelihood requirements.

Table 5. Local breeds conserved by the *Ganderia* community

Name of the breed	Body colour and other attributes	Wool productivity	Matured ram weight (in kg)	Climate variability adaptive capacity
<i>Makar</i>	Spotted black and white	I	20-28	Very good
<i>Lohi</i>	Red	III	25-30	Good
<i>Hanna</i>	Spotted red and black	I	15-22	Very good
<i>Chawar</i>	Black body and white tail	II	20-25	Moderately good
<i>Gondar</i>	Legs white and black body		22-28	Very good
<i>Hasar</i>	Black mouth, golden body with black spots	III	22-30	Fair
<i>Tikaska r</i>	Red stomach part, remaining body black	II	25-30	Very good
<i>Chanuv -slot</i>	White head, and remaining body black	III	20-25	Good
<i>Kajar</i>	Black mouth and remaining body parts white	I	28-32	Good
<i>Sokkan</i>	Golden mouth, and remaining body parts black	IV	18-22	Good
<i>Kasar</i>	Golden stomach, and remaining body parts black	III	20-25	Very good

Note: Rank was calculated by *Ganderia* community in a focus group discussion health, body weight, wool capacity and migrating ability of sheep as criteria. Meat is sold out @ Rs. 250/kg.

Similarly, we also learned that *Dewasi* of RS and *Ganderiya* of UP still conserve indigenous breeds of sheep (Table 5). These breeds are collectively conserved by more than 4-5 households and during flood or acute drought, they sale these breeds in local market to generate income. On average, wool productivity from one indigenous sheep in one year was observed to range from 750 to 900 gm depending upon age and health of a breed. About 200-250 gm wool is harvested in one harvesting and three to four harvesting of wool is done in one year. Based on this criterion, rank was calculated by *Ganderia* community in a focus group discussion. Although, *Gandeirya* community do not consume the sheep meat, but they sale their animal in local market during extreme climate for meat purpose @ Rs. 250/kg.

Discussion and conclusions:

Four key findings emerged from our results: (i) landless pastoral communities are increasingly experiencing the climatic variability and they are relatively more vulnerable than other livestock owners, (ii) vulnerability of these communities is further compounded by other socioeconomic, cultural and institutional factor, (iii)) the adaptive practices of pastoral communities are mostly location specific, helps in sustaining ecological goods and services, and based on community knowledge and ethical practices, and (iv) in the face of climate, ecological and socioeconomic changes, the livelihood security of these communities could be sustained with integrated planning and policy support. Adaptation of pastoral practices by different communities of Ar P, RS and UP is an ecological compulsion (Singh 2013)

leading to the emergence of the livestock based eco-cultural systems in fragile ecosystems of Indian subcontinent. Gradually, the spatial and temporal climatic variations in Ar P (Bhattacharyya *et al.*, 2007), RS and UP have increased the vulnerability of pastoralists (Gupta 1991). Given their migratory nature, sheep and yak based pastoral practices seems to be more uncertain when socio-political system is changing at unprecedented rate. The cost of inputs such as veterinary care for animals, and economic value of animals during extreme events decide whether to retain an animal or to sale it. The chronic poverty among pastoral communities and uncertain climate increases debt and drudgery of pastoralists. The natural capital maintained by rich social and ethical practices, and sum of intellectual capital of these



Fig. 6. Grazing ground and an accidentally burned pine forest at Naga-GG area of Dirang (West Kameng)

communities are the guiding forces in determining adaptation strategies (Gupta 1991, Gupta 2013).

Adaptive practices of pastoral communities not only help to sustain their life support system, but also maintain ecological goods and services. But, inappropriate policy decision and ignorance of local adaptation may hamper the ecological sustainability. For example, the *Brokpa* community has evolved the unique practice of controlled burning of the leaf litters in the pine forests every third year to reduce the thickness of pine needles on surface and modify the microclimate for better germination of grasses for their yak. This traditional practice has been helpful in sustaining forest ecosystem goods and services. With an institutional policy, the Forest Department in West Kameng district banned this traditional practice about 10 years back. As a consequence, an accidental forest fire in the year 2000 destroyed about 300 hectares of pine vegetation (Fig. 6). This situation suggests that the pastoral communities with their animals and location specific ecological knowledge maintain the ecological sustainability.

It has been now recognized that not always every response to climate is sustainable (Eriksen *et al.*, 2011). The initiatives and technology offered to resource-poor communities needs to be tailored on recognizing the context of vulnerability, acknowledgment of different values and social systems is necessary and integration of local knowledge is necessary (Eriksen *et al.*, 2011). It is highly desirable to invite and involve the pastoral communities while land use planning and conservation of natural resources. From beginning of problems identification to designing and implementation of projects of conservation and developments of pastoral system, participation of pastoral communities may enhance the sustainable adaptation (Pearce *et al.*, 2007). This could be done only when policy makers and practices are willing to have radical change in the policies adaptations for changing socioeconomic, climate and ecology livelihood security of these communities could be sustained with integrated planning and policy support. From foregoing learning with three different communities, we learned that climatic variability has adversely affected pastoral communities in many ways. Vulnerability has been further accentuated with the loss of native resources—particularly the indigenous animal biodiversity (which are considered to be more resilient to climate variability) and the changing landscape having indigenous flora.

Acknowledgements:

We are grateful to all the pastoralists who were part of this study and have provided their valuable knowledge. Authors are grateful to Central Soil Salinity Research Institute, ICAR for providing financial and logistic support for collection of data from Rajasthan and Uttar Pradesh. The editorial inputs received from Dr. Anshuman Singh, CSSRI is appreciated and acknowledged.

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Seasonal Variation in the Semen Quality of Buffalo Bull

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Buffalo is the main milch animal in our country and its milk is liked more, hence it fetches more prices as compared to cattle. Buffalo contributes more than 50 per cent (62.3 MT) of total milk (121.8 MT) production in India though its population is 105.3 million heads (2007, livestock census) about half that of the cattle population (199.1 M) (FAO, 2010; DAHD, 2011-12).

We have the best buffalo breeds of the world. However, its reproductive behavior and physiology have not yet been fully understood. Buffalo (both male and female) shows a seasonal pattern of reproduction. As buffalo sometimes doesn't or expresses weak estrus symptoms and poor conception rates especially during the summer months i.e. April to August due to which it's sometimes called seasonal breeder; on the other hand, the reproductive efficiency in buffaloes is more during September to February (>40% CR) which is known as favorable season for buffalo breeding.

Similarly quality of fresh and frozen buffalo semen also varies between different seasons; semen with satisfactory freezability (~45% post thaw sperm motility) is produced during September to February, whereas initial semen quality and freezability both remain poor during April to August (<40% post thaw sperm motility). In general, to accept or reject a semen sample for cryopreservation, initial semen quality is assessed by evaluating its ejaculate volume, mass motility, sperm concentration and individual sperm motility and to accept a semen sample post freezing, periodically assessment of sperm motility, viability, morphology, acrosome status, HOST etc are recommended before using for AI.

Artificial insemination is being used as a tool for genetic improvement in buffalo also, like in other species of livestock. Buffalo bull's artificial semen collection, cryopreservation and its use for AI is being done worldwide for more than three decades but the quality of the frozen semen and then conception rates (buffalo CR 35-40% at organized farms) limits the wide application of AI in this species. Further a little emphasis/attention on research on males' reproduction in general and on semen cryopreservation in particular, has been given. Inspite of

that the demand of buffalo frozen semen doses (80 million in demands vs. 15 million doses available) for AI has increased enormously during last 4 to 5 years in the country.

In general, in northern part of our country summer is regarded as the season exerting relatively more adverse effect on the overall reproductive performance of buffaloes in comparison to other seasons. In this season, high ambient temperature is relatively of a long spell, high mean of sunshine, hot blast of wind and a continuous stream of radiation impinging directly and indirectly through reflection from floor and shed on the animal's body and thereby precipitating a really distressing challenge to the animal's body and thermoregulatory mechanism. Further, a typical monsoon season in this region consists of a high ambient temperature and high degree of vapor pressure which limits the scope of heat dissipation through evaporative channels cause another great stress on animal body and its reproductive performance. Winter in this region does not consider extreme; whereas spring is considered the best season for buffalo quality semen production followed by autumn, winter, rainy and summer.

Seasonal variation in initial semen quality:

A seasonal pattern of reproduction in both male and female buffaloes is well established (Roy *et al.*, 1962; Sengupta *et al.*, 1963; Idris and Afify, 1979; Ramadan *et al.*, 2009). High heat stress during summer is known to depress the physiological functions of the animal which consequently reflected in poor libido, semen quality and low fertility of breeding bulls. It has been observed that in northern part of the country the ambient temperature remain high during May to September; the semen harvested during this season is of poor quality. Sengupta *et al.* (1963) were the pioneer workers to work on seasonal aspects of buffalo semen quality, in India. They found that initial quality of buffalo bull semen varies between the seasons; buffalo bull produces best quality semen during the spring season and the worst in summer. The similar findings were also reported by Sidhu and Guraya (1979). Seasonal trend in spermatogenic activity was observed in buffalo bulls, which was maximum in spring and minimum in rainy season (Sinha *et al.*, 1966). Sperm concentration and viability were the highest in spring and lowest in autumn in Surti and Nili Ravi buffaloes (Majeed *et al.*, 1961). The seasonality was also observed by Gill *et al.* (1974) in semen quality of buffalo semen; the quality was excellent during autumn and poorest in winter. Seasonal variation in semen quality parameters have also been observed by several other workers (Greesh Mohan *et al.*, 1977; Gangwar and Bhaga, 1979).

Lowest ejaculate volume in summer and the highest in winter in buffalo bulls were obtained by Bhavsar (1986). Dhami and Kodagali (1987) found excellent semen quality in cold and hot seasons and poor in wet season of Surti buffalo bulls.

Seasonal variation in freezability of semen:

Freezability of buffalo semen can also be affected by the season of collection i.e., by environmental factors like temperature, humidity and day length in a particular season. When the semen sample is not found of desired quality during collection, processing and evaluation is rejected. For the first time Tuli and Mehar (1983) observed the seasonal variation in freezability of buffalo semen. They found that post-thaw sperm motility was significantly higher in winter than summer season. Heuer *et al.* (1987) also found the effect of season on in vivo fertility of frozen semen in Nili Ravi buffalo. They reported that semen collected in November (winter) produced significantly higher conception rate than semen collected in June (summer) over a total of 3220 inseminations in both seasons (40.9 vs 34.0%) were made. They attributed 40% of the observed seasonality of buffalo fertility to the male. Period during September to February was found to be the most conducive for frozen semen production in buffaloes (Bhosrekar *et al.*, 1988; 1992). Bhavsar *et al.* (1989a) also found the monthly variations in freezability and fertility of buffalo bull semen. In their findings, the fertility of semen collected, frozen (July to January, post thaw sperm motility (55.6%) and February to June (52.3%)) and inseminated during July to January (monsoon or late wet summer to autumn and winter) was significantly higher (47.3%) than ejaculates processed and inseminated during February to June (spring to early dry summer) (44.3%). Sagdeo *et al.* (1991) reported the seasonal variations in freezability of buffalo bull semen. They found from the data of over a 4-year period that the season significantly affected the post- thaw sperm motility, and values being highest in ejaculates frozen in the winter and lowest in summer. Similarly, Bahga and Khokar (1991) studied the seasonal variations in freezability of buffalo bull semen. They found that post-thaw semen motility was significantly affected by season of collection, being lowest (28.92%) in summer and highest (43.32%) in winter (December–January). Younis *et al.* (1998) studied the freezability of semen collected during the low breeding season (May–July) and the peak breeding season (September–November). They reported that post- thaw motility and livability of spermatozoa were significantly higher in adult bulls during the peak breeding season. In addition, the sperm

abnormalities and deleterious enzymatic activity in frozen–thawed semen were significantly higher during the low breeding season than in the peak breeding season.

Possible basis of seasonal effects on semen quality:

Seasonal effect on quality of frozen–thawed buffalo spermatozoa diluted in Tris-based buffer was studied (Koonjaenak *et al.*, 2007a). They compared post-thaw sperm quality over three seasons of the year (rainy: July–October; winter: November–February; and summer: March–June), with distinct ambient temperature and humidity. It was concluded that post-thaw plasma membrane integrity and stability were significantly better in ejaculates processed during winter than in samples processed during the other seasons of the year. They further investigated in their study (Koonjaenak *et al.*, 2007b) that the frozen–thawed buffalo sperm nuclear DNA fragmentation by flowcytometry and head morphology over three seasons in tropical Thailand (the rainy season, July–October; winter, November–February; and summer, March–June). They found that the DNA fragmentation index (DFI) values varied statistically among seasons, being lower in the rainy season than in winter or summer, and were affected by the year of semen collection and processing. The proportion of morphologically abnormal sperm head shapes was low, with no significant differences between seasons. However, DFI was significantly related to the proportion of loose abnormal sperm heads. It was concluded that frozen–thawed buffalo sperm chromatin is not critically damaged by cryopreservation or affected by the seasonal variations in temperature and humidity seen in tropical Thailand. There is possibility that a seasonal variation in the biochemical composition of seminal plasma and / or spermatozoa may occur as it does in other farm animals (Cabrera *et al.*, 2005; Argov *et al.*, 2007; Koonjaenak *et al.*, 2007a). Argov *et al.* (2007) have reported that cattle semen samples collected during the summer and considered to be of good quality had alterations in lipid concentration, fatty-acid composition and cholesterol level. In addition, they provided the first evidence for the existence of a very low density lipoprotein receptor (VLDLR) in bovine sperm, suggesting a mechanism for sperm utilization of extracellular lipids. Interestingly, the expression of VLDLR was three fold greater in samples collected during the winter than in those collected in the summer. Therefore, it is suggested that such modifications may explain, in part, the reduced freezability of buffalo semen collected during the summer. Few scattered reports are available that describe the differences in chemical composition of buffalo seminal plasma and spermatozoa under different climatic conditions (Singh *et al.*, 1969; Mohan *et al.*, 1979; Sidhu and Guraya, 1979). However,

the information given in these studies are insufficient to explain the variation in freezability of buffalo spermatozoa during the different seasons. Therefore, detailed studies should be carried out to ascertain the biochemical or structural alteration in seminal plasma, spermatozoa, or plasma lemma, which might be influencing the freezability of buffalo spermatozoa during the different seasons.

Conclusion:

From the above discussion, it is evident that there is seasonal variability in buffalo semen initial quality and then in freezability. There is a higher loss of viability and fertility during the process of cryopreservation in summer, thus confirming that vitality of buffalo spermatozoa remains comparatively poorer during this season. As standard classification of semen for cryopreservation is based primarily on morphological features without any specific consideration of the time or season in which the semen was collected. Perhaps semen collected during the summer and morphologically classified as being of good quality is, in fact, less competent to survive freezing and to fertilize. Moreover, use of inferior semen might be one of the multiple factors involved in the low fertility of buffaloes during the hot season. Therefore, there is need to conduct detailed studies to ascertain the biochemical or structural alteration in seminal plasma, spermatozoa, or plasma lemma, which might be influencing the freezability of buffalo spermatozoa during the different seasons for making. This will help in overcoming the seasonal variation in semen quality and freezability of buffalo bulls.

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Livestock as a Cause and Sufferer of Climate Change

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Livestock are important to the food security of millions of people today and to millions more in the coming decades. Globally, 1426, 195, 1093 and 924 millions of cattle, buffalo, sheep and goat with other livestock are present in 2011. They contribute around 12.9 percent of global calories and 27.9 percent of protein to 7.1 billion human beings directly through providing meat, milk, eggs and offal, and contribute to crop production through the provision of transport, draft power, manure and current assets for a vast majority of rural households (FAO, 2009). The meat, milk and eggs provide proteins with a wide range of amino acids that match human needs as well as bio-available micronutrients such as iron, zinc, vitamin A, vitamin B12 and calcium in which many malnourished people are deficient.

Increasing concentration of Greenhouse Gases (GHGs) like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorocarbons in the atmosphere has contributed to an increase in the earth atmospheric temperature, an occurrence known as global warming (Gerber *et al.*, 2013) which further leads to climate change. Inter-Governmental Panel on Climate Change (IPCC) reported that CO_2 , CH_4 and N_2O concentration in the atmosphere has increased from pre-industrial values of 280 ppm, 715 ppb and 270 ppb in 1750 to 379 ppm, 1774 ppb and 319 ppb, respectively in 2005 (IPCC, 2007). More than 70% of this increase has occurred in the last 50 years where fossil fuel and land use remains as the primary sources of CO_2 , whereas agriculture including livestock is the main source of CH_4 and N_2O . The global warming potential of N_2O and CH_4 are about 298 and 25 CO_2 -equivalent units making their presence more harmful in the atmosphere when measured as a function of 100 years. Emission of CH_4 is responsible for nearly as much irraditative effect as all other non- CO_2 GHG combined. Indian Meteorology Department, New Delhi and Indian Institute of Tropical Meteorology, Pune projected that by the end of 21st century rainfall and mean temperature will increase by 15-40% and 3°-6°C, respectively (NATCOM, 2004). According to National Aeronautics and Space Administration, the first decade of the 21st century was the warmest on record, with 2010 and 2005 as the hottest years on record.

Climate change poses formidable challenge to the development of livestock sector in India and World as it has to reduce its GHG emissions while responding to a significant demand growth for livestock products (projected to be +70% between 2005 and 2050), driven by a growing world population, rising affluence and urbanization. The livestock sector that will be a sufferer of climate change is itself a large source of CH₄ and N₂O emissions through enteric fermentation, animal wastes and other services enumerated in Table 1. The anticipated rise in temperature between 2.3 and 4.8°C together with increased precipitation resulting from climate change is likely to aggravate the heat stress in dairy animals, adversely affecting their productive and reproductive performance, and hence reducing the total area, where high yielding dairy cattle could be economically reared. Given the vulnerability to rise in sea level, the impact of increased intensity of extreme events on the livestock sector would be large and devastating for the low-income rural areas. The predicted negative impact of climate change on Indian agriculture would also adversely affect livestock production by aggravating the feed and fodder shortages. The impacts of temperature increases at even the lower end of this range will be far-reaching and the broad trends will be over shadowed by local differences, as the impacts of climate change are likely to be highly spatially variable.

Livestock: a significant contributor to climate change:

Greenhouse gases emission from ruminants are mainly based on enteric fermentation and manure management whereas in non-ruminants hindgut fermentation and manure management predominates. The major sources of GHG emissions from livestock are described in Table 1. Livestock sector emits 7.1 Gt CO₂-eq per annum, representing 14.5% of human-induced global GHG emissions mainly via enteric fermentation, manure management, land use and land-use change (directly for grazing or indirectly through production of feed crops); thus responsible for climate change (Gerber *et al.*, 2013). Species wise GHG emission in terms of CO₂ equivalent (million tonnes) showed that cattle, buffalo, sheep and goat, pig and poultry amount for about 4600 (65%), 618 (9%), 575 (6.5%), 668 (9%) and 606 (8%) million tonnes, respectively. Feed production and processing, and enteric fermentation from ruminants are the two main sources of emissions, representing 45 and 39% of livestock sector emissions, respectively, whereas manure storage and processing represent 10% and the rest is attributable to the processing and transportation of animal products (Gerber *et al.*, 2013).

Table 1. Sources of GHG emissions from livestock sector (Gerber *et al.*, 2013)

Supply chain	Activity	GHG s	Sources
UPSTREAM	Feed production	N ₂ O	<ul style="list-style-type: none"> Application of synthetic N Application of manure Direct deposition of manure by grazing and scavenging animals Crop residue management
		CO ₂ , N ₂ O, CH ₄	<ul style="list-style-type: none"> Energy use in field operations Energy use in feed transport and processing Fertilizer manufacture Feed blending Production of non-crop feedstuff (fish meal, lime and synthetic amino acids) CH₄ from flooded rice cultivation Land-use change related to soybean cultivation
	Non feed production	CO ₂	<ul style="list-style-type: none"> Embedded energy related to manufacture of on-farm buildings and equipment Production of cleaning agents, antibiotics and pharmaceuticals
ANIMAL PRODUCTION UNIT	Livestock production	CH ₄	<ul style="list-style-type: none"> Enteric fermentation
	Manure management	N ₂ O	<ul style="list-style-type: none"> Direct and indirect N₂O from manure management
		CO ₂	<ul style="list-style-type: none"> Direct on-farm energy use for livestock (e.g. cooling, ventilation and heating)
DOWNSTREAM	Post farm gate	CO ₂ CH ₄ HFCs	<ul style="list-style-type: none"> Transport of live animals and products to slaughter and processing plant Transport of processed products to retail point Refrigeration during transport and processing Primary processing of meat into carcasses or meat cuts and eggs Manufacture of packaging On-site waste water treatment Emissions from animal waste or avoided emissions from on-site energy generation from waste Emissions related to slaughter by-products (e.g. rendering material, offal, hides and skin) Retail and post-retail energy use Waste disposal at retail and post-retail stages

On commodity-basis, beef and cattle milk are responsible for the most emissions, respectively, contributing 41 and 20% of the sector's overall GHG outputs. These followed by pig meat (9%), buffalo milk and meat (8%), chicken meat and eggs (8%), and small ruminant milk and meat (6%). The remaining emissions are source to other poultry species and non-edible products. Feed production and processing, and enteric fermentation from ruminants are the two main sources of emissions, representing 45 and 39% of sector emissions, respectively. Manure storage and processing represent 10%. The remainder is attributable to the processing and transportation of animal products. Included in feed production, the expansion of pasture and feed crops into forests accounts for about 9% of the sector's emissions. The consumption of fossil fuel along the sector supply chains accounts for about 20% of sector emissions (Gerber *et al.*, 2013).

Emission intensities (i.e. emissions per unit of product) are highest for beef (almost 300 kg CO₂-eq per kg of protein produced), followed by meat and milk from small ruminants (165 and 112 kg CO₂-eq.kg respectively). Cow milk, chicken products and pork have lower global average emission intensities (below 100 kg CO₂-eq/kg). At the global level, specialized beef meat production is most emission intensive (67.8 kg CO₂-eq/kg), followed by small ruminant meat (23.8 kg CO₂-eq/kg) and dairy meat (18.4 kg CO₂-eq/kg), and the emission intensities are consistently lower in most affluent countries (Gerber *et al.*, 2013). Monogastric production not only contributes a smaller share of total emissions, it is also much less emission intensive: the average emission intensities of chicken and pig meats are 5.4 kg CO₂-eq and 6.1 kg CO₂-eq per kg meat, respectively. Enteric emissions and feed production (including manure deposition on pasture) dominate emissions from ruminant production. In pig supply chains, the bulk of emissions are related to the feed supply and manure storage in processing, while feed supply represents the bulk of emissions in poultry production, followed by energy consumption. About 44 percent of livestock emissions are in the form of CH₄. The remaining part is almost equally shared between N₂O (29%) and CO₂ (27%). This means that livestock supply chains emit: 2 Gt CO₂-eq of CO₂ per annum, or 5% of anthropogenic CO₂ emissions, 3.1 Gt CO₂-eq of CH₄ per annum, or 44% of anthropogenic CH₄ emissions and 2 Gt CO₂-eq of N₂O per annum, or 53% of anthropogenic N₂O emissions (IPCC, 2007). All manure emissions were accounted for approx. 1.8-2.2 Gt CO₂-eq and land use change accounted for 0.65-2.4 Gt CO₂-eq.

The huge animal populations, especially the ruminants, are a big source of CH₄ because mainly raised on fibrous feeds. The oil cakes fed to ruminants as protein source, especially rape/

mustard and groundnut cake are highly degradable in rumen. In the absence of sufficient supply of starchy feeds like grains in their diet, the rumen microbes are unable to capture the large quantities of ammonia produced in rumen for protein synthetic process. The excess ammonia is thus, absorbed from the rumen wall and partly excreted out as urea. The loss of N as urea is not only a wastage of dietary proteins, but even it may pollute the environment through N_2O emission. Apart from that, both CH_4 and N_2O are emitted through the burning of dung cakes and crop residues.

Indian livestock scenario:

India possesses the largest livestock population in the world and accounts for the largest number of buffaloes (113 m, 57.8%) and goats (157 m, 17.0%) along with second highest number of cattle (211 m, world share 14.8%) and sheep (75 m, 6.8%) in the world in 2011 (FAOSTAT, 2013). Livestock production systems are mostly based on low cost agro-byproducts and traditional technologies. Resource-poor small and marginal farmers and landless labourers maintain majority of the ruminants (DAHD, 2012). India emerged as the largest contributor to the livestock CH_4 budget, simply because of its enormous livestock population, although the emission rate per animal in the country was much lower than in the developed countries. For instance, the annual CH_4 production per animal was estimated to be 95 kg for the dairy cows in Germany, nearly threefold higher than 35 kg for the Indian cattle (Crutzen *et al.*, 1986). The differences in per head emissions are due to lower level and poor quality of feed intake in India. The estimates of total enteric emissions from Indian livestock vary widely from 6.17 to 11.75 Tg/year (mean about 10 Tg/ yr; differences in reference year and emission rates used; ALGAS, 1998; Mohini and Singhal, 2010; INCCA, 2010; Chhabra *et al.*, 2013). Bovines contribute a bulk of the CH_4 emission from enteric fermentation i.e. cattle and buffalo, followed by small ruminants like goat and sheep and a negligible emission from other categories. Among cattle, although the emission coefficients for indigenous dairy and non-dairy animal is lower than exotic animals, but the emissions from indigenous dairy and non-dairy cattle is higher than exotic dairy and non-dairy cattle owing to their higher population. Dairy buffalo have higher population as well as emission coefficient as compared to non-dairy buffalo, resulting to nine fold higher CH_4 emissions. The CH_4 emission from manure management accounts for 9-10% of the total livestock based CH_4 emissions. The proportional contribution of dairy buffalo, indigenous dairy cattle, indigenous non-dairy cattle, exotic dairy cattle, other livestock, non-dairy buffalo and

exotic non-dairy cattle is 37.8, 22.2, 19.8, 7.8, 7.3, 4.2, and 1.04%, respectively for the year 2003 (Chhabra *et al.*, 2009).

Emissions from manure management:

The total global CH₄ emissions from livestock manure management have been estimated as 9.3 Tg/year (Scheehle, 2002), of which the developed countries contribute about 52%. The sharply different manure management practices in India, as compared to the western countries, lead to much lower CH₄ emissions from manure. Cattle and buffalo manure is extensively used in the country as fuel and is largely managed in dry systems. The emissions for India are estimated to be 0.905 Tg/year in 1990 (ALGAS 1998; Scheehle, 2002), and about 0.27 Tg in the year 2003 (Mohini and Singhal, 2010). Indian livestock contributed 0.99 Gg/year for the year 1997 (Swamy and Bhattacharya, 2006), 1.42 Gg/year during 2003 (Chhabra *et al.*, 2009), 1.09 Tg (Chhabra *et al.*, 2013) of N₂O per year. The latter also reported that poultry, pigs, indigenous cattle and exotic cattle contribute 86.1, 7.3, 5.7 and 1.0% to the total N₂O emissions.

Effect of global climate change on livestock:

The organ systems of animals respond to physical, chemical, biological and climatic stimuli from their surroundings and work in concert to perform the essential body functions. Climate change, directly and indirectly, alters different environmental components like temperature, radiations, humidity and wind etc. which in turn adversely affect performance (e.g., growth, milk and wool production, reproduction), health and well-being of livestock.

Main effects:

- Change in livestock feed, fodder and water availability
- Change in the distribution of livestock diseases and pests
- Direct effect of environmental factors on animal health, production and reproduction performances
- Increased intensity of extreme events will harm livestock population

Heat stress:

Global warming will alter heat exchange between animal and environment, and consequently affecting feed intake, mortality, growth, reproduction, maintenance, and production of animals. Near the thermal comfort zone (5–25°C) there are no significant changes in feed intake and numerous physiological processes. Hot and humid conditions can cause heat stress in livestock inducing behavioral and metabolic changes, including reduced feed intake and thus a decline in

productivity. There have been several occasions in the last twelve years when heat waves have caused substantial mortality in livestock population (Sirohi and Michaelowa, 2007). The vulnerability of livestock to heat stress varies according to species, genetic potential, life stage and nutritional status. Increasing temperatures at higher latitudes are generally going to have greater impacts on livestock than at lower latitudes, where local livestock breeds are often already quite well-adapted to heat stress and drought. Increasing intensification of dairy systems in the developing world through the use of temperate-breed genetic stock could lead to greater vulnerability to increasing temperatures, however. There is strong correlation among heat stress, physical activity and feed intake (Mader and Davis, 2004; Baumgard, and Rhoads, 2013). In addition, high temperatures as well as reduced feed intake put a ceiling on dairy milk yield irrespective of feed intake, and in the tropics, this may be between half and one-third of the potential of modern cow breeds (Parsons *et al.*, 2001). Increased energy deficits may decrease cow fertility, fitness and longevity (King *et al.*, 2006). Chase (2006) indicated that the maintenance energy requirement of a dairy cow is increased by 22% at 32°C compared with the energy requirement at 16°C; for the same temperature increase, predicted DM intake decreases by 18% and milk yield decreases by 32%. Heat stress reduced the conception rates of *Bos taurus* cattle (Amundson *et al.*, 2005). Similarly, the impacts of increased frequencies of extreme heat stress on existing livestock breeds are not known, nor do we know if there are critical thresholds in the relationship between heat stress and physiological impacts. Over this longer term, ongoing genetic improvement through both natural and artificial selection should allow a certain degree of adaptation to gradual changes in climate to occur. Estimates of livestock production efficiency suggest that negative effects of hotter weather in summer outweigh positive effects of warmer winters. The largest change occurred under a 5°C degree increase in temperature, when milk yields fell by 10-15% in cattle and buffaloes. Short-term extreme events (e.g., summer heat waves, winter storms) can result in the death of vulnerable animals. The conception rates of dairy cows are also reduced by as much as 36% during summer season. With global warming, an additional decline in milk production of about 5–14% (beyond expected summer reductions) may occur particularly in the hot/hot-humid southern regions of the United States.

Effect on quantity and quality of feeds:

Climatic conditions affect the quality and quantity of forage produced as evidenced in many parts of India and around the globe. Climate change may exert several impacts on feed crops and

grazing systems like changes in herbage growth pattern due to changes in atmospheric CO₂ concentrations and temperature, changes in the composition of pastures (i.e. ratio of grasses to legumes), changes in herbage quality etc (Hopkins and Del Prado, 2007). The impact of climate change on pastures and rangelands may also include deterioration of pasture as a result of warmer temperature and less frost. Simulations of the impact of climatic change on rice and wheat yields indicated that in north India, a 2°C rise in mean temperature would reduce potential grain yields of both the crops by about 15-17% (Hundal and Kaur, 2007).

At higher latitudes, rising temperatures may prolong the growing season, whereas in lower latitudes may result in more water stress for plants. Small changes in temperature can alter the species composition of grasslands significantly and often result in changes in livestock productivity. Increased temperatures increases lignifications of plant tissues and therefore reduce the digestibility and the rates of degradation of plant species. Projected increased temperature and reduced precipitation will lead to increased loss of domestic herbivores during extreme events in drought-prone areas. The feed and fodder deficit in India is 22% for dry fodder, 62% for green fodder and 64% for concentrates (GOI, 2002). These shortages would be further aggravated by the adverse effects of climate change. Worse consequences would be seen in regions where high temperature is associated with decline in rainfall, increased evapotranspiration or increase in the incidence of droughts, e.g. a drought in the arid state of Rajasthan in 1999–2000 affected about 34.5 million cattle and damaged 7.8 million ha of cropped area and fodder availability fell from 144 to 127 million tons (CSO, 2000). The shift of animal feed will further go towards crop byproducts, residues and cultivated green fodder.

Effect on livestock diseases and disease vectors:

Microbial agents and their vector organisms are sensitive to factors such as temperature, humidity, precipitation, surface water, wind and change in vegetation. There may be several impacts of climate change on the vectors of disease (midges, flies, ticks, mosquitoes and tse-tse are all important vectors of livestock disease in the tropics) (Baylis and Githeko, 2006). Changes in rainfall and temperature regimes may affect both the distribution and the abundance of disease vectors, as can changes in the frequency of extreme events. The livestock systems are susceptible to changes in the severity and distribution of livestock diseases and parasites as potential consequences of global warming. Climate change is affecting the pattern of disease outbreak as the vector borne diseases (blue tongue) and new areas are coming into the net of some diseases

unknown to the area like emergence of mosquito borne diseases (e.g. Rift valley fever) in the areas where previously they could not breed effectively to be present there (Jones *et al.*, 2008). Humid and tropical conditions are more prone to stress and infectious diseases than temperate regions. Mastitis which accounts for about Rs. 28 billion at 1994 prices may be increased due to heat stress and greater fly population associated with hot-humid conditions. The meteorological parameters like temperature, humidity and rainfall have been found to explain 52 and 84% variations in the seasonality of foot and mouth disease (FMD) in hyperendemic division of Andhra and meso-endemic region of Maharashtra states, respectively (Ramarao, 1988). As crossbred animal's population is increasing, which are less tolerant to heat, climatic variables and diseases, the challenges still remain very evasive. Emergence and reemergence of infectious diseases like FMD and diseases transmitted by ticks, flies, mosquitoes, midges and other arthropods are of prime importance as transmission cycle of most of these infestations are vulnerable to climate change as exhibited by *Fasciola* and *Schistostoma* spp. (De La Rocque, 2008). Viral diseases are more vulnerable to climate change as they can undergo rapid change in their adaptation leading to variation in disease pattern as exhibited by African horse sickness, blue tongue, FMD and West Nile virus (De La Rocque, 2008).

Climate change may also affect the abundance and/or distribution of the competitors, predators and parasites of vectors themselves, thus influencing patterns of disease. The patterns of international trade, local animal transportation, farm size and land use may also affect disease transmission and could give rise to new mixtures of species, thereby exposing hosts to novel pathogens and vectors and causing the emergence of new diseases (Baylis and Githeko, 2006). Singh *et al.* (1996) reported higher incidence of clinical mastitis in dairy animals during hot and humid weather due to increased heat stress and greater fly population associated with hot-humid conditions. In addition, the hot-humid weather conditions were found to aggravate the infestation of cattle ticks like, *Boophilus microplus*, *Haemaphysalis bispinosa* and *Hyalomma anatolicum* (Basu and Bandhyopadhyay, 2004; Kumar *et al.*, 2004).

Alterations of temperature and precipitation regime may result in a spread of disease and parasites into new regions or produce an increase in the incidence of disease, which, in turn, would reduce animal productivity and possibly increase animal mortality (Baker and Viglizzo, 1998). Certain existing parasitic diseases may also become more prevalent and/ or their geographical range may spread, if rainfall increases (Epstein and Mills, 2005). The viral

infection of bluetongue disease for example was once only a threat in Africa, now affects cattle and sheep in the whole of Europe. Warming and changes in rainfall distribution may lead to changes in spatial or temporal distributions of those diseases sensitive to moisture such as anthrax, blackleg, hemorrhagic septicemia, and vector-borne diseases (Baylis and Githeko, 2006).

Impact on production performance:

Climate change is causing more ambient stressful conditions and discomfort to the animals resulting in stress in the animal and subsequently production, reproduction and health status infliction. Animal performances depend upon the genetic makeup of the individual and the ambient environment including both biotic and abiotic factors. The major climatic factors affect the animal performance directly through neuro-endocrine system influencing the homeostasis of the body including body heat balance, thermoregulation, chemical balance of water and electrolytes and blood flow (Thornton *et al.*, 2009). Increase in solar radiations increase the heat load on animals thereby reducing feed intake and energy metabolism, leading to decreased performance and increased expenditure on maintenance.

Animal growth:

Climate change negatively affects animal's growth and delays time to attain puberty. The purebred exotic and crossbred calves are more affected than the native and slow growing ones as the former are observed to be more sensitive to rise in THI than either Zebu cattle or buffaloes. Upadhyay *et al.* (2008) reported that time to attain puberty may be prolonged by 5 to 17 days due to decline in growth rate at high temperatures.

Milk production:

There is normally a decrease in milk production for cattle/ buffaloes (10 to >25%) under heat stress (Hahn *et al.*, 1992; Singh and Upadhyay, 2008, 2009) which can be transitory or long term depending on the length and severity of heat stress. The negative impact on total milk production for India has been estimated about 1.6 million tonnes in 2020 and more than 15 million tonnes in 2050. High producing animals will be affected to the greater extent than low producing animals. The decline in milk production will be higher in crossbreds (0.63%) followed by buffalo (0.5%) and indigenous cattle (0.4%; Upadhyay *et al.*, 2008). If heat stress lowers milk production in early lactation of dairy animals, potential milk production for the lactation will decreased. Studies have shown that milk yield of crossbred cows in India (e.g., Karan Fries, Karan Swiss

and other Holstein and Jersey crosses) to be negatively correlated with temperature-humidity index (Kulkarni *et al.*, 1998; Mandal *et al.*, 2002a). The average daily milk yield of the crossbred animals in the hot-humid eastern part of the country was significantly reduced by the rise in minimum temperature, as rise in minimum temperature crossed the critical temperature of comfort while the maximum temperature was already above the comfort zone (Kale and Basu 1993). The influence of climatic conditions on milk production is also observed for local cows that are more adapted to the tropical climate of India. The rising temperature decreased the total dry matter (DM) intake and milk yield in Haryana cows (Lal *et al.*, 1987). The productivity of Sahiwal cows also showed a decline due to increase in temperature and relative humidity (Mandal *et al.*, 2002b). An increase in temperature humidity index by one unit above the normal could decrease the annual milk production by about 7.85 million tonnes at the current state of knowhow and size of dairy population by 2030 (Sirohi and Sirohi, 2010).

Reproductive performance:

Heat stress decreases reproductive performance in dairy animals by decreasing length and intensity of the estrus period; decreasing conception (fertility) rate; decreased growth, size and development of ovarian follicles; decreased fetal growth and calf size; increased risk of early embryonic deaths; increased number of artificial insemination per conception and increased incidence of silent heat especially in buffaloes (Upadhyay *et al.*, 2009). Amundson *et al.* (2005) reported a decrease in pregnancy rates of *Bos taurus* cattle of 3.2% for each increase in average THI by 70, and a decrease of 3.5% for each increase in average temperature above 23.4°C. Clearly, increases in temperature and/or humidity potentially affect the conception rates of domestic animals not adapted to those conditions. The morphological and anatomical characteristics of buffaloes make them well-suited to hot and humid climates, but heat stress has detrimental effect on the reproduction of buffaloes (Kaur and Arora, 1982; Tailor and Nagda, 2005). Heat stress delays puberty in both male and female livestock and in addition affects fertility traits like prolongation of estrous cycle, weakening of estrous signs, decreased gestation period and increased fetal death rate. Temperature rise due to global warming may negatively affect reproductive functions of buffaloes and increase the incidence of silent estrus, short estrus and change reproductive rhythm and production efficiency of buffaloes (Upadhyay *et al.*, 2012).

Water:

The agricultural sector is the largest (70%) user of fresh water resources. Water scarcity is a globally significant due to its uneven distribution and resulting in problems with food/feed/fodder production, health status and economic development. By 2025, 64% of the world's population will live in water-stressed basins, compared with 38% today (Rosegrant *et al.*, 2002). Water use in the livestock sector includes not only the water used at farm level for drinking and the growing of feed crops, but also other servicing and product processing roles. The negative effects of increased precipitation variability will temper the beneficial impacts of increased annual runoff in other areas and seasonal runoff shifts on water supply, water quality, and flood risks. Climate change will also affect groundwater recharge rates. Water intake is about 3, 5 and 10 kg/kg DM intake in *Bos indicus* and 3, 8 and 14 kg/kg DM intake in *B. taurus* at ambient temperature of 10, 30 and 35°C, respectively (NRC, 1981). Some of this water intake comes from forage, and forage water content itself will depend on climate-related factors like species and weather conditions. Thus, effect of climate change on water intake and its usage in livestock sector will have considerable spatial variation.

Biodiversity:

Globalization and climate change are already having substantial impacts on biodiversity. The 2007 FAO report on animal genetic resources indicates that 20% of reported breeds are classified at risk, and that almost one breed per month is becoming extinct and the intensity is more in regions having highly specialized livestock industries as their production is dominated by a small number of breeds (CGRFA, 2007). A 2.5°C increase in global temperature above the pre-industrial level may see major biodiversity losses as about 20–30% of all plant and animal species would be at high risk of extinction with such a temperature rise. The impacts of such losses are difficult to imagine, and the problems that will be caused by the loss of genes for disease and pest resistance, for environmental adaptation, and for other desirable traits, cannot be overstressed (Steinfeld *et al.*, 2006). Pastoralists and smallholders are the guardians of much of the world's livestock genetic resources and they will be most affected by climate change (CGRFA, 2007).

Conclusions:

Livestock is an integral part of human civilization as it plays a very important role in the economic progress of the nearly 1.3 billion people worldwide. It contributes to the climate

change by emitting GHGs through enteric fermentation, waste disposal and various related activities and at the same time livestock is very sensitive to climate change posing an increasingly formidable challenge to animal production and well-being, thereby the development of the livestock sector. Responding to the challenge of climate change requires formulation of appropriate adaptation and mitigation options like development of management strategies, efficient utilization of dietary carbohydrate and nitrogen, enhancement of feed efficiency and animal productivity, along with reducing global warming effects of emitted gases. Therefore, awareness programmes and more research work is needed in this direction to suggest the appropriate feeding, managemental and breeding practices for livestock to overcome the expected impact of climate change on animals production in coming years (2050/ 2100) as to ensure a sustainable development and coexistence.

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Feeding Strategies for Cattle and Buffalo under Climate Change Scenario for Sustaining Productivity

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In tropical and subtropical regions, heat stress, if not properly managed, can have a significant deteriorating impact on the production and health of dairy cattle and buffaloes. These adverse climatic conditions not only lead to reduction in feed intake which directly affect level of milk production, but also adversely affect the reproductive performance. Lactating dairy cows create a large quantity of metabolic heat and accumulate additional heat from radiant energy. Due to the increased heat production along with accumulation, cooling capability of the animal are compromised because of environmental conditions thus add heat load in the cow even to the extent that body temperature rises, intake declines and ultimately the cow's productivity declines. With improved balanced feeding systems the effects of summer stress can be reduced, ultimately improving animal performance, thus lowering the exposure of financial risks.

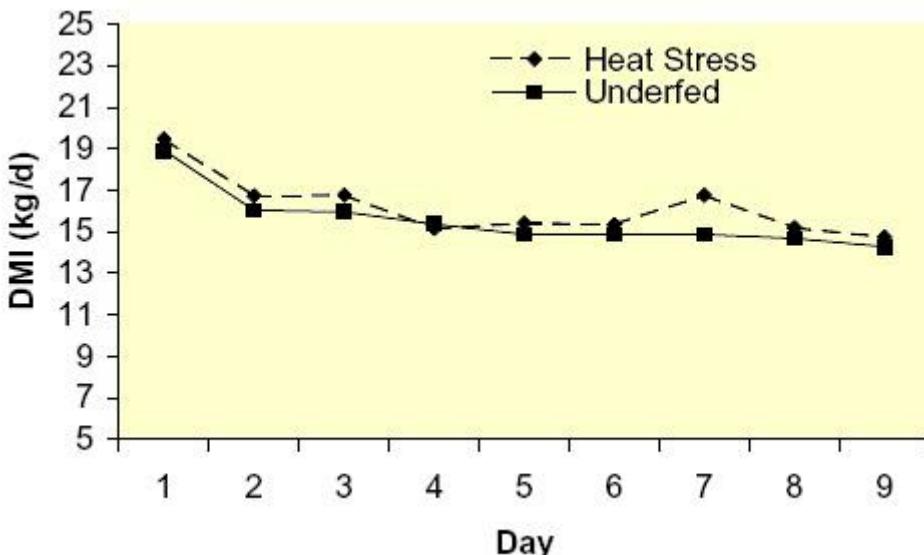
The effects of the ambient environment on cow performance have been measured by establishing critical ambient temperatures for the cow an equivalent temperature index incorporating temperature, humidity, and air velocity and temperature humidity index (THI), which has the combined effects of temperature and relative humidity. Studies established that milk yield and DMI exhibited significant declines when maximum THI reached 77 with significant negative correlation between THI and DMI. Effect of THI is probably mediated through the effects of increasing body temperature on cow performance.

Three management strategies to minimize the effects of heat stress are often recommended: (1) physical modification of the environment (shading, cooling), (2) improved nutritional management practices, (3) development of heat-tolerant breeds, and based on current knowledge, it appears that a combination of these practices may be necessary to optimize production of dairy cows in hot, humid climates. In this chapter, feeding strategies are discussed.

Metabolic effect of heat stress on DMI and energy balance:

Although the effect heat stress on production and reproduction can partly be explained by reduced feed intake; but it also includes altered endocrine status, reduction in rumination and

nutrient absorption, and increased maintenance requirements (Collier and Beede, 1985; Collier *et al.*, 2005) resulting in a net decrease in nutrient/energy availability for production. Considerable evidence suggests increased maintenance costs up to 10 to 15 % are associated with heat stress (ICAR, 2013) which is as a result of large energetic cost of dissipating stored heat. Due to non consideration of a heat stress correction factor it results in overestimating energy balance; thus inaccurately predicting energy status. Decreased reductions in feed intake, availability of energy and increased maintenance costs results in reduction in energy balance (EBAL), thus (reduced gut fill also contributes) have an explanation for loss of body weight in heat stressed animals. The problem gets aggravated in a majority of lactating cows entering into negative energy balance (NEBAL), and this is likely independent of stage of lactation. 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 (Goff and Horst, 1997; Drackley, 1999), decreased milk yield, and reduced reproductive performance (Lucy *et al.*, 1992; Beam and Butler, 1999; Baumgard *et al.*, 2002; 2006). It is likely that many of the negative effects of heat stress on production, animal health, and reproduction indices are the outcome of the negative energy balance. However, it is not clear how much of the reduction in performance (yield and reproduction) can be attributed to or accounted for by the biological parameters effected by heat stress (i.e. reduced feed intake vs. increased maintenance costs). It is indicated in studies by Wheelock *et al.* (2006) that heat stressed cows had an immediate reduction (~5kg/d) in dry matter intake (DMI) with the decrease reaching nadir at ~ day 4 and remaining stable thereafter (Figure 1). As expected and by design, thermal-neutral pair-fed cows had a feed intake pattern similar to heat stressed cows (Figure 1).



Effects of heat stress and pair-feeding thermal-neutral lactating Holstein cows on dry matter intake (DMI); (Rhoads *et al.*, 2007)

During heat stress cattle will have reduced feed intake and with severe heat stress dry matter intake (DMI) can decrease by up to 50%.

The Effect of DMI at Differing Temperatures:

Temperature °F	Expected DMI (% compared to 68 °F)
68	100.0
86	93.5
95	91.7
104	56.0

With a decrease in DMI and Poorer feed efficiencies, animal performance adversely affected in heat stress cattle. High producing cows are the animals most sensitive to heat stress because of their high feed intake. Dry matter intake starts to drop (8-12%) and milk production losses of 20-30%, besides milk production, conception rate is lower due to less activity during estrus, reduced follicular activity, and early embryonic death. It is associated with difficult births, heat exhaustion, fatty liver in fresh cows, and mastitis, as well as adverse reactions to vaccinations leading to abortions and death. Heat stress can result in sick cows which require prolonged care.

Results of the experiments conducted by Wheelock *et al.* (2006) suggests that heat stressed cow's exhibited the similar energy balance or retention pattern as that of under fed ones.

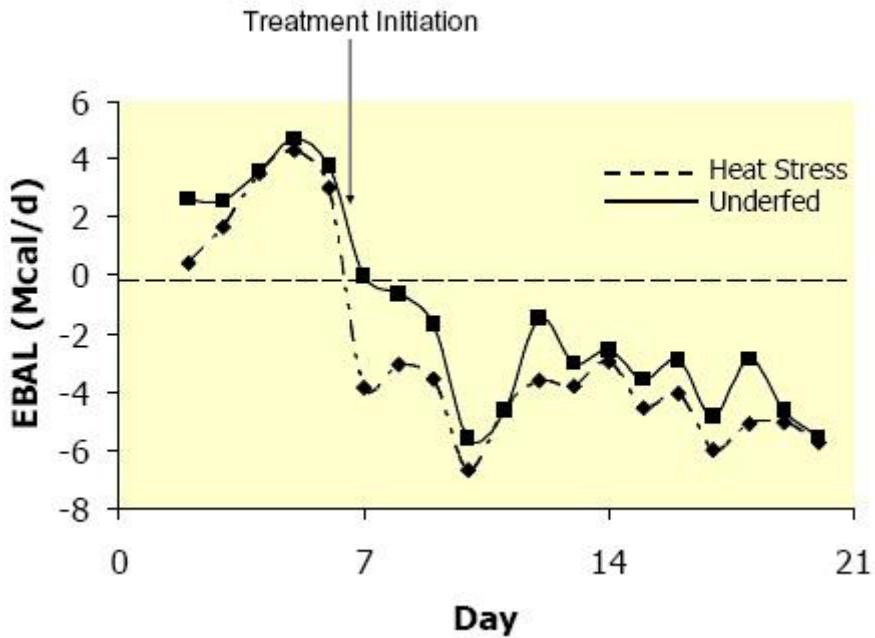


Fig 1: Effect of heat stress and pair-feeding thermal-neutral conditions on calculated net energy balance in lactating Holstein cows (Wheelock *et al.*, 2006.)

Effect of heat stress on rumen health:

Apart from reduction in feed intake, thermal stress has an adverse affect rumen health. It is the normal phenomenon that animals lose heat from the body via conduction/convection, radiation, evaporation. As ambient temperatures rise, the respiratory rate increases with panting progressing to open-mouth breathing. Due to increase in respiration rate, more amount of carbon dioxide (CO_2) is being exhaled. A consequence respiratory alkalosis occurs resulting from a rapid loss of carbon dioxide. For an effective blood pH buffering system, 20:1 ratio of bicarbonate (HCO_3^-) to CO_2 is preferred. In order to combat decrease in blood CO_2 concentration, the cow compensates it by increasing urinary output of bicarbonate, which affects rumen buffering capacity by decreased salivary bicarbonate pool thus adversely affect rumen pH.

In addition, panting cows drool and drooling reduces the quantity of saliva that normally would have been deposited in the rumen. Not only these reasons, but also due to reduction in feed intake, heat-stressed cows tend to ruminate less and therefore generate less saliva. The reductions in the amount of saliva produced, as well as reduced salivary HCO_3^- content and decreased amount of saliva entering the rumen make the heat-stressed cow much more susceptible to sub-clinical and acute rumen acidosis (see review by Kadzere *et al.*, 2002).

When cows begin to accumulate heat, there is a redistribution of blood to the extremities in an attempt to dissipate internal energy. As a consequence, there is reduced blood flow to the gastrointestinal tract and nutrient uptake may be compromised. Therefore, fermentation end products (volatile fatty acids; VFA) probably accumulate and contribute to the reduced pH.

Due to the reduced feed intake during heat stress and the heat increment associated with fermentation and utilization of roughages , the alternative of choice is to increase the energy density of the ration by supplementation with extra concentrates followed by reduction in forages especially the dry ones . However, this changes are to be made with utmost care as such modifications can be associated with a lower rumen pH and the cow's reduced ability to neutralize the rumen (because of the reduced saliva HCO_3^- content and increased drooling) directly increases the risk of rumen acidosis and indirectly enhances the risk of negative side effects of an unhealthy rumen (i.e. laminitis, milk fat depression, etc.).

Metabolic adaptations for combating reduction in feed intake due to heat stress:

In order to metabolically adapt to state of lowered feed intake, the animals draw the nutrients/energy from their body reserves. This condition of the deficit energy is normally seen in lactating animals in their early phases. Negative energy balance is associated with a variety of metabolic changes that are implemented to support the dominant physiological condition of lactation (Bauman and Currie, 1980). Conspicuous alterations in both carbohydrate and lipid metabolism ensure partitioning of dietary derived and tissue originating nutrients towards the mammary gland. A report suggests that many of these changes are mediated by somatotropin hormone, which is increased during periods of NEBAL (Bauman and Currie, 1980). Besides, there is a reduction in circulating insulin as well as-systemic insulin sensitivity. The reduction in insulin action allows for adipose lipolysis and mobilization of non-esterified fatty acids i.e NEFA (Bauman and Currie, 1980). Higher concentration of NEFA is typical in transition animals which is a significant source of energy (and are precursors for milk fat synthesis) for cows in NEBAL. Post-absorptive carbohydrate metabolism is also altered by the reduced insulin action during NEBAL, with the net effect of reduced glucose uptake by systemic tissues (i.e. muscle and adipose). The reduced nutrient uptake, along with the net release of nutrients (i.e. amino acids and NEFA) by systemic tissues, are major homeostatic mechanisms adopted by dairy animals in NEBAL to support lactation (Bauman and Currie, 1980). The thermal-neutral cow in NEBAL is

metabolically flexible, in that she can depend upon alternative fuels (NEFA and ketones) to spare glucose, which can be utilized by the mammary gland to copiously produce milk.

Wheelock *et al.* (2006) applied glucose tolerance test to demonstrate that glucose disposal (rate of cellular glucose entry) is greater in heat-stressed compared to thermal-neutral pair-fed cows (Figure 2). The workers also observed NEFA concentration pattern during heat stress and compared with underfed animals (Figure 3).

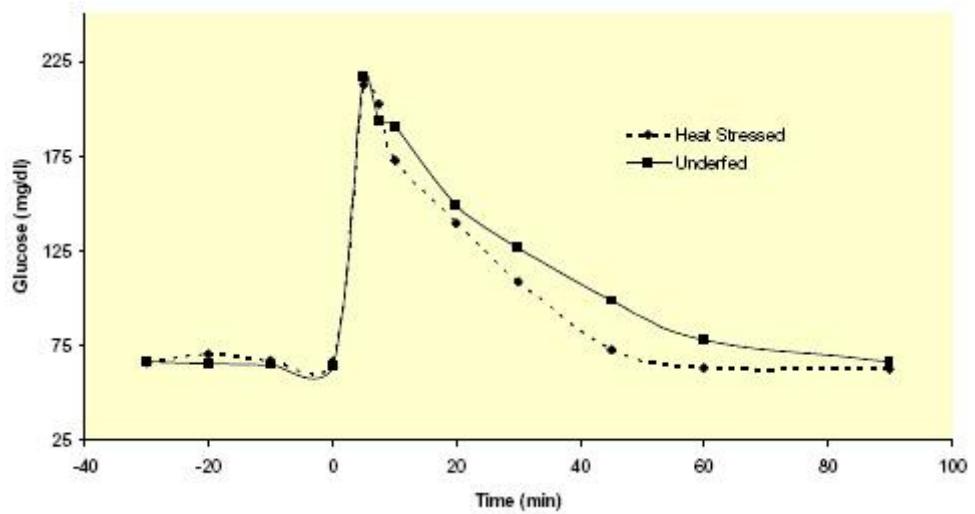


Fig 2: Effect of heat stress and pair-feeding thermal-neutral conditions on plasma glucose response to a glucose challenge (Wheelock *et al.*, 2006)

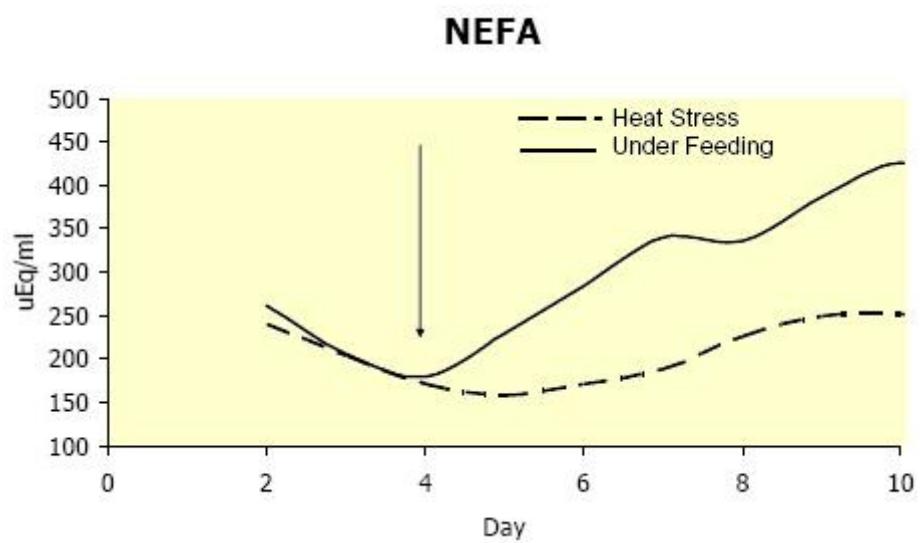


Fig 3: Effect of heat stress and pair-feeding thermal-neutral conditions on circulating nonesterified fatty acids (NEFA) in lactating cows (Wheelock *et al.*, 2006)

Effect of heat stress on production:

Heat stress reduces both feed intake and milk yield. The decline in nutrient intake has been a major cause of reduced milk synthesis (Fuquay, 1981); however, the exact contribution of declining feed intake to the overall reduced milk yield remains unknown. Glucose is required by the mammary gland for lactose synthesis and lactose production is the primary osmoregulator and thus determinant of milk yield. However, in an attempt to generate less metabolic heat, the body (mainly skeletal muscle) appears to utilize glucose at an increased rate. As a consequence, adequate amounts of glucose are not available for the mammary gland thus mammary lactose production and subsequently milk yield is reduced. This may be the primary mechanism which accounts for the additional reductions in milk yield that cannot be explained by decreased feed intake (Figures 4).

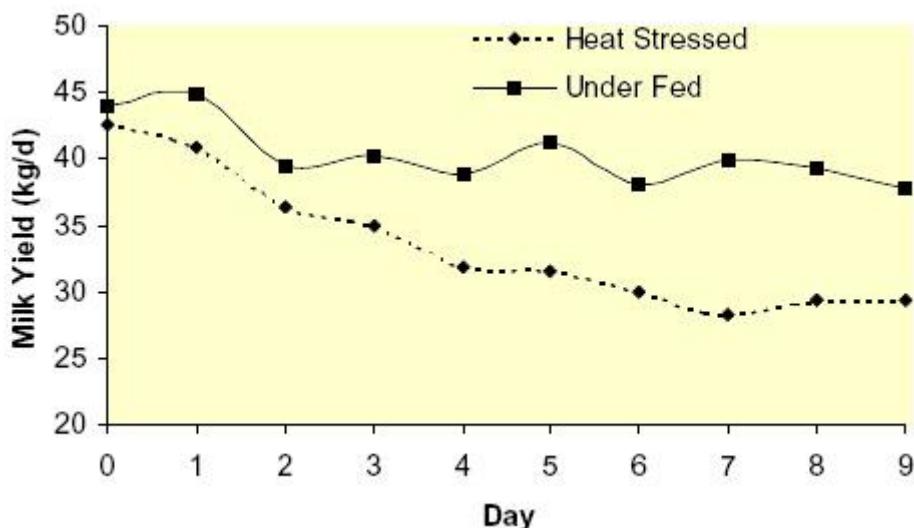


Fig 4: Effect of heat stress and pair-feeding thermal-neutral conditions on milk yield in Holstein cows (Rhoads *et al.*, 2007)

Nutritional strategies for alleviation of thermal stress:

Feeding management:

Reduced feed intake, followed by slug feeding when the temperature cools down, can cause acidosis which is considered a major cause of laminitis. Heat stressed cattle eat less frequently during hotter hours so feeding during cooler times of the day is one option. Increased frequency of feeding (an extra feeding or two), time of feeding (cooler time of day), adequate feed bunk space (all cows can eat together without crowding) are the important considerations.

In order to maintenance homeostasis during heat stress the animals may alter their requirements for some additional nutrients and energy. The following measures could be taken for maintaining productivity during heat stress.

Water and its requirement:

Clearly, water intake is vital for milk production (milk is ~90 % water), but it is also essential for thermal homeostasis. Cold drinking water in rumen increases feed intake by 24%. It also helps in lowering both rectal and tympanic membrane temperatures. As milk production and DMI increase, water intake also increases. At ambient temperature above body temperature, there is decrease in water intake, mainly due to reduced DMI and lower muscular activity. The animals fulfil their water requirement from (1) metabolic water which is produced as a result of tissue oxidation of organic substrates (2) water contained in ingested feeds and (3) drinking water. The latter is quantitatively most significant in fulfilling the needs of thermal-stressed animals. Water content in feed and fodders are highly variable, depending on fodder crops, season and feeding management system. Increased water consumption is a major response to thermal stress (McDowell, 1972). Drunk water has pronounced effect on animal comfort by direct cooling in the reticulo-rumen (Bianca, 1964), and by serving as the primary vehicle for heat transfer and dissipation through sweating and panting. McDowell (1972) showed how total body water balance was affected during thermal stress in animals. . Keeping water tanks clear of feed debris and algae is a simple and cheap strategy to help cows remain cool.

Energy:

As explained earlier, the heat-stressed cow is prone to rumen acidosis and many of the lasting effects of warm weather (laminitis, low milk fats etc.) can probably be traced back to a low rumen pH during the summer months in which high energy feeds i.e fats are to be increased and fiber which is culprit for increasing heat increment has to be restricted (however both the alterations are to be made judiciously; discussed below)

Fat:

The advantage of adding fat in the diet during hot weather, improved efficiency of energy use and greater energy intake as fats have 2.25 times greater in energy than carbohydrates. Fat is more efficiently utilized as a source of energy than other feeds as it produces less heat (heat increment) during digestion and utilization. Supplementation of fat more than 6% of dry

matter intake may affect rumen fermentation adversely. Thus, in ration of very high producing animals, fat supplementation must be more than 6-8% of ration.

This supplemental fat can also be made from whole seeds such as cottonseeds or soybeans, tallow, rumen inert sources, or combinations. Most diets will contain about 3% fat (dry basis) without any high fat feeds. The next 2 to 3% fat can come from whole seeds. This results in rations with 5 to 6% fat. Anything above this should be added as rumen inert fat or bypass fat (commercial sources are available). Rations should not exceed 7 to 8% of the overall dry matter as fat. Because fatty acids reduce the intestinal absorption of calcium and magnesium, requirements for these two minerals increase when fats are fed. Supply .9% calcium and .35% magnesium in the ration when fats are fed. Also avoid feeding excess fat especially in hotter weather. Overfeeding can cause problems with rumen function and can reduce dry matter intake.

Nutritional technologies such as formation of calcium soaps of fat viz., bypass fat helps in maintaining the level of milk production in lactating cows and buffaloes during hot and humid season when the animals are unable to consume enough energy which leads to drastic reduction in milk yield. In production systems where considerable forage is incorporated into diets, addition of lipids might be one of the ameliorative measures to maintain production by maintaining the energy levels of animals during heat stress (Kundu *et al*, 2008).

Fiber:

Feeding low fiber diets during the hot dry summer and hot humid season will improve DMI, milk production and help in reduction of negative effect of heat stress. However, a minimum amount (18% acid detergent fibre) of effective, high quality fiber needs to be fed to maintain normal rumen function. High quality forages are the best source of digestible fiber that is effective and produces minimum heat when fermented in the rumen. Feeding forages in a Total Mixed Ration (TMR) is recommended during heat stress, so that cattle consume sufficient forage intake relative to grain and concentrates when given a choice. Shojaeian *et al.* (2007) supplemented exogenous fibrolytic enzymes to the concentrate part of the diet of dairy cows and reported an increase in milk production of around 10-12% in treatment group over control group.

Protein:

It is best to feed a ration balanced for ruminal and post ruminal digestion. Overfeeding protein during hot weather should be avoided because it takes energy to excrete excess nitrogen. Rumen degradable protein should not exceed 60 percent of total protein. A possible reason why high degradable protein diets are deleterious during heat stress is rumen motility and rate of passage decline allowing for a longer protein residence time in the rumen and more extensive degradation to ammonia. Any ammonia in excess of what is normally needed for metabolism in the body is an energy cost and increases heat production as it is metabolized to urea and excreted in the urine. Metabolism studies indicated that acutely heat-stressed animals were in negative nitrogen balance, largely due to reduced intake. Feeding of formaldehyde treated mustard cake showed 15% increased in milk production over control in crossbred cows (Walli *et al.*, 2005). Increasing protein content especially of bypass nature in diet of thermal-stressed animals would be advantageous. However, because of reduced energy consumption and increased energy maintenance requirement during heat stress, supplemental natural protein may also be metabolized to meet energy requirements of the animal.

Vitamins:

Thermal stress caused reduction of around 30% in hepatic vitamin A stores of animals. Reduction of liver vitamin A content occurred whether animals were in sufficient vitamin A status before being subjected to thermal stress, or if they were first depleted by feeding vitamin A deficient diet for 105 d before thermal stress was imposed. Direct effects of heat stress on requirements of other vitamins and related potential changes in intermediary metabolism have not been characterized. However, with reduction in feed consumption associated with thermal stress, supplementation of vitamins to absolute amounts prescribed for normothermic animals, at least, would seem warranted. Further assessment will be required to ascertain if thermal stress increases requirements for various vitamins and emphasizes additional supplementation above normal recommendations. Some nutritionists have suggested raising levels of supplemented vitamins during heat stress. However, supplementing 100,000 international units (IU) of vitamin A per day, 50,000 IU of vitamin D, and 500 IU of vitamin E, it would not appear that more would do any good. Cows can manufacture vitamin D with exposure to sunlight and summer is a time where we might need less supplementation. Se in concentration with Vitamin E are the best antioxidants which prevent the formation of peroxides from unsaturated fatty acids and maintain the structural

and functional integrity of biological membranes and are more effective during heat stress (Sandhu and Singha, 2003).

Minerals:

Hot weather increases the need for certain minerals (Harris, 1992). This is due to increased sweating and urination resulting in more minerals being excreted. The electrolytes i.e. sodium (Na) and potassium (K) are important in the maintenance of water balance, ion balance and acid-base status of the heat stressed animals. Unlike humans, bovines utilize potassium (K^+) as their primary osmotic regulator of water secretion from their sweat glands thus results in a considerable loss of K. One percent K in the dietary DM appears to be too low during heat stress. Milk production increase by 3 to 9% and increase in DMI intakes have been recorded when K level in the dietary DM were 1.2% or higher. Increased Na in diets to 0.45% or greater improved milk production (7 to 18%) more than increasing K during heat stress periods. Animals reduce their voluntary feed intake during thermal stress and therefore the mineral intake may be less than optimum required for productivity. Also, associated nutritional-physiological ramifications may affect macro mineral needs (Schneider *et al.*, 1986). Climatic chamber studies indicated an increased sweating during hyperthermia also increased loss of K in skin secretions (Jenkinson and Mabon, 1973). Under natural subtropical conditions, it causes a fivefold increase in relative K loss from skin which was measured during peak thermal stress. Additionally, Jenkinson and Mabon (1973) noted marked increases in rates of loss of Na, Mg, Ca and Cl, but not P, and significant correlations of these losses with sweating rate. For lactating cows fed complete mixed diets, supplementation of K (Mallonee *et al.*, 1986) and K and Na (Schneider *et al.*, 1986) above current recommendations (NRC, 2001). During prolonged exposure to 35°C in climate rooms, plasma aldosterone concentrations of non-lactating Holstein cows were 40% lower than at 20° C. Thermal stress also caused reduction of serum and urinary K concentrations. It was suggested that the fall in serum K may have been related to depressed aldosterone secretion, possibly reducing urinary K losses. Reductions in serum and urine K concentrations may be related to increased need for K in sweat also. Urinary Na excretion also increased. Therefore, under heat stress, cattle increase Na excretion while decreasing K losses. These physiological events are consistent with the premise of increased K and Na requirements during thermal stress. In addition, dietary levels of sodium (Na⁺) and magnesium (Mg⁺) should be increased as they compete with K⁺ for intestinal absorption (West, 2002).

Feeding of dietary Acid-Base balanced rations:

Alteration in acid-base balance during thermal stress is very common phenomenon in cattle. It appears that keeping the DCAD at a healthy lactating level (~ +20 to +30 meq/100 g DM) remains a good strategy during the warm summer months (Wildman *et al.*, 2007). Ramifications of this balance may include blood acid-base imbalance plus a decrease in the salivary bicarbonate pool available for ruminal buffering. Rumen pH is lowered during thermal stress (Niles *et al.*, 1980). Schneider *et al.* (1986) showed enhanced lactational performance of heat-stressed lactating cows fed high concentrate (60 to 70%) diets by providing 0.85 to 1.0% dietary sodium bicarbonate, presumably buffering the rumen and maintaining a higher ruminal pH. During thermal stress, lactating dairy cows were extremely resilient to challenges to acid-base balance by dietary or environmental stressors. Schneider *et al.* (1986) indicating higher blood pH and decreased blood partial pressure of carbon dioxide and bicarbonate concentration, indicative of thermally induced respiratory alkalosis. Kronfeld (1979) suggested supplementation of an acetogenic agent (e.g., ammonium chloride or ammonium sulfate) to correct alkalosis. In thermally stressed ruminants fed sodium bicarbonate (to buffer the rumen), dietary addition also of an acetogenic agent might assist in maintaining blood acid-base homeostasis.

Buffers:

Feeding of buffers can be beneficial during heat stress periods for two ways. I: if fiber content of the diet is minimized and/or animals are selecting against eating forages, buffers can help to prevent a low rumen pH and rumen acidosis problems and II: the most common macro mineral in a buffer is usually Na; exception of K in KHCO_3 , which when increased in diets fed during heat stress has increased DMI and milk production. In case of lactational stress and heat stress, buffers help to make the transition to high concentrate rations after calving. The risk of acidosis is greatest at this time, where in the latter case, it can reduce dry matter intake and affect electrolyte balance. Buffers can help restore feed intake and replace lost electrolytes. e.g. Sodium bicarbonate. They increase the dry matter intake and stabilize rumen pH. Usually fed for 120 days postpartum with diets that are high in wet rations (over 55% moisture), lower fiber ration (<19% ADF), little hay (<2.5 kg), finely chopped forage, pelleted grain and during heat stress conditions.

Fungal cultures:

Arizona researchers have shown feeding *Aspergillus oryzae* reduced heat stress in cows through lowering rectal temperatures. It stimulates fiber digesting bacteria, stabilize rumen pH, and reduce heat stress. It is fed to cows during high grain diets, low rumen pH conditions, and under heat stress and for calves receiving a liquid diet, at the rate of 3 g per day.

Niacin:

Feeding niacin during the summer, increased milk production across all cows by an average of about 1Kg/day but cows producing over 34Kg/day increased over 2Kg/day per day. Milk components didn't differ. The response to niacin has generally been greatest in early lactation and may be related to the role of niacin in preventing ketosis and in lipid metabolism and to its role as a coenzyme in energy metabolism.

Yeast culture and yeast:

Feed additives that have been somewhat successful in hot weather are yeast (improved fiber digestion), fungal cultures such as *Aspergillus oryzae*, and niacin (improved energy utilization). However, all of these additives would not usually be used together. Most commonly used feed additive. It stimulates fiber digestion bacteria, stabilize rumen environment, and utilize lactic acid. It is included at the rate of 10 to 120 g depending on yeast culture concentration (Dann *et al.*, 2000). It is fed for two weeks prepartum to ten weeks post partum and during off- feed and stress conditions.

Probiotics (bacterial direct-fed microbes) and prebiotics:

They produce metabolic compounds that destroy undesirable organism and provide enzymes improving nutrient availability, or detoxify harmful metabolites fed to calves on liquid diet, transition cows, and during stress conditions. They exhibit strong bioactivity and the ingestion showed to reduced infection and stress (Sartor, 2004).

Usage of glucose precursors:

Based upon some of data, maximizing rumen production of glucose precursors (i.e. propionate) would be an effective strategy to maintain production. However, due to the rumen health issue, increasing grains should be conducted with care. A safe and effective method of maximizing rumen propionate production is with monensin. In addition, monensin may assist in stabilizing rumen pH during stress situations (Schelling, 1984). Proplyene

glycol is typically fed in early lactation, but may also be an effective method of increasing propionate production during heat stress. With the increasing demand for biofuels and subsequent supply of glycerol, it will be of interest to evaluate glycerol's efficacy and safety in ruminant diets during the summer months.

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Feeding Schedule for Reduction of Enteric Methane Emission from Indian Livestock

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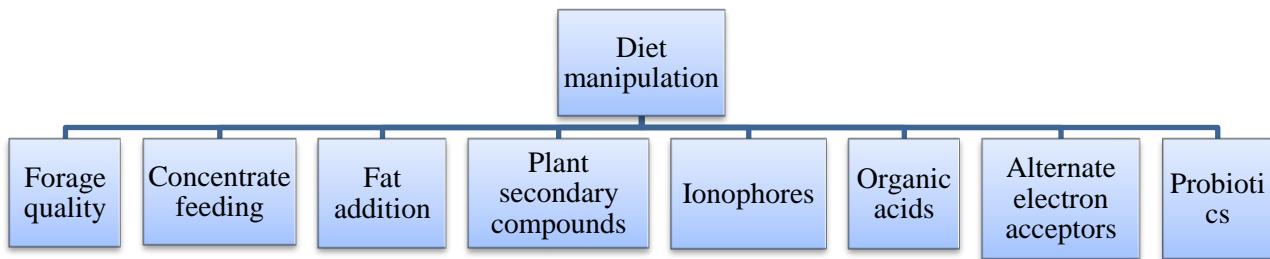
Introduction:

Green house gases (GHG) like carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) producing due to anthropogenic activities were one of the serious concerns now a days as these contributes highly to the phenomenon of global warming and other climatic changes. CH_4 is the second most important GHG after carbon dioxide, and contributes ~15 % to the global warming (IPCC, 2007). CH_4 remains in the atmosphere for ~9–15 years and is about 21 times more effective in trapping heat in the atmosphere than CO_2 over a 100-year period (FAO, 2006). Global atmospheric concentration of CH_4 has increased by a factor of 2.5 since preindustrial times, from 720 [695 to 745] ppb in 1750 to 1803 [1799 to 1807] ppb in 2011 (IPCC, 2013). Anthropogenic emissions account for 50 to 65 % of total emissions. Livestock is one of the major contributors of CH_4 . Livestock emissions from CH_4 had two components ie from enteric fermentation and dung CH_4 . About 37 % of anthropogenic CH_4 is attributed to enteric fermentation by ruminants as part of their normal digestive processes (Scheehle and Kruger, 2006).

India ranks first with 13% of the world total livestock population. Methane emission from Indian Livestock has been estimated by several workers from time to time. It is estimated that about 10.6 Tg methane is emitted from enteric fermentation (SNC, 2012). CH_4 emissions in ruminants also account for a 2 to 12% of gross energy loss of feeds depending upon the type of diets (Johnson and Johnson, 1995). Therefore, CH_4 mitigation strategies need to be followed to improve the utilization of feed energy for production purposes. Moreover, production of livestock products like milk, meat has increased rapidly. This will stimulate industrialization of livestock farming and exacerbate GHG problems in the absence of adequate mitigation measures. So there is an urgent need for the mitigation of CH_4 and other GHGs. CH_4 mitigation from livestock can be done by different methods like diet manipulations including feed supplements, animal manipulations (breeding based on residual feed intake, (Vimlesh, 2013),

management systems) and rumen manipulations (defaunation, vaccination). Some of the practical nutritional interventions for effective CH₄ reduction in ruminants are:

Feeding strategies for CH₄ reduction



Forage quality:

Improving the quality of forage had a highly positive effect in reducing the enteric CH₄ production. Feeding of forage with low fibre and higher soluble carbohydrates, using C3 grasses and less mature pasture can reduce CH₄ production (Beauchamin *et al.*, 2008). CH₄ emissions can also be lowered by using legume rich forages in diet because of its lower fibre content, higher passage rate and condensed tannin content (Malik *et al.*, 2010). Substitution of timothy hay by lucerne decreases CH₄ emissions by 21% (Benchaar *et al.*, 2001). Certain plant species also found to reduce CH₄ emission like *sulla*, *sainfoin*, *lotus*, *lespedeza* (Patra, 2012). Prusty *et al.*, (2013) analysed CH₄ emissions from different green fodders from river buffaloes and observed that chicory and berseem fed buffaloes emit 12.2 and 5.2% less CH₄ than oats fed animals.

Forage preservation also found to affect the production of CH₄. Methanogenesis tends to be lower when corn forages are ensiled than when they are dried (Boadi *et al.*, 2004). Mixing of maize fodder, berseem fodder and Lucerne fodder along with wheat straw reduced the CH₄ production (Singh and Mohini, 1999; Malik *et al.*, 2010).

Forage processing methods like chopping, grinding and pelleting causes a decrease in CH₄ emission. Chemical treatment of wheat straw using urea, ammonia, calcium hydroxide, sodium hydroxide, addition of urine etc are found to be effective in CH₄ mitigation. Biological treatment of straw like fungal treatment (*Pleurotus spp*, *Caprinus spp*, *Crinipellis spp*) of straw also showed some positive results (Mahesh and Mohini, 2013).

Concentrates:

Increasing the level of concentrate in the diet decreases CH₄ production. It was found that the relation between CH₄ production and concentrate proportion in the diet are curvilinear. More concentrate in diet increases the feed intake which in turn results in improved rumen fermentation and accelerated feed turnover, which causes large modifications of rumen physico-chemical conditions and microbial populations. A shift of VFA production from acetate to propionate occurs. This results in lower CH₄ production because the proportions of hydrogen sink increases. Lower rumen pH that is produced during concentrate feeding also causes inhibition of methanogens.

According to the work of Mathot *et al.* (2012), there is a significant reduction in enteric CH₄ emission per kilogram of live weight gain because of the usage of high concentrate (80: 20) in the diet compared to low concentrate diet (20:80). When the starch content in the concentrate increases, it had a positive effect in CH₄ reduction (Singhal *et al.*, 2008). So usage of more starchy concentrates like wheat, maize and barley are recommended. Addition of grain to forage diet increases starch and reduces fibre intake, reducing the rumen pH and favoring the production of propionate rather than acetate in the rumen (McAllister and Newbold, 2008).

Fat addition:

Dietary fat is one of the best options for mitigating CH₄ production in ruminants. Their effect has been assessed by equations provided by Giger-Reverdin *et al.* (2003) and by Eugene *et al.* (2008) who reported a mean decrease in CH₄ of 2.2% per percentage unit of lipid added in the diet of dairy cows, independently of the nature of fatty acid (FA) supply. Addition of fat in the diet inhibits the growth of methanogens and protozoa population and reduces ruminal organic matter (OM) fermentation and hydrogenation of unsaturated fatty acids (acting as an alternative H₂ sink) in the rumen. Dietary fat do not undergo fermentation in the rumen that also adds to reduced CH₄ production (Martin *et al.*, 2010).

Fat particularly at concentrations above 6–7% of dietary DM can significantly diminish DM digestion particularly fiber components and DM intake (Beauchamin *et al.*, 2008). High level of fat can reduce the milk fat percentage. Besides the level of fat in diet, the source of fat is also important. Normally medium chain fatty acids like coconut oil, myristic oil, canola oil, palm kernel oil etc shows greater result in reduction of CH₄. Bairagi and Mohini (2005) had studied

the effect of addition of groundnut oil and mustard oil in wheat straw, concentrate based diet and found that it reduces CH₄ emissions significantly.

Grainger *et al.* (2011) reported that reductions in CH₄ emissions could be maintained for up to 3 months only with the addition of lipids. It appears that proper supplementation of fat is a promising technology for mitigation of CH₄ on consistent basis without affecting production.

Plant secondary compounds:

Many plant secondary metabolites like tannins, saponins, essential oils show CH₄ mitigation effect (Patra, 2012). These metabolites lessen CH₄ production through a direct effect on methanogens and/or elimination of protozoa, reduction of OM digestion, and modification of fermentation in the rumen (Patra and Saxena, 2010).

Saponins are glycosides found in many plants that have a direct effect on rumen microbes. Saponins, decrease protein degradation and favour at the same time microbial protein and biomass synthesis (Makkar and Becker, 1996). Saponins can inhibit growth of protozoa in rumen. Effect of saponins on CH₄ reduction was found to be more effective in defaunated animals. Saponins ability in CH₄ mitigation also depends upon the level of saponin in diet. Tea saponin decreased methanogenesis (8%) as well as the protozoal abundance (50%) (Martin *et al.*, 2010). Saponins from *Sapindus saponaria*, *Acacia concina*, *Sesbania sesban* and fenugreek (Rejil *et al.*, 2008) are proved effective.

Tannins are of two types- condensed and hydrolysed. Anti-methanogenic activity has been attributed mainly to the group of condensed tannins. Hydrolysable tannins, although they affect methanogens, are usually considered more toxic to the animal. A direct effect on ruminal methanogens and an indirect effect on hydrogen production due to lower feed degradation. Many plants contain tannins, and these are often tropical shrub legumes. Animal trials with plants or extracts of condensed tannin-containing *Lotus corniculatus*, *Lotus pedunculatus* and *Acacia mearnsii* reduced CH₄ production in ruminants. Patra *et al.* (2010) observed that *Terminalia chebula* extracts reduce CH₄ by 13.17% in sheep.

Essential oils and organosulphur compounds had the ability for CH₄ reduction potential. Thymol (400 mg L⁻¹), a main component of EO derived from *Thymus* and *Origanum* plants, was a strong inhibitor of CH₄ *in vitro*, but acetate and propionate concentrations also decreased

(Martin and Nisbet, 1990). Coriander, garlic, ginger, fennel, clove, eucalyptus are found to be effective in CH₄ reduction (Sirohi *et al.*, 2009).

Dietary supplements:

Ionophores:

Among feed additives, ionophore antibiotics such as monensin, rumensin and lasalocid, typically used to improve efficiency of animal production, are known to decrease CH₄ production. These ionophores at the doses prescribed do not affect methanogens, however their effect on other microbes, inducing a shift in fermentation towards propionate synthesis, is the most likely mode of action. Ionophores also affect protozoa; the reduction and subsequent recovery in protozoal numbers perfectly matched CH₄ abatement. Addition of rumensin (100mg/day) to concentrate and wheat straw based diet had reduced CH₄ emission by about 35% (Singh and Mohini, 1999). The effectiveness of monensin at suppressing CH₄ production appears to be dose-dependent. Inclusion at 24 to 35 mg/kg caused a 4 to 13% reduction in CH₄ (g/day) in dairy cattle (Beauchemin *et al.*, 2008) in addition to improvement in reproductive performance (Dharmbir *et al.*, 2008).

Organic acids:

Dicarboxylic acids, like fumarate, malate, and acrylate, are precursors to propionate production in the rumen and can act as an alternative H₂ sink, restricting methanogenesis. Eckard *et al.*, (2010) reviewed studies that showed around 75% reductions in CH₄ achieved by feeding fumaric acid. However, at the relatively high doses required, dicarboxylic acids are prohibitively expensive as an abatement strategy. Green leguminous fodders are the good source of malic acid.

Chetan *et al.* (2013) deduced that supplementation of fumaric acid at 2.5 and 5.0% in the diets of goat's reduced CH₄ production by 18.45 and 14.97%. Sirohi *et al.* (2012) noticed that malic acid could make 47 to 65% reduction in CH₄ production in whet straw sorghum based diets.

Alternate electron acceptors:

Some rumen microorganisms capable of reduction of nitrate to nitrite and then nitrite to ammonia use hydrogen or formate or both as the common electron donors; thus, methanogenesis may be lowered by the addition of electron acceptors such as nitrate (Pankaj Jha, 2013) and

sulfate (Nisha *et al.*, 2011). It has been generally suggested that CH₄ production could be diminished by 10% for each 1% inclusion of potassium nitrate in a diet (Leng, 2008). Nitrate can be used as a nitrogen supplement also. However, under some nutritional conditions/feed management, nitrate becomes toxic because of the accumulation of nitrite in the rumen.

Probiotics:

Probiotics are directly fed microbials (DFM) that have claimed health benefits when consumed. Limited information is available on the effect of probiotic cultures such as *Saccharomyces cerevisiae* and *Aspergillus oryzae* on CH₄ production and most of all are *in vitro*. Addition of *S. cerevisiae* to an *in vitro* system suppressed CH₄ formation by 10% initially, though this was not sustained (Mutsvangwa *et al.*, 1992).

Future scope:

Some other methods like defaunation, use of vaccines for immunization, bacteriocins, bacteriophages can also be tried for effective reduction of CH₄ in ruminants. A preliminary study in Australia suggested that vaccination against methanogens can reduce methanogenesis, with a 7.7% (DMI basis) reduction in CH₄. Biological control strategies, such as bacteriophages or bacteriocins, could prove effective in directly inhibiting methanogens and redirecting H₂ to other reductive rumen bacteria, such as propionate producers or acetogens (McAllister and Newbold, 2008). However, most of these options are in the early stages of analysis and still require significant research over an extended period to deliver commercially viable vaccines or biological control options that will be effective over a range of production systems and regions.

Conclusion:

Strategies that can mitigate emissions, predominately CH₄, from ruminant production systems, not only improve production efficiency, but also help stabilize GHG emissions. Lipid supplementation is one of the most effective methods for CH₄ mitigation but its dietary inclusion percentage is limited. Accounting the cost of technology and the price of the product gain, supplementation of diets with monensin (rumensin), concentrates, and urea molasses mineral block could be cost-effective for high-yielding animals in Indian situation. Depending on all these works it can be concluded that diet containing optimum fat level, green legumes, immature

fodder, some tree leaves are the best to reduce methane emissions in addition digestive mixture (ginger, clove, jeera, arfotida) should also be fed on daily basis.

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Application of Proteomics to understand Effect of Heat Stress in Farm Animals

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Genes are transcribed into mRNA, mRNA is translated into proteins, and proteins form and build each cell. Therefore, the gene sequence, transcription, translation, and post translation are all points in a gene product's expression that are controlled. Genome projects have given us the gene sequence information, techniques such as real-time PCR and microarray are giving us gene expression data, and proteomics is giving us information regarding protein expression and posttranslational modifications. Together these techniques have brought animal research to a molecular level. Gene expression profiling helps to elucidate the genes that are likely to be important in the stress response. The major hurdle to these studies is that the expression at the gene and protein level is not necessarily correlated, thus the increased gene expression may or may not reflect an increase in its protein counterpart.

Consequently, evaluating the changes occurring in the proteome profile of heat stressed cattle would help us to assess the variation in a real time manner. Proteomics is the large-scale study of protein expression, protein-protein interactions, or posttranslational modifications. Unlike other methodologies that analyze a few proteins at a time, proteomics can analyze thousands of proteins in a single experiment. This ability to analyze thousands of proteins gives the field of proteomics a unique capability to demonstrate how cells can dynamically respond to changes in their environment. Therefore, a goal of proteomics is to identify new and potentially unexpected changes in protein expression, interaction, or modification as a result of an experimental treatment. Generation of large proteomic data sets is expected to demonstrate the interdependence of cellular processes important for normal cell growth or a cell's response to abnormal or disease conditions. Proteomic approach enables an investigator to step back and view the whole picture of cellular functions instead of one particular action of one protein. Few researchers have explored the proteomic approaches (2D gel electrophoresis and MS based technologies) to identify the proteins after exposure to thermal stress in different species. Most of the studies on thermal stress responses reported so far are conducted *in vitro* (cell culture) or on prokaryotes or at the most on laboratory model organisms. Nevertheless, studies focused on evaluating the changes in the system at protein

level using non-model organisms like cattle will allow us to understand the consequences of changes in thermal habitat.

Proteomic strategies for the identification and analysis of proteins:

Protein separation strategies:

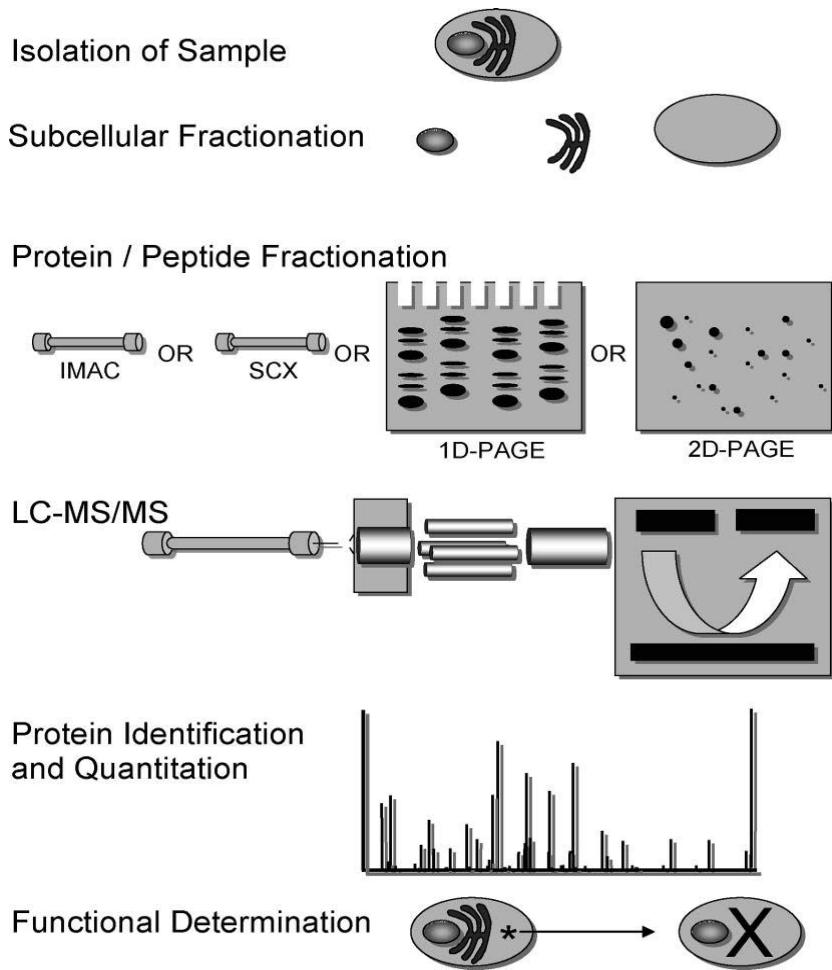
One of the rate-limiting steps in any proteomic analysis study is obtaining, and then handling, sufficient quantities of target protein(s) from its original biological source. The classical method of quantitative and qualitative expression proteomics combines protein separation by high-resolution 2D gel electrophoresis with MS (Mass Spectrometer) or MS/MS identification of selected protein spots. Because, even the best 2D gels can routinely separate no more than 1500 proteins, this technique is limited to the most abundant proteins if a crude protein mixture (whole-cell lysate) is used. 2D electrophoresis is limited by the amount of material that can be applied to the first-dimension immobilized pH gradient gel (~150 µg to low milligram quantities). Hence, 2D gels have limited ‘scale up’ capability. For this reason, it is often desirable to “trace enrich” for a particular subclass of proteins. By analyzing proteins in a cellular compartment or organelle, it is possible to reduce the complexity and differences in abundance of a subset of proteins within a cell.

Two-dimensional SDS-PAGE:

This separation method has become synonymous with proteomics and remains the single best method for resolving highly complex protein mixtures. Two-dimensional SDS-PAGE is actually a combination of two different types of separations. In the first, the proteins are resolved on the basis of isoelectric point by IEF. In the second, focused proteins are then further resolved by electrophoresis on a polyacrylamide gel. Thus, 2D-SDS-PAGE resolves proteins in the first dimension by isoelectric point and in the second dimension by molecular weight. Dedicated 2D-SDS-PAGE systems are available that use immobilized pH gradient (IPG) strips and relatively foolproof hardware to facilitate the transfer of proteins from the IPG strip into the SDS-PAGE slab gel. The IPG strip is based on the use of immobilized pH gradients, in which polycarboxylic acid ampholytes are immobilized on supports to reproducibly create stable pH gradient. The use of narrow pH ranges facilitates the separation of proteins with highly similar isoelectric points. Proteins separated by 2D gels are visualized by conventional staining techniques, including silver, Coomassie, amido black stains and fluorescent staining. Silver-staining and newer fluorescent dyes are the most sensitive.

Liquid chromatography:

The most common form of peptide separation in proteomic research is liquid chromatography. Peptides can be separated into fractions based on their hydrophobicity in the case of reverse-phase chromatography (RP-HPLC) or based on their charge state in the case of strong cation exchange chromatography. Capillary or nanobore columns (25 to 100 μm) with flow rates less than 200 nL/min and the volatile nature of the solvents used in RP-HPLC allows this type of chromatography to be directly linked to mass spectrometers (LC-MS). Therefore, peptide separation and peptide detection can be simultaneous. Other fractionation techniques can be used upstream of LC-MS to provide greater separation. In what is commonly referred to as multidimensional protein identification technology (Mud-PIT) two liquid chromatography techniques are used in tandem. Linking strong cation exchange chromatography and LC-MS, researchers have been able to detect nearly 1,500 proteins from a yeast proteome. When chromatography is linked to the mass spectrometer, peptides will elute from the column in finite peaks. A mass spectrometer can sample and sequence only one peptide at a time (approximately 1 peptide every 1.5 s). Many peptides may be missed if the sample is too complex. For example, if a whole proteome (30,000 proteins) is digested into 10 peptides each and was separated evenly over a 1-h chromatography gradient, the average number of peptides eluting off the column would be over 80 peptides per second. For this reason, multiple separations are necessary to reduce the number of peptides per second introduced into the mass spectrometer for the analysis of as many peptides as possible.



General Proteomic Experimental Design: Multiple separation methodologies can be employed in a proteomic experiment to maximize the number of proteins identified. This figure illustrates some potential separations that may be used singly or in combination. IMAC = immobilized metal ion affinity chromatography. SCX = strong cation exchange chromatography. 1D-PAGE = 1-dimensional PAGE. 2-D-PAGE = 2-dimensional PAGE. LC-MS/MS = liquid chromatography linked to a tandem mass spectrometer.

Mass spectrometry:

Current mass spectrometers can detect and identify peptides in the femtomole (10^{-15}) to attomole (10^{-18}) range. There are many types of mass spectrometers that can be used for proteomic studies, and each accomplishes the task of peptide identification in a slightly different way. However, the basic process of identifying a protein using a mass spectrometer is consistent between the various types. After initial protein digestion typically with trypsin, peptides must be ionized to enter the mass spectrometer. Peptides are then detected, isolated, and finally, fragmented and sequenced by the mass spectrometer.

Ionization of peptides is the first step in mass spectrometry of proteomes. The two frequently used ionization methods are electro spray ionization (ESI) and matrix-assisted laser desorption ionization. One advantage of ESI is that this method of ionization allows for the direct linkage between liquid chromatography and mass spectrometry because of the volatility of the HPLC solutions. Charged gas phase peptides are generated by ESI when the acidic HPLC solution containing peptides is sprayed from a tip, and the solution evaporates. The MALDI requires mixing of the peptide with a UV-absorbing molecule and the formation of crystals. When a laser strikes the crystalline structure, the results are the sublimation of the matrix and ionization and release of the associated peptides. The peptides are then analyzed by the mass spectrometer and the peptide mass determined. The peptide's mass is typically expressed as a ratio of mass divided by the charge of the peptide (m/z). Both ESI and MALDI cause peptides to gain protons. The same peptide population may gain a different number of protons. Therefore, a peptide with a mass of 1,000 Da will be detected on a mass spectrometer with an m/z of 1001 if it gained 1 proton, 501 if it gained 2 protons, and 334.3 if it gained 3 protons during ionization.

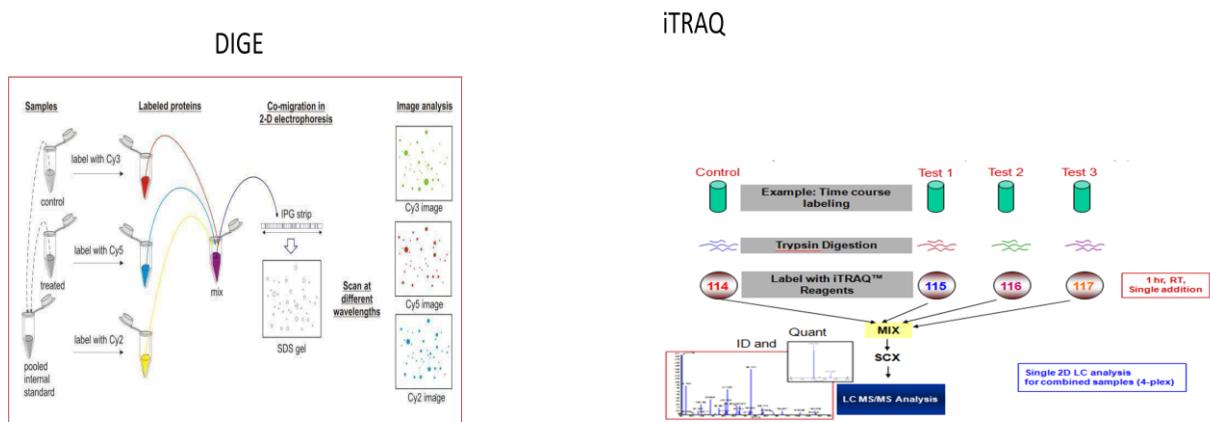
There are 2 basic mass spectrometry methods for the identification of proteins. The first called peptide fingerprinting is often associated with 2D-PAGE protein separation scheme. Individual spots from the 2D-PAGE gel are isolated and the proteins digested with trypsin. These proteins are typically analyzed with a MALDI time of flight mass spectrometer. The mass spectrometer will record all the peptide m/z detected in the gel spot. Identification of a protein is based on the measurement of multiple peptides that can come from that protein. For example, after a mass spectrometer has determined the m/z for the peptides from a gel spot, this information will be matched to a protein database. A successful protein match will be based on the number of peptides matched to the protein and the accuracy of the matches. The second method to identify proteins involves use of tandem mass spectrometers that allow for sequencing of individual peptides.

Differential display proteomics:

A fundamental aspect of proteomic research is the determination of protein expression levels between two different states of a biological system (e.g. relative quantification of protein levels), such as that encountered between a normal and diseased cells or tissues. This is referred to as differential display or comparative proteomics. This can be done in two ways such as running and comparing samples in 2D-SDS-PAGE or with LC-MS and Isotope tags.

For differences in the protein-expression profiling, we compare 2D-gels from two different samples for differences in the occurrence or intensity of protein spots. This approach provides a useful means of comparing proteomes. However, identification of protein is cumbersome and difficult by this procedure. Application of peptide mass fingerprinting and LC-MS-MS analysis now makes it possible to identify essentially any protein one can detect by staining the gel. Therefore, the critical task in comparative proteomics with 2D gels is identifying the features that differ between the gels.

The LC-MS approach to proteome comparisons is conceptually the opposite of the 2D gel approach. Whereas the 2D gel approach separates proteins and begins with an image comparison, the LC-MS approach separates peptides and ends with data mining to assess differences between samples. Two protein samples are treated with reagents to “tag” them. The tags are chemically identical, except that one contains heavy isotopes (e.g. ^2H , ^{15}N , ^{13}C , ^{18}O etc...) and the other contains light isotopes. The samples are digested and the peptides are analyzed by LC-MS-MS. Analysis of the MS-MS data allows identification of the protein present. Examination of the full-scan spectra corresponding to each MS-MS scan then allows measurement of the ratio of the light- and heavy- isotope tagged peptides. This ratio corresponds to the ratio of that protein in the two samples. This approach provides a relative quantification of the level of a particular protein in two samples.



Summary of the protocol for DIGE and iTRAQ

Differential expression analysis of serum proteins during heat stress:

Cattle, like other homeotherms, modulate internal body temperature by coupling the amount of heat produced through metabolism with the heat flow from the animal to its surrounding environment. Heat stress occurs as a result of an imbalance between heat production within the body and its dissipation. Heat stressed cattle experience reduction in food intake, growth,

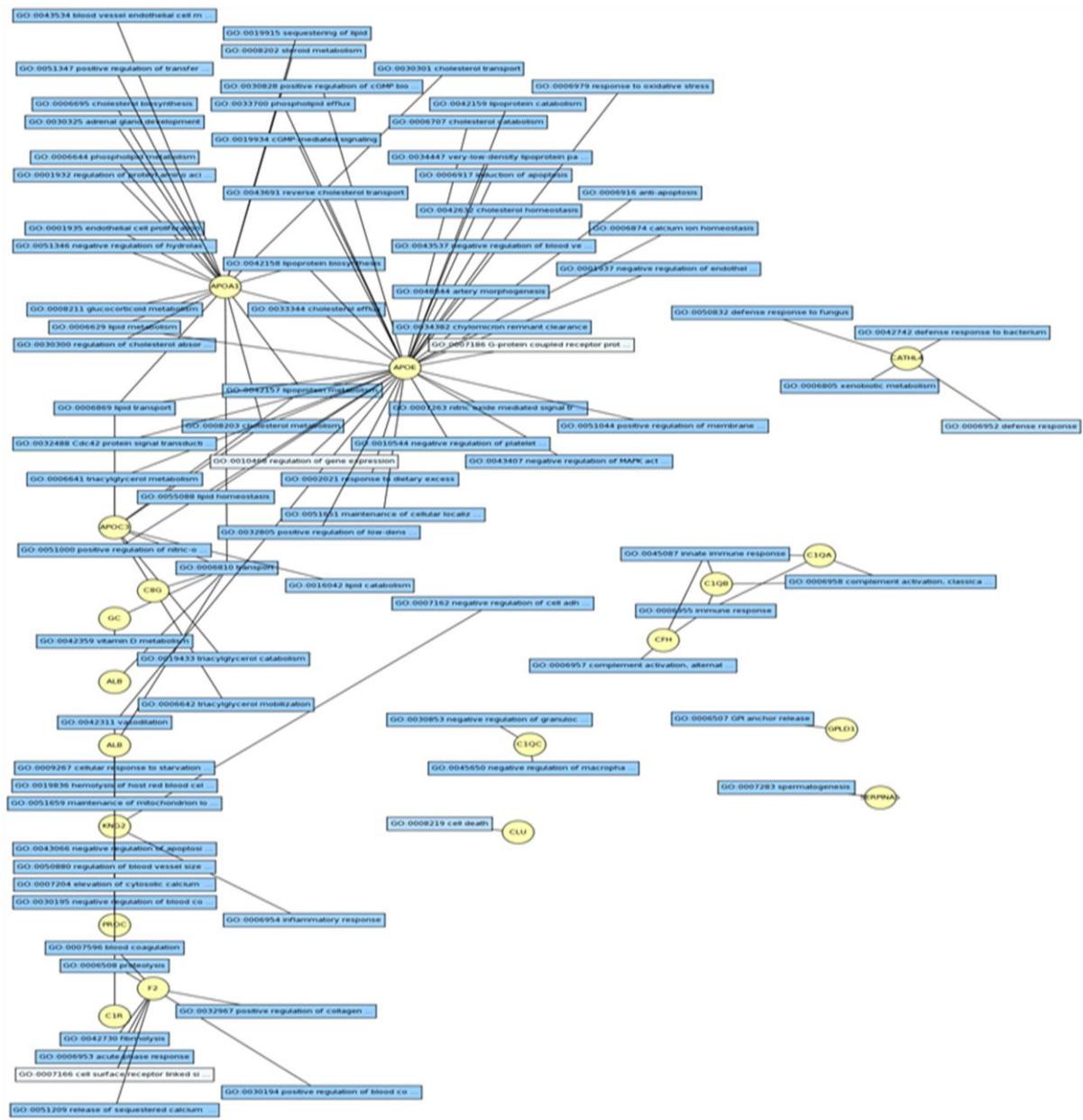
immunity, milk yield and reproduction efficiency. Environmental-induced hyperthermia jeopardizes animal welfare. The welfare of dairy cows can be evaluated on the basis of the temperature humidity index (THI) values. This index is usually used for evaluating the degree of stress on dairy cattle caused by weather conditions, as it comprises the effects of both ambient temperature and relative humidity in an index. In the critical range of THI of 70–72, performance of dairy cattle declines and cooling becomes desirable. At THI of 72–78, milk production is seriously affected. In the dangerous category at THI of 78–82, the performance is severely affected and cooling becomes essential. The detrimental effects of heat stress on animal welfare will likely become more of an issue if earth's climate continues to warm as predicted. According to the IPCC fourth assessment report, a warming of up to 2 °C above 1990–2000 levels would have significant impacts on many unique and vulnerable systems. Changes in extreme temperature will accompany continued global warming. Thus raising the question how organisms adapt to extreme temperature events.

Blood plasma/serum is the most sampled material for medical diagnosis as it represents the physiological and pathological condition of a well-being. Therefore, a resultant plasma/serum proteome profile will facilitate the identification of significant alterations in the plasma/serum proteins abundance in response to physiological and pathological conditions, opening new avenues for animal sciences. Unfortunately, the plasma/serum proteomics, a domain of the rapidly advancing field of proteomics having enormous application, is an analytical challenge. A major contributing factor to this analytical challenge is the large dynamic range of the serum proteome. Around 90% of the protein content of serum is dominated by only 10 serum proteins, with albumin comprising ~50% of the total protein content and regarded as the most abundant serum protein. Out of the remaining 10%, around 12 proteins make up 90% of this total and the low abundant proteins make up about only 1% of the entire serum proteome, thus 99% of serum protein content comprises of the 22 high abundant proteins (HAPs). These HAPs suppress the signal of numerous other low abundant proteins (LAPs), which as a consequence are not detected, therefore the proteins present in low copy numbers are masked by the HAPs during 2DE or identification by Mass spectrometer (MS). There has been a number of major advancements in finding solutions to these challenges in human. In farm animals like cattle and buffalos, it still remains a challenge. It is in general consensus that to allow detection of medium to low abundant proteins using the conventional proteomics tools, for digging deep into the serum proteome; the protocol necessarily requires a complex multidimensional fractionation approach to

increase the resolution of LAPs. The depletion of the HAPs or enrichment of LAPs from serum is an accepted technique as one of the prefractionation approach.

We have investigated the comprehensive proteome profile of serum samples of the Sahiwal cattle under normal condition, after hyperthermia as well as after returning to normalcy using gel based and gel free comparative proteomic approach i.e. DIGE and isobaric Tags for Relative and Absolute Quantification (iTRAQ). This study has helped us to gain insights into the changes observed in serum protein profile of Sahiwal cattle. Our main objective was to build a high confidence dataset of the differentially expressed proteins of the peripheral circulation after a brief exposure of 4 h to heat stress. These proteins once fully characterized can provide information about the biological processes and pathways that are affected during heat stress and can direct us towards understanding the probable role of various proteins that may be involved in adaptation to heat stress in Sahiwal cattle.

Out of a total 144 protein identified through iTRAQ approach, 93 proteins were common to both replicates, which represented 65% of the identified proteins. As compared to the control, 76 proteins were differentially expressed that included 18 up regulated proteins (13 characterized and 5 uncharacterized) and 58 down regulated proteins (31 characterized and 27 uncharacterized) after 4 h of heat stress. A total of 66 proteins were differentially expressed that included 21 up regulated (13 characterized and 8 uncharacterized) and 45 down regulated (24 characterized and 21 uncharacterized) proteins after 24 hours of recovery period. A total of 78 proteins were differentially expressed that included 16 up regulated (11 characterized and 5 uncharacterized) and 62 down regulated (36 characterized and 26 uncharacterized) proteins after 48 h in recovery period. From this list a subset of proteins were extracted showing fold change greater than 1.5 (up regulated) and lesser than 1.0 (down regulated). It was clearly observed that a majority of the proteins were down regulated in all 3 conditions while few proteins were up regulated. Prominent among the upregulated proteins are Serum Amyloid A, Inter-alpha (Globulin) H4, Alpha-1-antiproteinsase, Alpha-2-macroglobulin, Complement component C9, Hemoglobin subunits, Serpin A3-1, and Serpin D1. A majority of the proteins were down regulated in heat stressed conditions. Down regulation of a majority of proteins suggest that the translational machinery is severely affected when the animal is exposed to hyperthermia. Serine protease inhibitors, apolipoproteins and complement proteins are three major classes that exhibited differential regulation.



Activation of Pathways in Response to Heat Stress

The identified proteins were subjected to detailed bioinformatics analysis for their possible involvement in various biological processes including thermoregulatory mechanism and their probable role in various biological pathways. Various pathways such as fatty acid metabolism, protease insult, transport, immunity related and acute phase proteins, blood coagulation and chaperons were significantly affected. The findings in the present study significantly advance our understanding of the physiology of heat stress-induced injury in the cattle.

Genomic & Epigenomic Modulation of Mammalian Oocytes and Embryos: Using the Tools of RT-PCR, Competitive PCR and Bisulfite Sequencing

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The intensive research efforts on Embryo Transfer Technology *per se* and Embryo Biotechnologies in general have emerged as a potential tool for faster multiplication of superior germplasm and incorporating genetic modifications in livestock species. In spite of very exhaustive studies carried out to standardize an efficient procedure for in vitro production of embryos, the overall success rate of the technology has remained low, which presents a serious bottleneck for the successful implementation of high-end embryo biotechnologies. The maturation of the mammalian oocytes prior to fertilization and the molecular changes during preimplantation development is thought to play determinant role for successful fertilization and implantation of IVF produced embryos. The fine-tuning of gene expression and accumulation of crucial mRNA and proteins are important determinants of the future ability of an egg to become an embryo. Persistent alterations of the normal gene expression patterns under in vitro condition may be responsible for poor development competence of IVF embryos resulting into lower implantation rates. It has been recently found that the basic culture system along with the various kinds of supplementations had pronounced effects on transcript levels of a series of developmentally important genes and “*messenger RNA phenotyping*” and studying expression pattern of genes may provide a useful tool to assess the IVF process and optimizing the in vitro culture conditions for embryo production following IVF.

In mammals, the study of gene expression in the oocytes/ embryos have been difficult because the limitation of the paucity of the amount of starting material. Quantitative measurements of mRNA levels can be achieved using several approaches such as northern blotting, *in situ* hybridisation, RNase protection assay, reverse transcription polymerase chain reaction (RT-PCR). However, some of these approaches such as Northern blot analysis and RPA have been proved to be inadequate when working with oocytes/ embryos because of the less sensitivity and the large amount of starting material needed to obtain enough RNA to

perform these techniques. The RT-PCR in this regard provides a useful tool for exploration of gene expression because of its high sensitivity, suitability for quantifying rare transcripts and its ability to analyze a slight change in abundance of developmentally important transcripts. In our laboratory we are working on RT-PCR based methodologies to understand the differential expression level of developmentally important genes during the process of oocyte maturation and early embryonic development.

Quantitative RT- PCR (Q-RT-PCR): advantages and limitations:

q-RT-PCR offers one of the most sensitive methods for quantitative analysis of gene expression because it combines the advantages of Reverse Transcription (RT) and PCR, which involves amplification of specific mRNA of even very low copy number mRNA species available from minimal tissue samples. Since it has been evolved this technique has been used very widely either for the relative quantification of transcripts to determine the changes in the steady state expression of genes or for the absolute quantification of the number of copies of specific RNAs present in per unit mass of tissue. However, although described as a sensitive method the quantitative RT-PCR often leads to error when employed for the relative quantification of transcripts in two or more groups of samples/ experiments due to differences in quantity and quality of initial sample amount, variation in RNA recovery, possible RNA degradation of sample material, contamination of genomic DNA in RT product, tube to tube variation in RT reaction, variation in sample loading / pipetting, errors and variation in cDNA synthesis efficiency which significantly contribute to source of variations among observed differences in the levels of transcripts. To compensate for these variations requirement of a standard is important which could be used to normalize the sample to sample variation.

Using an internal or endogenous standard is used most frequently for relative quantification of transcripts which involves the co-amplification of an endogenously expressed RNA that is present in the sample mixture along with the target mRNA. Ideal endogenous standards are the ones whose expression does not vary in response to the experimental treatments or over the time of experimentation (temporal variation). Experimental samples are then normalized with respect to the endogenous RNA standard to allow comparison of target mRNA levels and the results are expressed as percent increase or decrease in the expression of target RNA as compared to the standard. But endogenous standard has problem of analysing transcripts of widely differing abundance, necessity to use multiple primers for the same

reaction and expression level of some housekeeping genes have been reported to vary during experimental treatments. In this strategy, several of the housekeeping genes have been used as standards. However, the use of endogenous standards in RT-PCR method for mRNA quantification is again limited by two factors. 1) The expression pattern of the endogenous gene decided to be used as standard may vary thereby making the comparison biased and 2) two different sets of primers used for the co-amplification of target RNA and the standard may exhibit different amplification efficiencies. Nevertheless, instead of these limitations, after properly calibrated for a particular tissue type, the endogenous control approach for mRNA quantification has been used by many workers for their genes of interest.

Designing a q-RT-PCR experiment:

Performing the RT-PCR can be the most technically challenging, often requiring substantial pre-experimental planning and design. One of the principal disadvantage/ limitation of this method is that it eventually determines the level of expression of a gene in terms of end point analysis of PCR product generated by a logarithmic amplification of mRNA. The amplification and accumulation of PCR amplified products in a reaction tube are constantly affected by available ratio of essential PCR ingredients and at one point of time it becomes a limiting factor for further amplification of products. This may incorporate an error in the estimation procedure. The critical parameters, which affect the RT-PCR efficiency, can be enumerated as follows.

1. PCR cycling conditions: Number of cycles, time and temperatures of annealing, and extension.
2. Concentration of oligonucleotide primers, MgCl₂, reaction templates, DNA polymerase.
3. Target and control oligonucleotide composition: Length, sequence, specificity, priming efficiency. (Innis *et al.*, 1990; Volkenandt *et al.*, 1992)
4. Contamination: As PCR being highly sensitive, even very small amount of genomic DNA contamination in RNA preparation may serve as template for amplification and produce misleading results.

PCR cycling conditions:

The method of Q-RT-PCR principally involves detection and quantification of amplification product at the end of defined number of PCR cycles, which often represents a plateau phase of amplification. Quantification done in the exponential phase of PCR seems to be more accurate. At plateau, the Taq polymerase also loose activity or cDNA begins to compete for primers and DNA amplification product concentration can increase to the point where single stranded products re-anneal each other rather than with primer. Optimisation of the plateau phase for a given reaction condition thus form an integral part of any Q-RT-PCR procedure.

Time and temperature of annealing and extension:

The standard PCR has three steps defined by temperature of denaturation annealing and primer extension. The first step i.e. denaturation occur when reaction is heated to 92^0C - 96^0C . At this step the template DNA becomes single stranded and the Taq enzyme has no measurable polymerase activity using these denatured single strands. Stability of the Taq activity also becomes a limiting factor in PCR. Taq polymerase loose 50% of its activity at 95^0C for 40 minutes. Annealing temperature of oligonucleotide primers to template DNA varies from 45^0 - 65^0C depending upon the homology of the primers for the target sequence as well as the base composition of the oligonucleotide. The oligonucleotide primer is in a 10^6 - 10^{10} fold molar excess to target sequence and the sequence complexity of the target DNA is at least 10-fold greater than that of nucleotides. Optimisation of annealing temperature is thus critical as too high temperature may result in no product and if it is too low, non-specific products accumulate which leads to the inaccurate quantification.

Concentration of oligonucleotide primer and MgCl_2 :

Particularly in the context of mRNA quantification using RT-PCR the effective concentration of magnesium cation is again crucial. MgCl_2 concentration for Taq polymerase has been reported to be 1.5mM-2mM. Chelating molecule such as EDTA, nucleotide tri-phosphate can reduce the effective concentration of Mg^{2+} . Too high Mg^{2+} concentration may yield non-specific PCR products whereas too low concentration will drastically reduce the efficiency of PCR. Any increase in the concentration of dNTPs will reduce the chelating effect of tri-phosphate thus; the change in dNTP concentration should be accompanied by a change in magnesium concentration in 1:1 molar ratio. Normally MgCl_2 concentration is tested in range

from 1.5mM to 5mM. Routinely 1.5mM MgCl₂ is used and a concentration of 4.5mM or 6mM has been reported to decrease non-specific priming in some cases. Because dNTPs and oligonucleotides bind Mg²⁺, the molar concentration of the cation must exceed the molar concentration of phosphate groups contributed by dNTPs and primers. In some cases increasing Mg²⁺ increases the specificity. Thus it has been recommended that for any Q-RT-PCR protocol the optimum concentration of Mg²⁺ is required to be determined empirically for each combination of primer and template.

DNA Contamination:

In the context of RT-PCR contamination of RNA with DNA will generate a PCR product that is same size as the PCR product generated by the target cDNA. This may also occurs if the target gene is part of a large gene family or if there are large number of pseudogenes such as the case with G3PDH and β-Actin. Specific strategies are therefore required to be adopted to rule out the DNA contamination in RT-PCR product. One option is to design an intron spanning primer that generates a bigger product when amplified from genomic DNA template against a shorter cDNA amplified product. Alternatively a Reverse transcriptase negative control may be included during the cDNA synthesis. In minus RT control, Reverse transcriptase enzyme is not added during cDNA synthesis and this negative reverse transcriptase control is used as a template for PCR along with the other experimental cDNA sample. A last option to rule out genomic DNA contamination is to treat the RNA with RNase free DNase during isolation of RNA. Whatever is the strategy used an experimental plan targeting mRNA quantification must contain a suitable control to establish the authenticity of the experiment.

Quantitative competitive PCR (QC- RT-PCR): an alternative approche:

As described above the most accurate quantification of mRNA transcripts following Q-RT-PCR is greatly hampered by a number of variables that can occur during sample preparation, or in the course of reaction, and minor variations in reaction are greatly magnified during the amplification process. These variations can be partly overcome by following a strategy called competitive PCR and competitive RT-PCR. This assay is based on competitive co-amplification of a target sequence together with known concentrations of a control (competitor) in the same tube. The competitor standard is designed with a strategy that it has to

share the primer recognition sites with the targeted template. The two other a-priory assumptions that are hypothesized for validity of this test are that both the specific template and external standard are amplified with same PCR efficiency and it must be possible to analyse the PCR amplified products of target template and external standard separately. Competition for amplification reaction between the target template and external standard template allows the determination of the equivalence point at which the control template and target template concentrations are assumed to be equal. The number of copies of the specific target template at the point of equivalence is assumed to be the same as that of added internal standard, enabling quantification of target molecule. During the process of competition a direct competition between the target sequence and competitor standard continues throughout the PCR, so the plateau effect does not interfere with quantification.

In competitive PCR to a large extent the validity and accuracy of quantification depends on the strategy of designing the competitive standards. Lots of reports have been published describing different competitive standard designing options. One way to classify the competitors is based on internal sequence as Homologous and Heterologous competitors. Homologous competitors have the same nucleotide sequence as the target DNA (RNA) but containing a deletion or an insertion or has a different restriction site introduced by site specific mutagenesis. Care should be taken to avoid the formation of heteroduplex which would interfere with subsequent quantification. Hetrologous competitors on the other hand have nucleotide sequence different from the target DNA (RNA) except for the sequence of primer annealing sites. Heteroduplex formation can be avoided using this strategy and since the primer sequences are same the difference in amplification efficiency between target and competitor can be considered as minor.

Designing of standard template competitor:

A common strategy for designing competitor template is to inset or delete a restriction enzyme recognition site within the internal sequence of the targeted template so that the products of the target and standard amplification can be easily resolved by electrophoresis after restriction digestion. Kephart, 1998 used another approach for the quantification of human β -Actin RNA. Using these approach three primers was designed. Two primers upstream (US) and downstream (DS) were designed to generate a 511 bp product during RT-PCR amplification of the β -Actin RNA. An additional upstream primer (USC) was required solely for construction

of the competitive template. The USC primer was a composite of the US primer sequence and a sequence region located 132 nucleotide downstream in the RNA. When the USC and DS primers are used to amplify total RNA sequences between the composite oligonucleotide are eliminated from the amplified product to generate the delta actin cDNA. The truncated product contains binding site for the US and DS primers but is truncated relative to the native sequence. The difference in size between the full length and truncated RT-PCR products allows discrimination between product generated from endogenous RNA and competitive RNA (cDNA) template. The full length and truncated amplification products were purified and an aliquot was ligated directly into the cloning vector. Competent cells were transformed and resulting recombinants were screened by blue/ white colony selection. Potential positive were rapidly screened by PCR using the upstream and downstream primer to check for insertion of the correct template sequence and with the downstream primer and a T7 primer to confirm the orientation of the inserted DNA. The RNA was purified and the concentration of RNA was quantified by spectrophotometric analysis. This strategy has been used to measure precisely the relative expression of α , β , γ fibrinogen mRNA in rat liver lobes using the synthetic RNA as an internal control (Zhang *et al.*, 1997).

However, the above strategies essentially require multiple step manipulations following PCR. A more simplified strategy avoiding the cloning and ligation steps have been described by O'Connell, 1998. Following this strategy the standard is derived from the target template by PCR using the regular antisense primer and a composite primer. The composite primer is essentially composed of the original upstream primer sequence plus another stretch of ~20 bases 90-100 bases downstream from the regular sense primer falling within the amplifiable target sequence. When the target cDNA is amplified with this composite sense primer and the regular antisense primer a truncated amplicon is derived that lack the upper 5' 90-100 bp region of regular amplicon but have the sense primer recognition site intact. This shorter product has been used as standard for competition. Once the standard is prepared it can be quantified with spectrophotometer and a dilution strategy could be decided that is helpful in finding the point of equivalence between standard and target amplification. This particular strategy was used in our laboratory for its efficiency in measuring 18S rRNA expression during in vitro maturation of oocytes in buffalo.

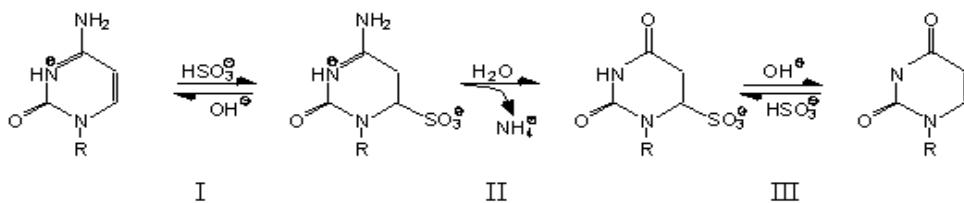
Mathematical considerations in quantification following competitive RT-PCR:

Q-RT-PCR being an indirect method of measurement, an understanding about the mathematical interpretation of competition result is essential to reach to a valid interpretation. Several mathematical models are described to explain the events in RT-PCR. In brief, the band intensity values for the target as well as the competitor product obtained by any densitometry software forms the basic input data to interpret. From the graph of the log of standard/native signal on ‘Y’ axis verses the log of input RNA/cDNA standard plotted on ‘x’ axis, the amount of initial native RNA/ cDNA can be found at the point of equivalence i.e at the point where the ratio of input and standard becomes 1 or close to 1 and the ordinate value will be zero. As with any standard curve, there must be standard amount above and below the equivalence point. The regression analysis of the values for standard as independent variable and the standard/native as dependent variable must obtain a linear trend to authenticate the competition. The formulae for calculating the number of copies of cDNA present for a given amount of purified DNA and subsequent extrapolation of results to reach to a value for number of copies of RNA is mentioned by O’Connel, 1998. In performing the competitive Q-RT-PCR when standards are added in varying concentrations in search for the point of equivalence the dilution series of the standard sometimes may not include the precise quantity to compete with the amount of target in a particular sample and as a result the exact point of equivalence is missed. Under such circumstances the target concentration can be estimated by calculating the concentrations from the target: standard ratios at either sides of the missed equivalence point and obtaining the average from these two estimates.

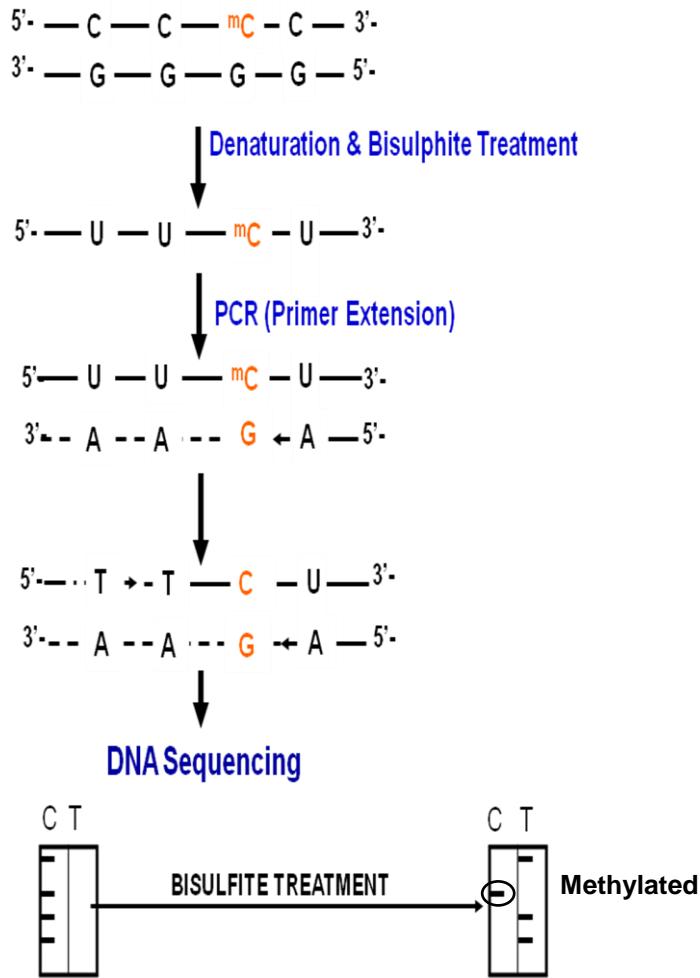
Bisulfite sequencing (BsS) as a method of studying epigenetic changes:

Methylation of cytosines in the 5'-position of the pyrimidine ring has been sited as an extremely important epigenetic modification of the eukaryotic genome that affects various cell processes. Such changes in DNA have been reported to be associated with compaction of chromatin, repression of transcription of the inactivated X chromosome of mammalian females, genomic imprinting of some specific genes and also with chromosomal stabilization. Methylation is also shown to play important role in regulation of gene expression during embryogenesis of mammals and further cell differentiation. Mapping of methylation patterns especially in CpG islands using bi-sulfite sequencing method is considered as a gold standard tool.

As principle, this method relies on the fact that Sodium Bisulfite preferentially deaminates cytosine residues of single stranded DNA to uracil, compared with a very slow rate of deamination of 5-methylcytosine to thymine. Thus the bisulfite reaction yields single stranded DNA strand products which are no longer complementary. PCR primers can therefore be designed, in such a way that it can only bind to one of the bisulfite-reacted DNA strand. Primers for each strand will differ in positions where there is a C or G in the original sequence (Frommer *et al.*, 1992). The chemistry of bisulphite reaction and steps involved in Bisulfite sequencing are shown below:



In the figure above step I represents sulphonation at the position C6 of cytosine. Step II represents irreversible hydrolytic deamination at the position C4 generating 6-sulphonate-uracil and step III represents subsequent desulphonation under alkaline conditions. Methylation at the position C5 impedes sulphonation at the C6 position at step (Frommer *et al.* 1992). The figure below (Fig 2) describes a summary of the BsS method. To start with the original DNA duplex is denatured to make them single stranded. On principle the bisulfite treatment is supposed to convert the unmethylated Cytosines to Uracil which on PCR yields a Thymine by complementary pairing, whereas the methylated Cytosine remains unchanged yielding a Guanine. Thus the sequence data for bisulfite treatment can easily distinguish between a methylated from unmethylated Cytosine.



Principle of Bisulfite Sequencing method

Parameters which require stringent standardization in working out a repeatable BsS protocol:

Incomplete conversion of C to U:

Since conversion of C to U requires the DNA to be single-stranded, incomplete denaturation of genomic DNA or its partial renaturation during bisulfite treatment can result in incomplete conversion of C to U, leading to false positive results. In this regard, the high salt concentration used (bisulfite reaction) favors renaturation. In addition, bisulfite treatment must be exhaustive in order to ensure complete conversion of Cs to Us in single-stranded DNA. In practice, complete bisulfite conversion is not feasible, even if the DNA is kept single stranded, because the DNA undergoes degradation with increased time of incubation. However, the

incomplete conversion may not pose a serious problem when the total PCR product is sequenced, the occasionally unconverted cytosines certainly under represents the total PCR amplified population and thereby not significantly affecting detection of the average extent of methylation at a specific site. Moreover, this problem can be overcomed by selectively designing PCR primers to anneal to sequences containing U in place of C, and by choosing sequences that originally contained several Cs.

Occasional conversion of 5 methyl cytosins to Uracil:

Although as a principle of bisulfite conversion it has been assumed by many workers that the 5mC will remain unchanged by sodium bisulfite treatment however on the contrary in occasional cases (about 2-3%) 5mC has been observed to react with bisulfite and get converted to uracil. As a reason for this it has been described that although bisulfite preferentially deaminates cytosine residues of single stranded DNA to uracil a slow rate of deamination of 5-methylcytosine to thymine cannot be absolutely ruled out. However, this problem is not significant when the total PCR product is sequenced directly and thereby giving opportunity to preferentially amplified product to be sequenced for interpretation of results instead of sequencing the cloned products and thereby risking inappropriate representation of methylation status.

Loss of DNA due to fragmentation:

The bisulfite treatment involves incubation of DNA at higher temperature (55°C - 95°C) and for a prolonged time (4-16 hrs) at lower pH (pH 5). Incubation of DNA under these conditions can generate apurinic sites which may pose problem during PCR amplification resulting into truncated products and incomplete PCR amplification. Thus, to overcome this problem the incubation time, temperature and salt (Sodium Meta Bisulfite) concentration require thorough standardization.

Conclusion:

The oocytes and embryos present one of the most interesting subject to study the events of gene expression because these cells farm the very basis of a highly specialized, accurate and orderly cellular development process leading to the formation of fully developed multicellular organism and any alteration during the course of the chronology of these events leads to either termination of the developmental process or more seriously to the development of an

malformed organism. Knowledge on gene expression profiling in oocytes and embryos is still expanding and lots of researches are going on to characterize the oocyte competence, genes responsible for healthy growth of post fertilized embryos and orderly development leading to successful implantation. This information will contribute to better standardize the procedure for IVF and will help optimizing acceptable rate of offspring produced following IVF. On the other hand there is tremendous change taking place in the molecular biology methodologies to explain functional behaviour and consequence of genes. As a result we are also going through a phase of continuously redefining our knowledge about the genetic control of early development processes. Studies on gene expression in oocytes and embryos particularly in buffaloes are a completely unexplored area and almost no information is available on this topic. Using the RT-PCR and competitive PCR strategies as presented above will provide valuable information on this aspect. However, more recent developments in quantification of differential gene expression are real time PCR and microarray strategies. Using these techniques will not only add to the authenticity of our knowledge but will help us to plan more physiologically valid experiments which will eventually help us to work out effective protocol for Artificial Reproductive Techniques (ART) and will help us to resolve many vexed questions in explaining the reproductive problems of animals.

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Genomic Technologies: A Way Forward for Learning Climate Resilience through Cellular Responses to Heat Stress

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1. Introduction:

In today's world, we are certainly living in an era of strange paradox, where economic development and impact of climate change to the planet earth have been burning issues for all the stakeholders, that is, the whole humanity. The Intergovernmental Panel on Climate Change (IPCC) projected that global average surface temperature may increase in between 1.8° C and 4.0° C by 2100. With increases of 1.5° C to 2.5° C, approximately 20 to 30 per cent of plant and animal species are expected to be at risk of extinction (FAO, 2007) with severe consequences for food security in developing countries. India, in this scenario, is faced with dual challenge of sustaining its rapid economic growth while dealing with the global climate change threat. What is the best way forward then? Both the proponents and opponents have to decide what the right balance between each one's priorities is?

According to the FAO, the animal agriculture sector that includes production of feed crops, fertilizer manufacturing, meat, eggs, and milk, is responsible for 18% of all greenhouse gases (GHGs) emissions, measured in CO₂ equivalent (Steinfeld *et al.*, 2006). On the other hand, it is also likely that there are severe negative impacts of climate change on livestock (Upadhyay *et al.*, 2008). The climate change can heighten the vulnerability of livestock systems and reinforce the existing factors that affect the livestock production systems. For farmers, losing livestock assets could prompt them to fall into chronic poverty and an adverse effect on their livelihoods. Animal agriculture is thus, both a victim of and a contributor to climate change. Both of these scenarios provide renewed and emerging challenges to animal scientists. They have to work both on GHGs mitigation strategies as well as to strengthen the adaptive capacity and production of climate resilient livestock.

2. Climate resilient livestock and heat stress:

Animal scientists have the impending challenge of enhancing the resilience of Indian livestock to climatic variability and climate change through development and application of improved production and risk management technologies. Abiotic stress is generally used to

describe non-living factors such as intense solar radiation in summer, extreme temperature, humidity, drought, flood, high winds, and other natural disasters, which adversely affect living organisms. As plants are affected by abiotic stress, animals too are vulnerable to them. Heat stress (HS) is the most stressful of all abiotic stressors in animals and is caused due to overexposure or overexertion in extreme environmental temperature. Temperature is a critical abiotic factor affecting organisms on ecological, organismal, cellular and molecular levels (Hochachka and Somero, 2002). Thermal stress is a unique and complex phenomenon that brings about numerous challenges beyond the animals baseline homeostatic mechanism causing alterations of the normal physiological mechanisms thus elicits a stressful response. At the cellular level, acute environmental change initiates the “heat shock” or cellular stress response. Changes in gene expression associated with a reaction to an environmental stressor involves acute responses at the cellular level (in most if not all cells) as well as changes in gene expression across a variety of organs and tissues associated with the acclimation response.

2.1. Heat stress and its effects on animal production:

There is a huge negative impact of heat stress on dairy industry every year. Heat stress (HS) shows negative impacts on all aspects of dairy cattle and buffalo reproduction (Rensis and Scaramuzzi, 2003; Hansen, 2007; Marai and Habeeb, 2010), milk production (West, 2003) and immune function (Elvinger *et al.*, 1992). Length of oestrous cycle and degree of expression of oestrus in buffaloes are affected by various factors, such as season, climate, photoperiod, temperature and nutrition (Beg and Toty, 1999). Heat stress can have large effects on most aspects of reproductive function in mammals. These include disruptions in spermatogenesis and oocyte development, oocyte maturation, early embryonic development, foetal and placental growth and lactation. These deleterious effects of heat stress are the result of either the hyperthermia associated with heat stress or the physiological adjustments made by the heat-stressed animal to regulate body temperature. Many effects of elevated temperature on gametes and the early embryo involve increased production of reactive oxygen species. Heat stress was found to suppress aromatase activity in granulosa cells and decrease the estradiol concentrations in the follicular fluid and plasma of dairy cows (Badinga *et al.*, 1993). Heat stress inhibits the FSH-R signalling pathway in the granulosa cells of growing follicles, causing decreased estrogen levels, which are known to produce increased follicular atresia. India is losing 2% of the total milk production, amounting to a whopping over Rs 2,661 crore due to rise in heat stress among cattle and buffaloes because of

the global warming". Heat stress downs the milk secretion by up regulating the activity of milk born negative feedback regulatory system (Silanikove *et al.*, 2000).

2.2. Genomic technologies for unravelling heat and abiotic stress towards climate resilient animals:

Heat and abiotic stress tolerance in animals is essential for improving productivity and prevents loss. Homeostasis, a set-value for metabolism under optimal conditions, is resultant of external stress factors *viz.* climatic, biotic, and nutrient imbalances.

During the past decade, genomics technologies have emerged and may be useful in addressing, in an integrated fashion, the multigenicity of the animal abiotic stress response through deciphering of genome sequences; cell-, organ-, tissue- and stress-specific transcript collections; transcript, protein and metabolite profiles and their dynamic changes; protein interactions etc. Genomic technologies that can be used for studying abiotic stresses such as heat stress in dairy animals is illustrated in the figure elsewhere in the text and can include the followings:

2.2.1. Gene discovery:

An important genomic approach to identify abiotic stress-related genes is based on ESTs generated from different cDNA libraries representing abiotic or heat-stress treated tissues collected at various stages of development or other physiological and pathological conditions. NCBI dbEST release 130101 summarizes about the ESTs for each organism and can be accessed at http://www.ncbi.nlm.nih.gov/genbank/dbest/dbest_summary/. For buffaloes, 1,857 ESTs are evident compared to 1,559,495 for cattle (as on 1st January, 2013). Therefore, specific sequencing programs based on cDNA libraries from heat –stress affected animal tissues and organs at many developmental stages are required to enrich dairy animals especially buffaloes EST datasets with stress-responsive genes.

2.2.2. Transcript profiling:

Genomic technological approaches such as MPSS, SAGE, quantitative real time PCR (qPCR) and array-based transcript profiling technologies allow us to perform an assessment of high-throughput expression of thousands of genes in control and stressor-treated tissues at various stages. Insights into gene expression patterns and functions coupled with stress tolerance can be explored by EST-based cDNA arrays. Gene expression profiling using cDNA macroarrays or microarrays can also be novel approaches to identify higher number of transcripts and pathways related to stress tolerance mechanisms. Studying co-regulation/ regulon identity for

condition-specific coregulation of overlapping sets of genes is also required to gain further insights. It has to be noted that genes reacting to a particular stress differ between organisms, species, breeds and even genotypes. It happens because certain organisms, breeds or genotypes have efficient stress signal perception and transcriptional changes that can lead to successful adaptive response and adaptations and eventually further tolerance.

2.2.3. Genome-wide transcriptome and methylome studies:

Identification of key molecules, regulators based on gene expression patterns related to multi-stress interactions can be achieved by genome-wide transcriptome analysis. This may prove an efficient tool for identifying biomarker for a particular abiotic stress. It has the ability to identify hundreds of genes encoding transcription factors that are induced or repressed by plethora of environmental stressors. Therefore, molecular cross-talk of gene regulatory networks among abiotic and heat stress treatments could be revealed.

2.2.4. Genome wide association studies (GWAS):

To reduce the magnitude of heat stress, we can opt to select animals that are genetically resistant to heat stress. In many species of livestock, thermo-tolerant strains or breeds have been developed (Hansen, 2011). In this context, GWAS can be used to identify genetic markers that can be used to select animal breeds and species having heat tolerance or resistant to deleterious effects of heat stress. For instance, GWAS can be used to identify quantitative trait loci (QTL) and single nucleotide polymorphisms (SNPs) associated with regulation of rectal temperature. Quantitative trait loci (QTL) can be identified for lowly-heritability traits and they used to improve reliability of genetic estimates despite the gain in reliability being less than the more heritable traits (Cole, 2011). In addition, GWAS can be useful for understanding the underlying biology of a trait by identifying candidate genes in physical proximity to QTL (Cole *et al.*, 2011; Berry *et al.*, 2012). One such study has been performed in Holstein cows and identified these genes as part of the QTL – SNORA19, RFWD2 and SCARNA3 in one contig and CEP170, PLD5 in another contig (Dikmen *et al.*, 2013). Therefore, SNPs could be proved to be useful in genetic selection and for identification of genes involved in physiological responses to heat stress.

2.2.5. Hapmap studies:

Haplotype refers to a combination of adjacent genetic markers that tend to be inherited together. Association analyses of haplotypes for heat stress can provide better reliability for an associated QTL region. Such haplotypes have been stored in hapmap projects of several

species including farm animals e.g. in the international bovine hapmap (IBHM) project study. A haplotype in HSP70A1A has been identified to be associated with increased risk during heat stress in Chinese Holstein cattle (Xiong *et al.*, 2013).

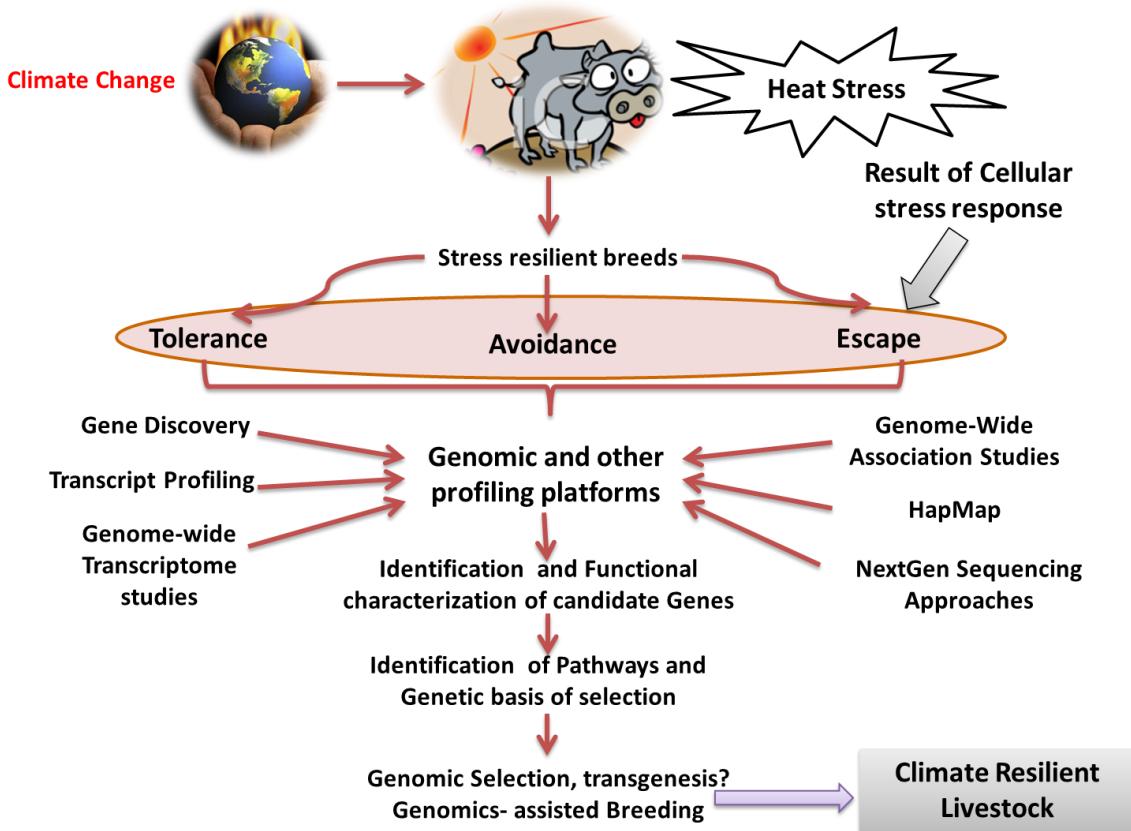


Figure: Genomic technologies for unravelling heat and abiotic stress towards climate resilient animals

2.2.6. Next generation sequencing (NGS) approaches: Newer genomics approaches like next-generation sequencing (NGS) hold great promise for accelerating search for genes related to heat tolerance-related traits. With the advent of NGS technology, the sequence-based transcriptome analysis is in many ways considered superior to microarrays since the sequencing-based method is applicable in real time, digital and highly accurate. The application of NGS technologies to gene expression analysis has catalysed the development of techniques like Digital Gene Expression TAG (DGE-TAG), DeepSAGE and RNA-Seq. RNA-Seq is a newer approach to transcriptome profiling that uses deep-sequencing technologies. RNA-seq based on NGS technologies has several advantages for investigating transcriptome fine structure including detection of allele-specific expression and splice junctions (Malone and Oliver, 2011) and may allow direct high-throughput sequencing of RNA from the heat-stress challenged tissues in different conditions. It can help to identify

candidate genes associated with climate resilience. NGS has been used to study variants in cattle to identify genes that contribute to heat tolerance, osmotic shock tolerance and other disease resistance.

3. Perspectives on our experiences on heat stress in dairy animals:

To understand the cellular responses upon heat-stress and to improve our insights into global gene expression in heat stressed dairy animals, a study was conducted in our laboratory to analyse the global gene expression profiling of peripheral blood leukocytes of indigenous cattle (Tharparkar). The genes that were found to be differentially expressed were further validated in cattle (Tharparkar and Sahiwal) and buffaloes (Murrah). The results showed that heat stress affect expression of a significant numbers of genes in peripheral blood nuclear cells of diverse biological functions. Further analyses are required to understand their functional role in livestock during heat stress.

4. Conclusions and future perspectives:

Comparative analysis of the response to abiotic (e.g. heat) stresses among diverse tolerant organisms, breeds and species can lead to the identification of evolutionarily conserved and unique stress defence mechanisms. Role of computational methods and integrated omics technology including protein dynamics and metabolome landscape in a systems approach will be instrumental in future. Challenges to be met for integrated knowledge of animal abiotic stress responses and tolerance in changing climate scenario will include identification of sensors and signalling pathways, understanding the molecular basis of interplay among stresses and developmental processes/stages, examining long-term responses under a plethora of abiotic stress conditions in nature and simulating the farm conditions. The road ahead to animal agriculture's adaptation to climate change should therefore integrate technological advances for climate resilient animals, policy, and finance options toward lowering emission and promoting inclusive growth.

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Impact of Physiologically Relevant Elevated Temperature on Oocytes/Embryos of Buffalo (*Bubalus bubalis*) Produced In Vitro

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Introduction:

Buffaloes are found only in certain regions of the world i.e., Asia, some Mediterranean, Eastern Europe and many Latin America countries. Since these regions are widely different in the geographical conditions, buffalo can thrive very well and be simultaneously economical. India has about 105 million buffaloes (www.dahd.nic.in) which is the highest figure in the world contributing about 55 million tons of milk to the country's total milk production. Like all other mammals, buffaloes are homoiotherms i.e., they maintain a constant body temperature regulating peripheral and internal body temperature with assistance of cutaneous sensors and internal temperature sensors (located in the hypothalamus) along with integration of endocrine system. Buffaloes are essentially shade and water-loving animals. However, they exhibit signs of great distress when exposed to direct solar radiation or when working in the sun during a hot weather. At the same time, although, generally, buffaloes are associated with hot humid tropics and sub-tropics, it has a greater tolerance of cold weather than is commonly supposed due to its exposure to cold stress in some of the cold areas in which it exists in the world. Buffalo and animal production economics are affected by many factors, particularly environment and nutrition. However, under the hot climate conditions, the combined effect of such factors may be more substantial due to the negative effect of elevated ambient temperature on appetite and accordingly on the feed intake that ends with slowing growth and impairment of reproduction. The ability of buffalo to tolerate high environmental temperature depends primarily on the capacity to dissipate heat. In buffaloes, the ability to sweat is deficient and the body attempts compensatory measures such as increased respiration rate and panting. An increase in body temperature by 1-2°C may result in detectable, deleterious effects in metabolism, tissue integrity and a significant depression in production. When the heat load of an animal is greater than its capacity to lose heat, a portion of the metabolizable energy typically used for production must be diverted to assure thermal balance. Therefore, selection for tolerance to environmental stress has traditionally resulted in reduced productivity.

The primary factors that cause heat stress in buffaloes are high environmental temperatures and relative humidity. Exposure to high ambient temperature is the major constraint on buffalo productivity in hot climatic conditions. This is due to the fact that it evokes

a series of drastic changes in the biological functions that include depression in feed intake and its utilization, disturbance in metabolism of water, protein, energy, mineral balance, enzymatic reactions, hormonal secretions and blood metabolites. Such changes result in impairment of productive and reproductive performance of buffaloes (Habeeb *et al.*, 1997). The effect of heat stress on cellular function includes inhibition of DNA synthesis, transcription, RNA processing and translation; inhibition of progression through cell cycle; denaturation and misaggregation of proteins; increased degradation of proteins through both proteosomal and lysosomal pathways, that lead to a net reduction in cellular ATP and changes in membrane permeability (Lacetera *et al.*, 2009). Thermal stress is a unique and complex phenomenon that brings about numerous changes beyond the animal's baseline homeostatic mechanism causing alterations of the normal physiological mechanisms that elicits a stressful response.

Heat stress and reproductive functions:

Females of a variety of species (mice, rats, rabbit, sheep, pig and cattle) experience reduced pregnancy rates when exposed to elevated ambient temperatures such as those during summer months. Reduced fertility during heat stress condition is largely because of hyperthermia. Exposure to elevated ambient temperature negatively impacts several stages of development to reduce pregnancy rates. These stages include the germinal vesicle (GV)-stage oocyte contained within antral ovarian follicles (Al-Katanani *et al.*, 2002), the ovulated oocyte near the time of fertilization (Stott and Williams, 1962), the zygote just after fertilization (Ju and Tseng, 2004), and early cleavage-stage embryos (Ealy *et al.*, 1993). The majority of early embryonic losses in cattle in the presence of environmental heat stress occur before day 20 of gestation (Hansen and Arechiga, 1999). During periods of heat stress, losses are more pronounced and may continue through day 40 to 50 of gestation (Cartmill *et al.*, 2001). After the embryo becomes a fetus (day 42), pregnancy losses are small (Jousan *et al.*, 2005). Effects of heat stress are particularly prominent during estrus (day-1) when the oocyte is undergoing meiotic maturation in preparation for fertilization (Cavestany *et al.*, 1985). While negative effects of high temperature exposure on fertility during meiotic maturation could result from effects on the maternal environment such as the follicle or hormonal profiles (Rensis and Scaramuzzi, 2003), direct (*in vitro*) exposure of bovine oocytes to elevated temperature during maturation reduced embryo development to a similar degree as that seen *in vivo*.

Impact of heat stress on buffalo oocytes/embryos *in vitro*:

Although the effects of heat stress on buffalo are well studied (Marai and Habeeb, 2010), to our knowledge, there are no reports on the effects of heat shock on buffalo oocytes or embryos. Of

the various models that have been employed in cattle to examine the effects of heat stress on the oocytes, the most successful and the feasible model is that of *in vitro* culture which includes *in vitro* maturation, *in vitro* fertilization and *in vitro* culture of buffalo oocytes up to the blastocyst stage. There are no reports available regarding the effect of heat stress on the buffalo oocytes except the study carried by Yadav *et al.* (2013). In this study, using the model of *in vitro* culture, buffalo oocytes/embryos were exposed to a heat shock of 2h every day at 39.5 and 40.5°C. The temperatures were chosen based on the earlier studies in which the highest recorded rectal temperature following exposure of buffaloes to solar radiation during summer was reported to be 40.2°C (Aggarwal and Upadhyay, 1998) to 40.6°C (Das *et al.*, 1999). The duration of exposure was kept 2 h once every day throughout IVM, IVF and *in vitro* culture (IVC) of oocytes as buffaloes were expected to find shade or a pond to wallow within 2 h of it being let out for grazing. Experiments were aimed at examining the effect of heat shock on the developmental competence and level of apoptosis in embryos. In another, experiment the effects of heat shock on the quantitative expression of some genes related to stress (HSPs), apoptosis and development were investigated by quantitative real-time PCR (qRT-PCR).

***In vitro* maturation, fertilization and culture of oocytes:**

Buffalo ovaries collected from a nearby abattoir in warm isotonic saline (32–37°C) fortified with antibiotics and were transported to the laboratory within 6 h at approximately 25°C. Follicular (2–8 mm in diameter) oocytes were aspirated with a 19-gauge needle attached to a 10-ml syringe. Only those cumulus-oocytes complexes (COCs), which had a compact and unexpanded cumulus mass having ≥2 layers of cumulus cells and with homogenous evenly granular ooplasm were used for IVM. Groups of 18–20 COCs were cultured in droplets overlaid with sterile mineral oil in 35-mm Petri dishes and cultured for 24 h in a humidified CO₂ incubator (5% CO₂ in air) at 38.5°C. The spermatozoa used for IVF throughout the study were from the same donor tested for IVF earlier. The spermatozoa were processed for IVF as described earlier (Chauhan *et al.*, 1998) with some modifications. The *in vitro* matured oocytes were then co incubated with spermatozoa in a CO₂ incubator (5% CO₂ in air) at 38.5°C for 18 h for IVF. At the end of sperm–oocyte incubation, the cumulus cells were washed off the oocytes by gentle pipetting. The oocytes were then cultured in IVC medium for 48-h post-insemination in a humidified CO₂ incubator at 38.5°C. The cleavage rate was recorded on Day 2 post-insemination, and the percentage of oocytes that developed to 4-cell, 8–16-cell, morula and blastocyst stage were recorded on Days 3, 4, 8 and 8 post-insemination, respectively. For each of the three experiments conducted under the present study, the immature oocytes were divided into the following three groups. Group 1 (Control): IVM, IVF and IVC were carried out at 38.5°C. The

oocytes/embryos of the experimental groups were exposed to a temperature of 39.5°C (Group 2) or 40.5°C (Group 3) for 2 h (11.00 AM to 1.00 PM) once every day during the course of IVM, IVF and IVC.

Effect of heat shock on the developmental competence of buffalo oocytes:

In the present study, the effect of heat shock on the developmental competence of oocytes/embryos was evaluated. Results indicated that the cleavage rate on Day 2 post-insemination and the percentage of oocytes that developed to 4-cell stage on Day 3 post-insemination were significantly lower ($p < 0.05$) for oocytes/embryos exposed to 39.5 or 40.5°C for 2 h once every day during IVM, IVF and IVC compared to those for controls (Table 1). The percentage of oocytes that developed to 8–16-cell stage on Day 4 post-insemination and to blastocyst stage on Day 8 post-insemination was significantly lower ($p < 0.05$) for the oocytes/embryos exposed to 40.5°C compared to those exposed to 39.5°C, which in turn was significantly lower ($p < 0.05$) than the corresponding value for controls. Not a single oocyte that had been exposed to 40.5°C was able to develop to morula or blastocyst stage. The present results are in accordance with the already ongoing studies in our lab in which the maturation at a slightly higher temperature (40.5°C) for complete 24h has an adverse effect on the buffalo oocytes (Ashraf *et al.*, 2011).

Table 1: Effect of heat shock on *in vitro* developmental competence of oocytes

Experimental Group*	Morula at 8 days post insemination	Blastocyst at 8 days post insemination
Group 1 (38.5°C, Control)	153 (14.2 ± 0.3) ^a	97 (9.0 ± 0.3) ^a
Group 2 (39.5°C)	75 (8.7 ± 0.9) ^b	24 (2.8 ± 0.5) ^b
Group 3 (40.5°C)	0 (0) ^c	0(0) ^c

(Yadav *et al.*, 2013)

Values are represented as mean ± SEM. Oocytes/embryos were exposed to 39.5°C or 40.5°C for 2 h once every day from 11.00 AM to 1.00 PM during entire culture conditions (i.e. IVM, IVF, IVC).

Roth and Hansen (2004) also studied the effects of heat stress on the oocytes of cattle during IVM and the negative effects could be readily observed on the cleavage and blastocyst production rate. Not only the heat stress but also the high oxygen conditions that are prevalent during *in vitro* culture conditions decrease the cleavage and the blastocyst production rates. The culture of bovine oocytes at an oxygen environment of 21% compared to the normal oxygen environment of 5% has been carried out (Balasubramanium *et al.*, 2007) and the results indicate the adverse effects of high oxygen environment (21%) compared to the 5% oxygen environment

on the culture of bovine oocytes. The adversity is because the concentration is nearly three to four times more in culture conditions than prevalent *in vivo* (5%). One of the mechanisms through which heat stress manifests in embryos/oocytes is apoptosis. Roth and Hansen (2004) concluded that in cattle, the proportion of apoptotic oocytes was higher after the oocytes were matured at a slightly higher temperature. Similar results were obtained by Jousan and Hansen (2004) in which the proportion of apoptotic blastomeres was higher when heat stress was applied on 8-16 cell stage embryos. These previous findings were in agreement with the study carried out in buffalo in our lab (Yadav *et al.*, 2013) in which the proportion of TUNEL-positive blastomeres was higher following the heat shock of 39.5 and 40.5°C for 2h each day (Table 2). The total cell number (TCN) was significantly higher ($p < 0.05$) in the control group blastocysts compared to the oocytes/embryos exposed to 39.5 or 40.5°C for 2 h once every day during IVM, IVF and IVC as determined by Hoechst staining.

Table 2: Effect of heat shock on the level of apoptosis in buffalo embryos produced *in vitro*

Number of TUNEL-positive cells in embryos		
Group*	8-16 cell stage	Blastocyst stage
Group 1 (38.5°C, Control)	4.18 ± 0.23^a (20)	4.94 ± 0.44^a (15)
Group 2 (39.5°C)	6.18 ± 0.30^b (21)	18.16 ± 0.78^b (18)
Group 3 (40.5°C)	11.54 ± 1.34^c (21)	-

Values are mean \pm SEM. *Oocytes/embryos were exposed to 39.5°C or 40.5°C for 2 h once every day from 11.00 AM to 1.00 PM during culture conditions (i.e IVM, IVF and IVC).

Blastocyst Quality Assessment (TCN) - Hoechst staining

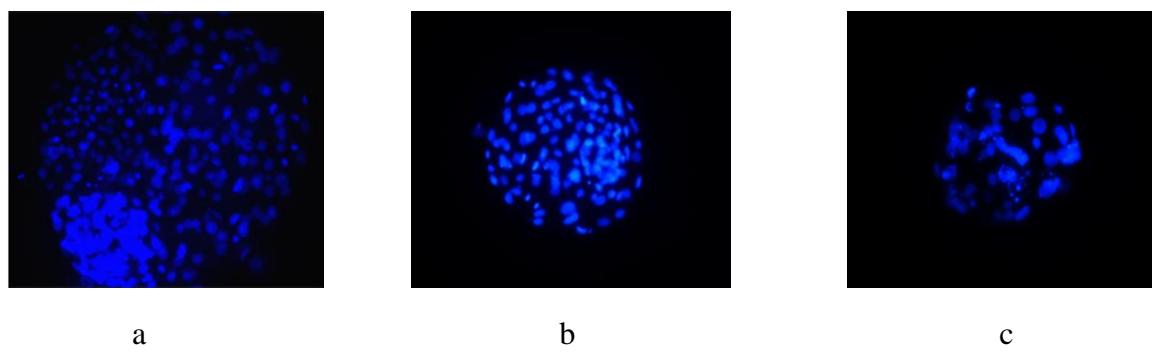


Fig 1: (a) Control group 38.8°C, (b) 39.5°C, (c) 40.5°C

This increase in apoptosis was also confirmed by higher relative abundance of pro apoptotic genes like caspases and Bcl-2 family transcripts in heat shocked embryos/oocytes. The results obtained here are in agreement with the previous results carried out by various

investigators (Jin *et al.*, 2007; Chang and Yang, 2000) in cattle. Another mechanism that can explain the adverse effects of heat stress on oocytes/embryos is the accumulation of free radicals or the reduced activity of antioxidant enzymes. The role of reactive oxygen species (ROS) and its association with apoptosis in mediating the effects of heat shock is not clear in oocytes/embryos. However, the increased abundance of free radicals in other cell types like fibroblast, lymphocyte has been reported by many investigators in buffaloes and other species. Hyperthermia is considered to cause oxidative stress in cells and tissues by generating the superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2) (Fisher *et al.*, 1991). In physiological conditions, the superoxide anion is degraded to H_2O_2 by SOD in the cytosol and mitochondria. Hydrogen peroxide is then removed by either catalase or glutathione (GSH) peroxidase and GSH transferase. When somatic cells, such as CHO cells and ovarian carcinoma cells, are exposed to heat shock, the SOD activity is increased in response to the stress (Loven *et al.*, 1985). Glutathione is a tripeptide derived from glycine, glutamate, and cysteine and is one of the redox buffers in living cells in which it serves as a free radical scavenger catalyzed by GSH peroxidase to remove peroxides. GSH is a ubiquitous intracellular free thiol that functions in many biological processes including DNA and protein synthesis, drug and chemical metabolism. GSH is thought to play critical roles in oocyte function including spindle maintenance and provision of reducing power needed to initiate sperm chromatin decondensation. In thermotolerant cells, GSH levels are higher than in thermal intolerant strains indicating that hyperthermia can activate the antioxidative defense system and enzyme synthesis. Without these enzymes, cellular injuries can occur in the forms of chromatin damage, lipid peroxidation of membranes, altered cytoskeletal structures, and other detrimental changes. When rat embryos were in hyperthermic conditions, the GSH level was increased compared to that of the control group (Harris *et al.*, 1991). When L-buthionine-S, R-sulfoximine (BSO), a GSH synthesis inhibitor, was used in culture, the thermotolerance of embryos decreased without affecting HSP or its mRNA. However, similar studies need to be carried out in buffalo oocytes/embryos so as to ascertain the role of antioxidant enzyme systems in hyperthermia and the possible heat stress mitigation strategies. The present study also suggests the decreased developmental competence of buffalo oocytes due to the effect of heat stress on the developmentally important genes and HSPs. Exposure of buffalo oocytes and embryos to heat shock increased the relative mRNA abundance of stress-related genes HSPs significantly at the 8-16 cell stage. It is quite possible that HSPs produced constitutively and those produced in response to heat shock may be involved in providing thermal resistance.

Thermotolerance of domestic animals can be altered by using modern biotechnological techniques such as transgenesis. Transgenic approaches and cloning of heat resistant genes or breeds may be one of the potential strategies to improve thermotolerance in domestic species particularly in sub tropical countries like India where high temperature and high humidity are major constraints in buffalo production processes. These approaches have already been employed to small animals such as mouse, hamsters etc. and have proven to be simultaneously beneficial. Microinjection of HSP mRNA was first reported to be effective in enhancing thermotolerance of mouse oocytes (Hendrey and Kola, 1991). Over-expression of the glutathione peroxidase gene in mice as well as HSP27 in hamsters (Hout *et al.*, 1991) also contributes to increased thermotolerance and resistance to oxidative stress.

In conclusion, the results of the present study demonstrate that exposure of buffalo oocytes and embryos to elevated temperatures for duration of time that is physiologically relevant severely compromised their developmental competence, increased apoptosis, and significantly decreased the relative abundance of important development-related genes. However, more studies need to be carried out in order to improve the thermotolerance of buffalo embryos/oocytes, since in India, buffalo forms the backbone of milk production contributing about 55% of the total produce. Buffalo not only contributes in milk production but also has a major share in the meat industry in Asian countries in general and in India in particular. Therefore, more and more strategies need to be formulated and adopted for mitigating the effects of heat stress on buffalo embryos/oocytes in order to enhance their reproductive efficiency and hence overall production.

Acknowledgement:

This work was funded by National Initiative on Climate Resilient Agriculture (NICRA, Dr. R.C. Upadhyay, PI) project.

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Present and Future Requirement of Water for Agricultural Crops and Livestock

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It is known fact that fresh water constitutes only 2.5% of the total water on the planet and remaining 97.5% is sea/salt water. Out of total global water reserves (~ 1400 MM Tr litres), the freshwater consists of only about 35MM Tr litres, half of which supports 86% of the population (Grail Research, 2009). Groundwater and surface water, which together constitute 30.5% of the freshwater reserves (~0.76% of the total water on the planet), are the most easily accessible and used sources of water. Every year, about 0.11 MM Tr litres of precipitation falls on land, 92% of which is lost due to surface runoff, evaporation, etc. It is estimated by UN as reported in World Water Development Report (UN, 2006) that by 2025, 3 billion people will be leaving below the water stress threshold. In low and middle-income countries about 85%, 10% and 8% of water is consumed in agriculture, industrial and domestic sectors, respectively. In India, these sectors consume 1658, 115 and 93 billion litres per day (in year 2000), respectively and is estimated to be (with CAGR 0.56%) 1745, 441 and 277 Bn litres per day, respectively in 2050. The consumption is expected to increase due to increase in population and urbanization; changing consumption patterns of the population towards use of water-intensive products; and rapid industrial growth.

India is endowed with large renewable water resources, but is faced with the issue of imbalance, poor seasonal distribution, and vulnerability to changing climates. As much as 139 Mha of land area receives $> 1000 \text{ mm yr}^{-1}$, of which 33 Mha receives $> 2500 \text{ mm yr}^{-1}$ of rainfall (Table 1).

Table 1. Rainfall received in different geographical regions of India (FAO, 2002)

Rainfall (mm yr ⁻¹)	Geographical area (10 ⁶ ha)	Rainwater received (10 ⁶ ha-m)
100-500	52.1	15.6
500-750	40.3	25.2
750-1000	65.9	57.6
1000-2500	106.4	205.9
>2500	~32.6	95.7
Total	297.3	400

Total annual rainfall received in India is ~ 400 million ha-m; of this, only 150 million ha-m (37.5%) infiltrates into the soil, 180 million ha-m (45%) is lost as surface runoff, and 17.5% as evaporation (FAO, 2002). Therefore, conservation, management, and recycling of rain water are crucial to reducing waste and to enhancing production of rain-fed agriculture.

Water resource in terms of rainfall and renewable water; and sector-wise utilization of water in India is shown in Tables 2 and 3 (Grail Research, 2009).

Table 2. Water resources in terms of rainfall and renewable water in India

Water resources	Quantity
Total renewable water resources (billion m ³ year ⁻¹)	1897
Per capita total renewable water (m ³ year ⁻¹)	1729
Total internal renewable water (billion m ³ year ⁻¹)	1261
Per capita total internal renewable water (m ³ year ⁻¹)	1149
Withdrawal for agriculture (%)	87
Average annual precipitation (mm)	1170

Table 3. Water utilization in different sectors of India

Water withdrawal and utilization sectors	Amount
Total water withdrawal (mi m ³)	761
Agriculture (%)	89.00
Municipality (%)	7.40
Industry (%)	2.23
Others (including losses) (%)	1.37
Source	
Ground water (%)	97.1
Sea water (%)	2.9

In India, population growth and overall economic development are expected to lead to increase in water usage across sectors. The production of water-intensive crops is expected to grow by 80% between 2000 and 2050. Virtual water consumed for production of wheat, rice and sugarcane increased by 88 trillion liters over the period 2000 to 2008; for wheat ~4 trillion litres, for rice~ 18 and for sugarcane ~ 66 trillion litres. This is based on water footprints, which has been calculated for respective crops to be 1654, 2850 and 15 thousand litres per ton, respectively (FAO, 2008). It is estimated that livestock industries consume 8% of the global water supply, with most of that water being used for intensive, feed-based production (Schlink *et al.*, 2010). In developing countries animals are not used solely for food production but also provide draught power, fibre and fertiliser for crops. In addition, animals make use of crops by-products that would otherwise go to waste. However, the livestock sector is the fastest-growing agricultural sector, which has led to increasing industrialisation. Average virtual water content of selected agricultural products in India and world is given in Table 4.

Water consumption in Indian agriculture:

The available water resources are likely to be overexploited with a rise in the consumption of water for irrigation because of rise in domestic demands of food grains (due to increase population)-will grow from 178 MM mt in 2000 to 241 MM mt in 2050 ; increase in exports (value of agricultural exports have tripled from \$5.6 Bn in 2000 to 18.1 billion in 2008); and change in consumption pattern of agricultural products (demand for agricultural products with high water footprints). Contribution of other than food-grain items (sugarcane, fruits and

vegetables) and animal products in daily food intake for an individual is expected to grow from 35% in 2000 to ~50% in 2050 (Grail Research, 2009).

Table 4. Average virtual water content of selected agricultural products in India and world (adapted from Hoekstra and Chapagain, 2007)

Product	Virtual water content of products (l/kg biologically available protein) in India	World average virtual water content of products (l/kg)
Soybean	14907	1789
Wheat	29726	1334
Maize	59290	-
Rice	56815	2291
Sorghum	54821	2853
Eggs (whole)	73067	3340
Milk	50629	990
Chicken meat	77125	3918
Goat meat	51712	4043
Sheep meat	66717	6143
Pork	61257	4856
Beef	164319	15497
Cotton (lint)	-	8242
Coffee (roasted)	-	20682

Future Scenario:

At present irrigation sector consumes as much as 83% of available water resources. Rice, wheat and sugarcane together constitute ~90% of India's crop production and are the most water consuming crops. The states with the highest production of these crops are expected to face groundwater depletion of up to 75% by 2050. The water demand as estimated by Standing Sub-Committee for assessment of availability and requirement of water for diverse uses in the country by the Ministry of Water Resources will be 1447 BCM in 2050 against 634 BCM in 2000 (Table 5). The World Bank, however, has projected that the annual water demand will be 1050 BCM by 2025 of which 69% will be from agricultural uses, 20% from industrial use, 5% from uses and 6% from environmental and others.

Table 5. Water demand (BCM) by various sectors at different period of time (MoWR)

Sector	2000	2010	2025	2050
Irrigation	541	688	910	1072
Drinking water	42	56	73	102
Industry	8	12	23	63
Energy	2	5	15	130
Others	41	52	72	80
Total	634	813	1093	1447

With the demand from other sectors rising at a fast pace, the availability of water for irrigation would reduce. It is, therefore necessary to improve the performance of existing system; diversifying the existing cropping systems; and adopting scientific water-use efficient technologies. Irrigation efficiency should be improved from the present average of about 35-40% to the maximum achievable (~60%). Wherever water is scarce, economically advantageous deficit irrigation may be practiced. The total annual replenish able ground water resources have been reassessed as 433 BCM and the stage of groundwater development is 58%. The strategy for the next Five Year Plan therefore, recommends a two-pronged approach for ground water:

- i) Promotion of groundwater development in areas having untapped / unutilized potential
- ii) A comprehensive act for regulation of groundwater development on sustainable basis.

Adaptation strategies for water management:

Already large numbers of efficient water and fertilizer management practices are available for paddy and wheat cultivation that reduce the consumption of water. Water harvesting and supplemental irrigation for drought proofing areas, improved drainage and flood control measures in high rainfall regions and techniques for conservation of resources are of immense importance for mitigating the water scarcity and adverse effect of climate change. These adaptations can be at the level of individual farmer, society, farm, village, watershed, or at national and regional level. Important technologies include: conservation agriculture (tillage, residue and nutrient management), *in situ* moisture conservation, rainwater harvesting and recycling, efficient use of irrigation water, energy- efficiency in agriculture, use of poor quality waters, alternate land uses and agro forestry on degraded lands. Some of these are dealt below in brief:

Conservation agriculture (CA):

The three key elements of conservation agriculture include minimum disturbance of soil, permanent soil cover, and innovative and economically viable crop rotations (FAO, 2009). CA systems are now practiced globally on about 125 M ha in all continents and all agricultural ecologies and the adoption has been low (4.72 M ha) in Asia, particularly in South Asia where the awareness and adoption of CA is on the increase (Friedrich *et al.*, 2012). Results from rain-fed and irrigated long-term trials have shown that not zero tillage as such, but the combination of zero tillage with retention of sufficient soil-surface crop residue resulted in increased physical, chemical and biological soil quality (Hobbs and Govaerts, 2010). Moreover, it has been reported that zero tillage without residue retention results in deteriorated soil beyond the conventional tillage practices. CA increases water stable aggregates, enhance water holding capacity and infiltration rate, hence reduced runoff resulting in lower soil erosion, increase soil penetrability of roots, and increase biological porosity, and increase microbial population including earthworms (Hobbs and Govaerts, 2010).

Resource conservation technologies (RCTs):

The term ‘resource conserving technologies’ (RCTs) has been coined to describe some of these intermediate steps towards the complete implementation of all the CA principles. RCTs encompass practices that improve resource- or input-use efficiency (including water, air, fossil fuels, soils, inputs, and people) and provide immediate and demonstrable economic benefits such as reductions in production costs, savings in water, fuel and labour requirements and timely establishment of crops resulting in improved yields. Laser land leveling, bed planting, zero tillage, direct drilling in residue, direct seeding rice (DSR), residue management to avoid burning, alternate wetting and drying in rice, site specific nutrient management, diversification/intensification, and alternate land uses/agroforestry are some innovative RCTs, which are able to quickly respond to critical needs that address the concerns (e.g. farm economics and climate change) faced by Indian agriculture (Saharawat *et al.*, 2012). The RCTs are increasingly being adopted by farmers in the rice-wheat belt of the Indo-Gangetic Plains (IGP) because of several advantages of labour saving, water saving, and early planting of wheat. In the IGP, in the wheat-rice cropping system, there is large adoption of zero-till (ZT) wheat with some 5 M ha, but only marginal adoption of permanent no-till systems and full CA.

Zero tillage:

Many studies conducted across the production systems under varied ecologies of India (Malik *et al.*, 2005; Jat *et al.*, 2010, 2011; Saharawat *et al.*, 2012) have revealed the potential benefits of above mentioned RCTs (Table 6), which also play important role in mitigating climate change. In India, the adoption of no-till practices by farmers has occurred mainly in the wheat portion of the wheat-rice double cropping system.

Table 6. Effect of different conservation agriculture-based crop management technologies on yield gain, water saving and increase in water productivity (WP) over conventional practice in Indo-Gangetic plains

Technologies	Crop/cropping system	Yield gain (kg ha ⁻¹)	Water saving (ha-cm)	Increase in WP (kg m ⁻³)
Laser leveling	Rice-wheat	750-810	24.5-26.5	0.06
	Rice	750	22.0	-
No till	Wheat	150-140	2-4	0.10-0.21
	Wheat	610	2.2	0.28
	Maize	150	8	0.21
	Rice-wheat	500	61	0.24
No till with surface residue	Wheat	410	10	0.13
Direct seeded rice	Rice	120	25	0.08
	Rice	510	13	0.09
	Rice	62	18	0.10
Raised bed planting	Maize	324	12	0.80
	Wheat	310	16	0.58
	Wheat	270	5	0.50

Source: Chauhan *et al.* (2012)

Alternate crop establishment methods:

Furrow irrigation-based bed system:

In raised bed, planting not only reduces irrigation water use by 12-60% (Gupta *et al.*, 2003) and improves drainage but will also reduce CH₄ emission. Singh *et al.* (2003) reported that compared with transplanted rice, DSR rice on flatland and on raised beds reduced total water input during

crop growth by 35–42% when the soil was kept near saturation and by 47% when the soil dried out to 20 kPa moisture tensions in the root zone. Most of the water savings were caused by reduced percolation losses. There are several reports of reduced irrigation amounts or time by up to 30-40%, with similar or higher yields for wheat on raised beds compared with conventional tilled wheat (Bhardwaj *et al.*, 2009; Ram *et al.*, 2011). In wheat-rice system in IGP, irrigation water saving ranged from 18% to 50% with adoption of bed planting system (Jat *et al.*, 2005).

Dry direct seeded rice (DSR):

Conventional puddled transplanted rice consumes a large quantity (120 to 180 cm) of water (Chauhan *et al.*, 2012). Dry direct seeded rice (DSR) is considered a viable agro-technology to cope with the looming crisis of water supply that threatens the sustainability of irrigated rice production systems. Overall, DSR needs 25-57% less water than conventional lowland rice, it has been reported to produce similar or lower grain yields in many studies conducted in India (Kumar and Ladha, 2011; Mahajan, 2011).

System of rice intensification (SRI):

System of rice intensification (SRI), an innovative rice production system, developed in Madagascar, is characterized by transplanting very young rice seedlings (8–12 days old) in single plants/hill, and widely spaced; applying intermittent irrigation to keep soil moist; applying compost; and hand or mechanical weeding, through which weeds are incorporated into the soil (Stoop *et al.*, 2002). A number of reports (e.g. Geethalakshmi *et al.*, 2011) have shown substantial (25 to 70%) reduction in irrigation water requirements with SRI compared to traditional method of rice cultivation while producing similar or higher yields. The irrigation water use efficiency was increased with SRI by about 90.0% compared with that of CT.

Precision laser land-leveling:

Precision land levelling resulted in irrigation water savings of 15-30% accompanied by increases in crop yields by 4-6% compared to traditional levelled fields (Sidhu *et al.*, 2010; Kaur *et al.*, 2012). Currently this technique is being practiced over 1.5 million ha in South Asia (Jat *et al.*, 2011).

Straw mulching:

In India, more than 140 Mt of crop residues are disposed of by burning each year. In IGP, rice–wheat is the main cropping system. There are few options for rice straw because of poor quality for forage, bioconversion, and engineering applications. Farmers burn the rice straw to establish the wheat crop rapidly while labour is limited. Presently, more than 80% of total rice straw (22 Mt) produced annually in Indian Punjab to clear the fields for timely sowing of wheat (Yadvinder-Singh *et al.*, 2010). Rice straw can be managed successfully *in situ* by allowing sufficient time between its incorporation and sowing of the wheat crop. Mulching in non-flooded crops has a significant effect on soil water conservation in reduced and no tillage systems and the effect was more pronounced in dry periods. Balwinder-Singh *et al.* (2011) reported that rice straw mulch in wheat in rice-wheat system in the IGP of India, reduced evaporation by 42-48 mm during wheat growing season. Chakraborty *et al.*(2010) reported that compared to no-mulch, mulches produced 13-21% higher grain yield and more roots (25 and 40% higher root weight and root length densities) in sub-surface (>0.15 m) layers, probably due to greater retention of soil moisture in deeper layers. Mulches were effective in reducing 3–11% crop water use and improved its efficiency by 25%.

Crop diversification:

Rice-wheat cropping system is the most important cropping system for food security in South Asia but the sustainability of the system is threatened because of the shortage of resources such as water and labour. The farmers have taken the initiative to diversify their agriculture by including short duration crops such as potato, soybean, black gram, green gram, cowpea, pea, mustard, and maize into different combinations (Gangwar and Singh, 2011). However, lack of profits, price support and marketing bottlenecks for the alternate crops are some constraints in adopting diversification. Inclusion of certain crops in sequential and intercropping systems has been found to reduce nutrient and water needs and also the population of some obnoxious weeds to a considerable extent. Further, inclusion of legumes in cropping system has been found to be effective in reducing nitrate leaching in lower soil profiles and legumes also play an important role in conserving groundwater and soil water (Chauhan *et al.*, 2012).

Replacing rice with cotton, maize and basmati rice in summer season and wheat with oil-seed (rapeseed mustard) crops and chickpea in winter season can lower ET and reduce irrigation requirement (Jalota *et al.*, 2009). Hira (2009) suggested reducing rice area in Punjab by about 1 million ha and cultivating BT (*Bacillus thuringiensis*) cotton, *kharif* maize, soybean and groundnut, which require 2-5 number of irrigations.

Developing climate-resilient genotypes:

The challenge is to develop water-efficient genotypes that produce higher yields with limited water supply, and equal or greater yields than current varieties under favorable growth conditions without stress. Developing short-duration varieties/hybrids is also an effective strategy for reducing seasonal transpiration and minimizing yield loss from terminal drought, as early maturity helps the crop to avoid the period of stress. Developing rice varieties for aerobic rice culture should possess moderate tolerance to moisture stress, improved lodging resistance and higher harvest index (Atlin and Lafitte, 2003).

Another important approach to minimize the occurrence of water stress is through development of a good root system that, in the case of drought, permits water to be accessed deeper in the soil and, in the case of heat, permits transpiration rates that better match evaporative demand, thereby permitting maximal carbon fixation with the added benefit of cooler plants. Much effort is currently being directed to developing molecular markers for various traits such as maximum rooting depth, the capacity of roots to penetrate hard pans, and ability of the plant to osmotically adjust to water deficit.

Alternate land use systems/agroforestry:

In the current scenario, agroforestry systems have attracted special attention in climate change mitigation and adaptation discussions as most of these farming systems yield multiple benefits, such as sustainable production, household nutrients requirement through diversified food products, resource conservation, groundwater recharge, employment generation, social equity, and environment improvement. Agroforestry has been recognized as having high potential for sequestering carbon, adaptation and mitigating climate change and above all resource conservation and sustainable productivity (Dagar *et al.*, 2012).

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Water Footprint of Cattle and Buffalo Milk Production

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The water resources are sine-qua-non for the survival of all life-forms on planet earth. The climate system and the hydrological cycle are intimately linked, and any variability in climate affects the hydrological cycle. A recent report of IPCC maintains that as per the evidence provided by the observational records and climate projections there is ample evidence that freshwater resources are vulnerable and have the potential to be strongly impacted by climate change, with wide ranging consequences on human societies and ecosystems (Bates *et al.*, 2008). The World Bank report on the water and climate change inter-linkages also reiterated that climate change could profoundly alter future patterns of both water availability and use, thereby increasing levels of water stress and insecurity, both at the global scale and in sectors that depend on water (Alavian *et al.*, 2009).

The rational and judicious use of water in the production process is therefore, in a way, an essential adaptation mechanism to cope with the potential threat of climate change. The total volume of freshwater used to produce the goods and services consumed by the individual, business or nation is known as the water footprint of an individual, business or nation (Chapagain and Hoekstra, 2004). The water footprint concept is closely linked to the virtual water concept (Chapagain and Hoekstra, 2004). The volume of water that is used to produce a commodity, measured at the place where the commodity is produced, is called the virtual water content of a commodity (Allan, 1993, 1994). The inverse of the virtual water content is known as the water productivity of the commodity. In line with the concept of virtual water, the concept of the water footprint has been introduced to create a consumption-based indicator of water use (Hoekstra and Hung, 2004; Hoekstra and Chapagain, 2007). From the perspective of a nation, the water footprint is defined as the volume of water needed for the production of the goods and services consumed by the inhabitants of a

country. From the commodity perspective, its water footprint is the Consumptive Water Use (CWU) in the process of production of a commodity (Hoekstra, 2003).

With the purpose of sensitizing about the issue of water use efficiency in livestock sector, this lecture note outlines the estimation methodology of water footprint of milk production and illustrates the CWU in milk production in the irrigated rice-wheat cropping system.

Water footprint: the concept

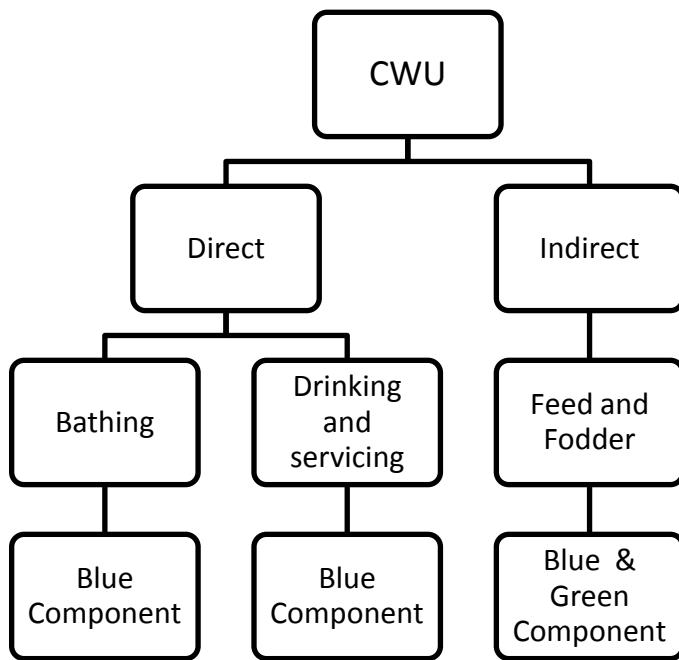
The water footprint or the CWU in the production process of primary commodities has three components, namely, blue, green and grey:

- i) The Blue water footprint is CWU from irrigation, both, groundwater and surface water
- ii) The Green water footprint is CWU from rainfall
- iii) The Grey water footprint is non-consumptive use due to deterioration in water quality

In milk production, the consumptive water use is both, direct (drinking, bathing and servicing) and indirect (through feed and fodder intake) (Fig. 1). In case of stall-fed animals, the direct water requirement is entirely met from groundwater and /or surface water sources; hence it comes under blue component of water footprint. For animals partly or fully under grazing system, rainwater may also partly account for water use by animals for drinking and bathing. But in the absence of data on the proportion of rain: ground water use, the entire volume of water is considered under the blue component. In feed and fodder production, there is CWU from irrigation (blue water) and effective rainfall (green water).

The milk production may also lead to pollution of water (grey water) through deterioration in water quality below drinking water standards due to contamination from animal wastes, excessive use of inputs like chemical fertilizers in production of feed and fodder crops. However, some of the polluted water may contain nutrients beneficial to crops.

Fig.1: Sources of Water use in Milk production



Estimation methodology:

Here the estimation of water footprint from the blue and green component is outlined in detail. Many of the parameters for estimating the third (grey) component; that is sometimes estimated as the quantity of freshwater required if the polluted water is to be brought to ambient (drinking) water quality standards (Hoekstra *et al.*, 2009); are not easy to collect and hence, its estimation is beyond the scope of this lecture note.

1. Estimation of direct water use (blue water):

Data on water required from drinking was collected (partly from observation and partly through farmer interview) on the volume of bucket / water trough used and number of times in a day same was filled by the farmer. The estimation of this component at the farmers' field is rather difficult but information on water use for drinking, servicing and bathing can be collected partly from observation and partly through farmers' interview or can be proxied from related information like, the diameter of water pipe used for serving and bathing, time period of water flow and number of animals in the enclosure at farm household. Similarly, data about drinking water can be approximated from the information on the volume of bucket/water trough used and number of times in a day same was filled by the farmer. On the research stations, controlled experiments can be carried out to ascertain the drinking water requirement of different breeds of dairy animals on the seasonal basis.

2. Estimation of Indirect Water Use (Blue and Green water footprint):

The calculation of indirect water footprint consists of calculation of water use of feed and fodder crops. The starting point in this methodology is the calculation of consumptive water use of a crop which is estimated as follows:

Calculation of crop water requirement:

The crop water requirement is the actual evapo-transpiration (ET_p) in four crop growth periods (initial, development, mid- and late stage) (Allen *et al.*, 1998)

$$ET_p = \sum_{i=1}^4 k_i \times \sum_{\text{month } j \text{ in } i^{\text{th}} \text{ growth period}} d_{ij} ET_0^{ij}$$

Where,

k_i = crop coefficient of i^{th} growth period

d_{ij} = Number of days of the j^{th} month in the i^{th} crop growth period

ET_0^{ij} = Reference evapotranspiration of the j^{th} month in the i^{th} crop growth period

If rainfall meets the full crop water requirement, then CWU from effective rainfall equals actual evapo-transpiration. If rainfall is insufficient to meet full crop water requirement, it is equal to the effective part of rainfall at the root zone (P_{eff}), where,

$$P_{\text{eff}} = \sum_{i=1}^4 \sum_{\text{month } j \text{ in } i^{\text{th}} \text{ growth period}} \frac{d_{ij}}{n_{ij}} P_{\text{eff}}^{ij}$$

$$P_{\text{eff}}^{ij} = \begin{cases} P75^{ij} \times (125 - 0.2 \times P75^{ij}) / 125 & \text{if } P75^{ij} \leq 250 \text{ mm} \\ 125 + 0.1 \times P75^{ij} & \text{if } P75^{ij} > 250 \text{ mm} \end{cases}$$

$P75^{ij}$ = daily 75% exceedence probability of rainfall

n_{ij} = total number of days in the j^{th} month of the i^{th} crop growth period

$$\text{CWU}^{\text{effective rainfall}} = \text{Min} (ET_p, P_{\text{eff}})$$

When irrigation meets part of the deficit crop water requirement, the irrigation CWU is the net evapotranspiration, that is the difference between the actual evapotranspiration (ET_p) and P_{eff}

$$CWU^{\text{irrigation}} = ET_p \cdot P_{\text{eff}}$$

Green Water Footprint is the CWU from rainfall = Min (ET_p, P_{eff})

Blue Water Footprint is CWU from irrigation = ET_p-P_{eff}

The meteorological parameters, like, the monthly data of ET₀ (reference evapotranspiration) and P75 (monthly 75% exceedence probability of rainfall) can be taken from World Water and Climate Atlas prepared International Water Management Institute (IWMI, 2000). This database gives the values for a large number of locations in India. The data on length of growing period, crop coefficient and yield of feed and fodder crops can be obtained from the regional agricultural stations situated in that particular area.

In India, as the livestock are fed on crop-residues (eg. wheat straw, rice straw, etc.) and by-products of crop (eg. oilseed cakes), the CWU in crops is apportioned on main and by-product. The data requirement for the same is (i) conversion ratios of main: by-product (ii) prices of main: by product.

The conversion ratios can be region and crop-variety specific. There is lot of difference in the prices of the main and by-products; hence the prevailing market price should be taken into consideration. The CWU (m³/ha) of by-product is

$$CWU^{\text{by product}} = (\alpha_i CWU^{\text{crop}})$$

$\alpha_i = q_i p_i / \sum_j q_j p_j$, and q_i and p_i are conversion ratios and prices of the main product or by-products (conversion ratio of the main product is 1).

Using the conversion ratios the yield of main and by-product can be worked out and dividing the CWU^{by product} by its respective yield gives the water use on per tonn basis.

Calculation of CWU of animals through feed and fodder:

Having estimated the blue and green CWU in prevalent feed and fodder crops, the next step is to collect data on their intake by the dairy animals. There are vast seasonal varieties in the fed and fodder intake and therefore, to capture the same, the data should be collected on seasonal basis, preferable for three seasons i.e. hot-humid/rainy, winter and summer. Intake of each type of feed and fodder is then multiplied by the CWU (in m³/kg) of respective crop to get the indirect water use by animals. Thus,

$$\text{Indirect Water Use} = \sum_i x_i \text{CWU}_i$$

Where, x_i is intake of 'i' feed/fodder by the animal and CWU_i is the consumptive water use of 'i' feed/fodder resource.

Estimates of water footprint of milk production:

Direct water use: The reported estimates of direct water use by dairy cattle vary widely from 64 litres/day (Chapagain and Hoekstra, 2003) to 100 litres /day (Singh *et al.*, 2004). Chapagain and Hoekstra (2003) have used the following averages of water consumption for drinking and servicing under the alternate dairy production systems (Table 1)

Table 1. Drinking and service water footprint of dairy cattle

Drinking water (litre/day)				Service water (litre/day)			
Grazing	Mixed	Industrial	Weighted average	Grazing	Mixed	Industrial	Weighted average
40	55	70	52	5	13.5	22	12

Note: grazing and industrial drinking and service water footprint from Chapagain and Hoekstra (2003). For mixed production system an average is used.

However, these are global average and not specific to India. The estimates of 100 litres/day given by Singh *et al.* (2004) for Gujarat have also been used by Amarsinghe *et. al.* (2010) for the study in Moga district of Punjab. The official norms of livestock water requirements used by the government in developing action plan for water resources in country are, however, very low at 20 litres /animal /day (GOI, 1999).

In order to resolve the uncertainty in estimates of direct water use, an attempt was made to estimate drinking and servicing water requirement of different breeds of dairy animals on the experimental herd at NDRI. The study was conducted during hot-humid, winter and summer seasons taking 10 lactating animals each from Murrah buffalo, Sahiwal, Tharparkar and Karan Fries A metabolism trial was conducted to determine water intake, feed intake and individual animal's daily milk yield was

recorded for by using circular dial type spring balance, with the capacity of 20 kg and accuracy of ± 0.05 kg. The results of the experimental data are as follows (Table 2):

Table 2. Season Wise Average Drinking Water Required by Cattles and Buffaloes at NDRI Research Farm (In Lt. /day)

Breed	Summer (Mar.-June)	Hot-Humid/Rainy (July-Oct.)	Winter (Nov.-Feb.)	Average
Karan Fries	40	25	45	35
Murrah	45	30	50	40
Sahiwal and Tharparkar	30	20	35	30

Besides the observations from the controlled experiment, data on drinking water use was also collected from the farmers' field, taking a sample of 120 dairy farmers from three villages in Karnal district. The average drinking water use based on the field data worked out to be 26 litres /animal /day (Table 3), which is somewhat lower than the average based on data from NDRI research farm. The average water use of servicing and bathing worked out to be 50 litres /day on the NDRI farm and marginally lower, i.e. 40 litres /day on the farmer's field.

Table 3. Blue Water footprints of milk production from direct CWU

Breed	Av. Milk Yield (Lt./day) (1)	Drinking water (Lt.) (2)	Bathing and Servicing (Lt.) (3)	Total Direct WU (Lt.) (4)	CWU (m ³ /ton) (5)= (4)/ (1)
<i>Organized Farm</i>					
Karan Fries	9.0	35	50	85	9.44
Murrah	7.4	40	50	90	12.16
Sahiwal and Tharparkar	7.2	30	50	80	11.11
<i>Unorganized Farms</i>					
Cross bred	8.3	26	40	66	7.95
Buffalo	5.2	26	40	66	12.76
Local Cow	4.5	26	40	66	14.64

In the estimation of direct water use the future studies can either take these results as default values or can work out the usage in their regions as per the data collection method outlined above.

Indirect water use: The estimates of water use and water footprint for the feed and fodder crops prevalent in Haryana are summarized in Table 4.

Table 4. CWU, Blue Water Footprint (BWP) and Green Water Footprint (GWP) of Feed and Fodder Crops in Haryana

	Crops	CWU (m ³ /ha)	GWP (m ³ /kg)	BWP (m ³ /kg)
Dry Fodder	Wheat straw	561.13	0.003	0.122
	Paddy Straw	380.33	0.030	0.069
Green Fodder	Jowar	2895.33	0.032	0.026
	Berseem	6530.44	0.001	0.086
	Maize	1121.04	0.009	0.013
	Oats	1825.58	0.001	0.040
	Mustard	1372.62	0.016	0.812
Concentrate	Mustard Cake	703.39	0.015	0.751
	Groundnut cake	1205.75	1.485	0.517
	Cotton seed cake	646.02	3.514	3.153
	Wheat Bran	2403.50	0.011	0.522
	Rice bran	6216.96	0.626	1.419

The dry fodder fed was wheat straw on the both, the organized and unorganized farms, except in some months (Jan.-March) where farmers also fed small amount of paddy straw. The composition of concentrates and the type of green fodder fed to animals on the organized (NDRI) and unorganized farms of Karnal is as follows:

Table 5. Composition of Concentrate and Green Fodder

Type of Farm	Concentrate	Green fodder		
		Hot-humid/rainy	Winter	Summer
Organized	<ul style="list-style-type: none"> • Maize- 33% • Groundnut cake (oiled)- 21% • Mustard cake (oiled)- 12% • Wheat bran- 20% • Deoiled rice bran- 11% • Mineral mixture- 2% • Common salt- 1% 	Maize and Jowar	Maize, Berseem and Oats	Maize, Oats and Jowar
Unorganized	<ul style="list-style-type: none"> • Mustard cake- 35 % • Cottonseed cake- 32% • Wheat flour- 33 % 	Maize and Jowar	Jowar, Berseem and Oats	Jowar, Berseem and Maize

Based on the monthly feed intake data of crossbred, buffaloes and local cows at NDRI farm and the seasonal (three seasons) data from the farmer's field the water footprint in milk production are as follows:

Table 6: Total water footprints (m³/tonn) of milk production

Breed	Av. Milk Yield (lt./day)	CWU (m ³ /tonn)		
		Blue (Direct+Indirect)	Green (Indirect)	Total
<i>Organized Farm</i>				
Karan Fries	9.0	996	216	1212
Murrah	7.4	1031	238	1269
Sahiwal & Tharparkar	7.2	1279	304	1583
<i>Unorganized Farms</i>				
Cross bred	8.3	1166	812	1977
Buffalo	5.2	1201	746	1947
Local Cow	4.5	981	563	1544

The water footprints will differ from place to place, for eg. Amarsinghe *et al.* (2010) calculated water footprint of milk production in Moga, Punjab to be 940 m³ /ton. Another study by Amarsinghe *et al.* (2011) and Chapagain and Hoekstra (2004) calculated all India average to be 1789 m³/ton and 1369 m³/ton respectively.

Concluding remarks:

The per capita freshwater availability in India is 1539 m³/year (AQUASTAT, 2012), below the global water stress threshold of 1700 m³/year (Falkenmark, 1989) and is anticipated to worsen in future due to rising demand and reduction in net water recharge due to climate change (Mall *et al.*, 2006). Thus, reducing water footprint or virtual water content of commodities produced in the country is important for coping with the water stress. The key to improving water use efficiency of a primary product lies in productivity enhancement through development and dissemination of region specific technologies.

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Carbon Credits from Livestock Production and Mitigation Strategies

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A carbon credit is a permit that allows the holder to emit one ton of carbon dioxide. Credits are awarded to countries or groups that have reduced their green house gases (GHGs) below their emission quota. Credits can be sold in the international market at the prevailing prices via certain credit exchanges at their current market price. Groups and organizations that stay below the emission quota can sell their balance quota as Carbon Credits certificates or just credit to another organization that has already gone or could go beyond the assigned cap. Under the Kyoto Protocol the caps are issued by National registry as 'Assigned Amounts' or 'Kyoto Units'. Formalised in the Kyoto Protocol, carbon credits help developing/underdeveloped countries as they traditionally have lower per-capita carbon emissions than developed countries and will need to emit CO₂ owing to increasing industrial growth. At this point though, these countries can sell their carbon credits to other countries and reap the economic benefits of not polluting the planet. The aim of the protocol is to lower the overall GHGs emissions.

Indian scenario:

India signed and ratified the Protocol in August, 2002 and has emerged as a world leader in reduction of GHGs by adopting Clean Development Mechanisms (CDMs) [The Clean Development Mechanism is an arrangement under the Kyoto Protocol allowing industrialized countries with a greenhouse gas reduction commitment to invest in emission reducing projects in developing countries] in the past few years. India is well ahead in establishing a full-fledged system in operationalising CDM, through the Designated National Authority (DNA). Other than Industries and transportation, one of the major sources of GHGs emission in India as follows:

- Paddy fields
- Enteric fermentation from ruminant
- Municipal Solid Waste

Green house gas emissions from Indian livestock:

Livestock is one of the major sources of CH₄ emission and also from agriculture sector as compare to N₂O emission. The emission of total GHGs from Indian livestock was estimated 247.2 Mt aggregate CO₂ equivalents which included 99.8 % CH₄ and 0.2 % N₂O emissions (Chhabra *et al.*, 2013).

Methane production:

Enteric methane:

Methane is produced as a result of the natural digestive processes in ruminant mammals (those that chew the cud), such as cattle, buffalo and sheep. Ruminants have a distinctive digestive system that enables them to eat fibrous plants and in the process of digestion they produce methane. Since, the digestion or enteric fermentation is only 50-60 percent efficient, some of the feed energy (i.e., 4-15 percent) is lost in the form of methane a potent greenhouse gas. Animals retained high fibrous diets in the rumen for a considerable period of time where, in the presence of a large and diverse microbial population, it is anaerobically fermented to form volatile fatty acids, ammonia, carbon dioxide, methane, cell material and heat. The balance of these products varies between animals and with dietary intake, being largely determined by the composition and activity rates of microorganisms present in the rumen. The gaseous waste products are mainly removed by the process of eructation or eliminated as part of the respiration process. Methane is released into the atmosphere from animals routinely and contribution of ruminants is more than non-ruminants. The enteric fermentation is a natural process of digestion in both ruminants and non-ruminants, where anaerobic microbes i.e., *methanogens* decompose and ferment fibrous feeds. Methane, a by-product of microbial digestion process, has 21-folds higher global warming potential than that of carbon dioxide. The level of methane production in rumen is affected by the quantity and quality of the feed. As the amount of feed consumption increases, the energy available for conversion into methane also increases. However, as the feed digestibility increases, the portion of energy that is converted to methane decreases. In a highly digestible feed, only 3-6% of energy would be converted to methane and as the digestibility decreases, the energy loss as methane increases to 9 percent or more. The effective measures to mitigate enteric fermentation in ruminant system would not only help to reduce methane emissions but also raise the productivity by improving the feed efficiency.

Manure management:

The anaerobic management systems of manure are mostly associated with intensive farming system of dairies or piggeries, where large numbers of animals are contained. The high density of animals leads to the production of large quantities of waste, which is typically washed out into tanks, lagoons or pits where it is stored as a liquid or ‘slurry’. It restricts the availability of oxygen to the waste, being liquid, air is excluded and waste decomposition takes place under predominantly anaerobic conditions, resulting in methane production. The amount of waste produced is dependent upon the number and types of animals, along with the quantity and quality of food consumed. The amount of methane produced from any given animal waste is dependent upon the quantity and composition of the waste, type of animal and feeding practices. As the temperature increases the biological activity of animal also increases, thereby enhancing methane production.

Table1: State-wise estimates of livestock methane, nitrous oxide and CO₂ equivalent emissions for the year 2003 (Chhabra *et al.*, 2013).

State/Union Territory	Livestock population (Millions)	Enteric Fermentation Methane emission (Tg/year)	Manure Management Methane emission (Tg/year)	Total Methane Emission (Tg/year)	Nitrous oxide emission (Gg/year)	CO2 equivalent emission (Mt)
Andhra Pradesh	48.19	0.88	0.0806	0.961	0.26	20.3
Arunachal Pradesh	1.26	0.01	0.0025	0.012	0.01	0.3
Assam	13.83	0.27	0.0791	0.349	0.07	7.4
Bihar	27.16	0.58	0.0253	0.605	0.05	12.7
Chhattisgarh	13.49	0.03	0.0532	0.083	0.03	1.8
Delhi	0.37	0.02	0.0006	0.021	0	0.4
Goa	0.21	0	0.0005	0.0005	0	0
Gujarat	21.65	0.58	0.0183	0.598	0.03	12.6
Haryana	8.88	0.33	0.005	0.33	0.04	6.9
Himachal Pradesh	5.12	0.1	0.0359	0.134	0	2.8
Jammu and Kashmir	9.9	0.15	0.0126	0.163	0.02	3.4
Jharkhand	15.83	0.3	0.0349	0.335	0.05	7
Karnataka	25.62	0.52	0.051	0.571	0.07	12
Kerala	3.48	0.09	0.0086	0.099	0.03	2.1
Madhya Pradesh	35.62	0.91	0.0936	1.004	0.04	21.1

Maharashtra	36.76	0.83	0.0814	0.911	0.11	19.2
Manipur	0.97	0.02	0.0034	0.023	0.01	0.5
Meghalaya	1.55	0.02	0.0044	0.024	0.01	0.5
Mizoram	0.28	0	0.0011	0.001	0	0
Nagaland	1.35	0.02	0.0047	0.025	0.01	0.5
Orissa	23.39	0.49	0.0523	0.542	0.06	11.4
Punjab	8.61	0.36	0.0323	0.392	0.03	8.2
Rajasthan	49.14	0.98	0.0911	1.071	0.02	22.5
Sikkim	0.34	0.01	0.0007	0.011	0	0.2
Tamil Nadu	24.94	0.43	0.0417	0.472	0.23	10
Tripura	1.46	0.02	0.0034	0.023	0.01	0.5
Uttar Pradesh	58.53	1.59	0.1631	1.753	0.06	36.8
Uttarakhand	4.94	0.13	0.0126	0.143	0.01	3
West Bengal	41.62	0.66	0.073	0.733	0.17	15.4
Andaman & Nicobar Islands	0.19	0	0.0005	0.0005	0	0
Chandigarh	0.031	0	0.01	0.01	0	0.2
Dadra Nagar Haveli	0.078	0	0.0003	0.0003	0	0
Daman & Diu	0.01	0	0.0181	0.0181	0	0.4
Lakshdweep	0.052	0	0.0002	0.0002	0	0
Pondicherry	0.13	0	0.0003	0.0003	0	0
All-India	485	10.65	1.09	11.75	1.42	247.2

Table 2. Category-wise population and Green House Gas emissions from Indian livestock
(Chhabra *et al.*, 2013)

LS category ^a	Population (millions)	Methane emission (Tg/year)			Nitrous emission (Gg/year)	Total CO ₂ equivalent (Mt)	% contribution
		EF ^b	MM ^c	Total			
Dairy Cattle							
Indigenous	82.96	2.32	0.29	2.61	0.05	54.83	22.18
Exotic	19.74	0.84	0.07	0.92	0.01	19.32	7.82
Sub-total	102.7	3.17	0.36	3.54	0.06	74.36	30.08
Non-Dairy							
Indigenous	77.53	2.12	0.21	2.33	0.03	48.94	19.8
Below 1 year	9.85	0.09	0.01	0.1	0.004	2.1	0.85
1 - 3 years	12	0.27	0.03	0.31	0.006	6.51	2.63
Above 3 years	55.68	1.76	0.17	1.92	0.02	40.33	16.31
Exotic	4.91	0.11	0.01	0.12	0.002	2.52	1.02
Below 1 year	1.9	0.02	0.002	0.02	0.001	0.42	0.17
1 - 3 years	1.14	0.03	0.003	0.03	0.0005	0.63	0.25

Above 3 years	1.87	0.06	0.005	0.07	0.0007	1.47	0.59
Sub-fataa	82.44	2.23	0.218	2.45	0.032	51.46	20.82
Dairy Buffalo	80.03	4.06	0.371	4.44	-	93.24	37.72
Non-Dairy	17.88	0.44	0.055	0.49	-	10.29	4.16
Below 1 year	7.37	0.06	0.013	0.071	-	1.49	0.6
1 -3 years	3.83	0.08	0.014	0.098	-	2.06	0.83
Above 3 years	6.68	0.29	0.028	0.321	-	6.74	2.73
Sub-total	97.91	4.5	0.43	4.93	-	103.53	41.88
Other Livestock							
Sheep	61.4	0.23	0.01	0.24	-	5.04	2.04
Goat	124.35	0.45	0.02	0.47	-	9.87	3.99
Horses and	0.75	0.01	0.001	0.01	-	0.21	0.08
Mules and	0.65	0.02	0.002	0.02	-	0.42	0.17
Camels	0.63	0.03	0.001	0.03	-	0.63	0.25
Pigs	13.52	0.01	0.06	0.07	0.1	1.5	0.61
Total livestock	485	10.65	1.1	11.75		246.81	99.84
Poultry	489.01	-	-	-	1.22	0.38	0.2
	Total			11.75	1.42	247.2	100

Livestock category, ^b EF Enteric Fermentation, ^c MM Manure Management

Mitigating emissions from livestock:

Emissions from livestock are a diffuse source and as such are not easily captured or quantified. Therefore mitigation strategies are focus on reducing the source of production. Methane production from animals is dependent on a number of factors, which can be calculated simply as: Total emissions =Number of animals X Lifetime of animal X Emissions per head per day. The total emissions can be reduced by reducing the numbers of livestock, reducing the emissions per animal, or achieving the high yielding animals instead of large numbers of animals. Methane is a waste product from animals, representing a loss of 2-12% dietary energy available to the animal. The production of methane both directly from enteric fermentation and from the decomposition of organic animal waste is also a reflection of inefficiency digestive process. Methane emissions can be reduced through productivity improvements that offer a potential cost benefits to the agricultural sector. This requires dietary adjustments, such as changes in diet, use of feed supplements, or genetic modifications. However, the applicability of these techniques in practice is limited by a number of factors, namely: risks to human health; consumer acceptability; animal welfare issues (particularly risks to animal health and development); ethical considerations; and cost effectiveness.

1. Dietary adjustments:

Methane emissions depend on the average daily feed intake, efficiency of rumen fermentation and the quality of the feed. The improvements of animal feedstuff quality in terms of digestibility and energy value or improvements of rumen efficiency help in methane reductions.

- (a) Altering and improvement of animal feedstuff for higher productivity: increased levels of starch, feeding supplementary dietary fat, and reducing the proportion of fibre in the diet are examples of potential methane reduction strategies. Increasing forage quality combined with the management of stocking rates and rotational grazing strategies have also been reported to reduce enteric methane emissions (Mirzaei-Aghsaghali and Maher-Sis, 2011).
- (b) Feed conversion efficiency: Certain type of feed have a higher conversion ration into product than waste, such as grain feeds are converted more efficiently than forages (grass) and they are relatively easy to digest and have a high energy.
- (c) Use of feed additives: Feeding of additives can manipulate rumen microflora populations. It can induce stable and modified rumen fermentation with lower methane emissions. The plant secondary compound like tannins and saponins are using as a CH₄ mitigation strategy. Legumes containing condensed tannin (lotuses) are able to lower methane production by 12-15% (Beauchemin *et al.*, 2008; Rowlinson *et al.*, 2008). Feeding of yeast culture (*Saccharomyces cerevisiae*) has also been suggested to reduce CH₄ production by rumen microflora (McGinn *et al.*, 2004).

2. Rumen efficiency:

Diet should contain essential nutrients for rumen microorganism for the efficient fermentative digestion. If nutrients such as ammonia, sulphate and phosphate are limited resulting in increased emissions of methane. Nutritional supplements can therefore be used to enhance dietary nutrition. The largest gains from nutritional supplementation are likely to be amongst animals on low quality feeds. A number of advanced supplements, in the form of chemical and pharmaceutical additives, have been developed which have the potential to deliver further efficiency or productivity improvements. This may not prove a popular option as the growing consumers are against the use of chemical additives in recent years due to health concern. Therefore a number of such substances have already been prohibited in the EU and elsewhere.

3. Herd management:

Management systems designed for high milk output per cow will tend to result in lower emissions per unit of milk produced. More extensive systems require more animals to produce a given quantity of milk, resulting in higher methane output per litre. The opportunities to reduce methane emissions by increased animal productivity are larger in the extensive systems compared to the intensive systems.

4. Manure management and treatment:

Helpful manure- management techniques include frequent and complete removal of manure from indoor storage, deep cooling of manure, and management of bedding and manure heaps to avoid anaerobic conditions (IPCC, 2006).

5. Improved productivity and efficiency as a mitigation of GHGs:

Higher productivity generally leads to higher gross efficiency and a given level of production (e.g. a national milk quota) can be achieved with fewer high yielding animals and followers. There has been an overall reduction of methane emissions of 28% from 1990 to 1999 in the UK (Defra, 2001) and the dairy sector in Canada has reduced its methane emissions by 10% since 1990 (Désilets, 2006). There is a decreased enteric methane production per day in animals selected for lower residual feed intake (Hegarty *et al.*, 2007). Reduced residual feed intake is akin to selection for high feed efficiency as an animal is eating less but maintaining a similar growth rate (high net feed efficiency) and therefore less feed is required to produce a unit of output. Use of such traits in selection is limited but this might be one of the possibilities for selection of reduced GHGs emissions.

6. Direct selection to reduce emissions:

It is quite difficult to measure the all sources of methane emission from individual animals (exhaled by the animal due to enteric fermentation, manure management and flatulence). However, expired air samples can taken from individual animals or groups of animals and samples can be analysed for their methane concentrations using infrared spectroscopy, gas chromatography, mass spectroscopy or a tuneable laser diode. Several techniques have been used to take air samples, such as, respiration chambers, head boxes, hoods, masks and polytunnels. The measurement indicates variation between animals, between breeds and across time (Herd *et al.*, 2002). This observation may help us for potential improvement through genetic selection. However, measuring methane directly from animals is currently difficult and direct selection on reduced methane emissions may prove difficult in practice.

Development of new measurement techniques, on direct and indirect emissions traits, will help to enhance the capability of reducing emissions through genetic selection.

7. Genetic improvements:

Selective breeding may help in significant improvements to the genetic characteristics of ruminants, such as digestive efficiency and productivity. High yielding animals are more or less associated with fertility problem, lameness, mastitis and metabolic disorders. Improvement in reproductive efficiency could significantly reduce the number of animals. So, Greater scope exists with improvements to reproductive efficiency which typically comprises a reproductive herd. There have also been suggestions that genetic engineering could achieve methane reductions from ruminants by increasing fermentation efficiency through adjustments to rumen micro-organisms. However, there remains strong public resistance to genetic engineering in the food chain due to both ethical objections and scientific uncertainty regarding associated risks and knock on effects.

8. Reducing livestock numbers:

Fewer numbers of animals will result in a reduction in methane emissions from both enteric fermentation and manure management. On the same way the demand for meat and animal products also be reduced, otherwise production and methane emissions will merely be taken over by other countries. Within the UK, as well as a number of other European countries and the USA, there has been a noticeable move away from red meat towards poultry, along with a growing vegetarian movement and support for organic produce. In the present scenario it seems to be minimised consumption of dairy products is more significant than minimising meat consumption, in terms of greenhouse gas emissions. The approach to reduce the number of livestock may not be possible across the whole agricultural sector. Some parts of the globe may increase their herds in order to supply the same level of consumer demand for meat and milk. So, neither enforced reductions in agricultural production levels or consumer demand are considered to be realistic or viable routes to long-term methane emission reduction.

Conclusion:

The best approaches to reduce GHGs emissions depend on local conditions and therefore vary from region to region. Carbon and methane emissions in India and a ways to cap these emissions have been essential. To mitigate the CO₂ emissions, it is necessary to encourage planting and maintenance of a number of fast growing carbon trapping trees on animal rearing concentration areas, and even on busiest streets across major cities within the state.

To mitigate CH₄ emissions, the public as well as the government should support waste management in all the states of India to have a safe disposal of organic matter from cattle by herdsmen. The adequate information, technologies, and educate the farmers are very essential. Contribute to GHGs mitigation and how to cope with climate change should properly be instructed to the farmers. This may be a helpful to prevent the amount of methane emission from carelessly release to the environment. The effect of climate change can be halted by applying mitigation strategies depending on the location.

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Efficient Management of Livestock Waste to Reduce Global Warming

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The Indian livestock sector is on a high growth trajectory and currently contributes 27 percent to the agricultural gross domestic product. Besides milk, the animals also supply a valuable product ‘dung’ which can be utilized as best source of energy and fertilizer. Dairy animal manure is an excellent source of fertilizer, but fresh dung has a disagreeable odor, high ammonia levels that may burn plants, and may contain excess salt. Manure can be (i) solid dung, feed wastes, soiled bedding etc; (ii) liquid-urine wash water. Under ideal managemental conditions solid manure is usually collected and removed from sheds twice daily. Provisions are to be made to carry off and store liquid manure as and when the same appears.

The livestock are responsible for 18 percent of greenhouse gas emissions, a bigger share than that of transport. However, livestock sector's potential contribution to solving environmental problems is equally large, and major improvements could be achieved at reasonable cost. It generates even bigger shares of emissions of other gases with greater potential to warm the atmosphere: as much as 37 percent of anthropogenic methane, mostly from enteric fermentation by ruminants, and 65 percent of anthropogenic nitrous oxide, mostly from manure.

The future of the livestock-environment interface will be shaped by how we resolve the balance of two demands: for animal food products on one side and for environmental services on the other". Since the natural resource base is finite, the huge expansion of the livestock sector required to meet expanding demand must be accomplished while substantially reducing its environmental impact.

Total emissions from global livestock: 7.1 Gigatonnes of CO₂-equiv per year, representing 14.5 percent of all anthropogenic GHG emissions. In terms of activities, feed production and processing (this includes land use change) and enteric fermentation from ruminants are the two main sources of emissions, representing 45 and 39 percent of total emissions, respectively. Manure storage and processing represent 10 percent. The remainder is attributable to the processing and transportation of animal products.

About 44 percent of livestock emissions are in the form of methane (CH_4). The remaining part is almost equally shared between Nitrous Dioxide (N_2O , 29 percent) and Carbon Dioxide (CO_2 , 27 percent).

For handling of manure/ waste material many alternatives can be used depending upon scale of production such as:

1. Compost
2. Biogas
3. Vermiculture

Compost:

Composting is a controlled and managed aerobic (“with air”) decomposition process for manure, bedding, and other organic materials (yard waste, food scraps etc.). It produces a stable, nutrient rich, humus-like material that can be used as soil amendment on fields and gardens.

Composting involves the proper mixture of materials, oxygen, moisture, area design, and maintenance to provide an environment for microbial activity. The microbes or beneficial bacteria and other organisms digest and process, the manure and bedding. Through this process, and with proper maintenance, compost pile temperatures should raise high enough to kill unwanted pathogens, weed seeds, residual hormones, antibiotics, and pesticides. Manure and bedding volume will reduce by about 60 percent, odor will be minimal, and nutrients will become concentrated.

Composting is a viable option for any size of farm operation. It is a flexible system that can be designed to meet the needs of any farm. However it requires upfront planning and good management. If odor issues from manure storage and land application occur due to the proximity of neighbors, composting may be a beneficial alternative. If off-farm uses cannot be found or hauling charges are high, composting may also prove to be a viable option, as it significantly reduces manure volume and provides a more marketable product. However, if off-farm options exist that are economical, then the time and labour required to maintain a compost system may not be worth it.

1. Select a location where you can build a 3- to 4-foot square pile and have room to turn it.
2. Spread a 3-inch layer of dry organic material on the square area. Spread two inches of manure on top of it. Continue layering until the pile is 4 feet tall. Water the pile as you build it so it is slightly damp all the way through. Cover with a layer of soil.
3. Turn the pile every 3 days. Keep the pile moist but not soggy. Check the temperature of the center of the pile when you turn it. It should be between 120 and 160 degrees Fahrenheit.
4. Use the compost when it stops heating in the center and is dark brown, crumbly, and has an earthy smell.

Increasing fertility:

Fresh cow manure contains about the same ratio of nitrogen, phosphorus and potassium as a balanced commercial fertilizer but in much smaller quantities. You will need to use 10 times as much fresh manure as commercial fertilizer to get the same amount of these macronutrients. It also contains calcium, magnesium, zinc, sulfur, copper, manganese and sodium. All of these are nutrients required for plant growth.

Organic matter:

One of the most important benefits of using manure is the addition of organic matter. Organic matter breaks down into small particles called humus. In sandy soil these particles get between the sand particles and act like sponges, holding both nutrients and water in the soil. In clay soils they help break apart the lumps of clay and make them more permeable to both water and the tiny feeder roots of the plants. Humus also reduces the pH of soil, which is a benefit on high pH western soil.

Microorganisms:

Cow manure can be up to 30 percent bacteria and other microorganisms. The majority of these microorganisms are beneficial. They help break down organic matter into humus, change nutrients from forms that are unavailable to plants to forms that are available, retain nutrients in the soil and form partnerships with plants to increase the growth and health of the plant. They can also break down pollutants in the soil and play a vital role in the nitrogen cycle.

Disadvantages of fresh cow manure:

While the majority of the microorganisms in the manure are beneficial, some of them may be pathogens. There is a slight chance that fresh manure can spread diseases if used on food crops. Manure can also contain weed seeds. Compost fresh manure before using it on the vegetable garden to kill pathogens and weed seeds. Some manure may also contain too much sodium for healthy plant growth, depending on how the cattle were fed. This is not a problem where rainfall is high but the sodium can build up and cause problems in arid or semiarid areas.

Bio Gas:

Definition: A mixture of methane and carbon dioxide produced by bacterial degradation of organic matter and used as a fuel.

What are the Components of a Biogas System?

Biogas technology is a manure management tool that promotes the recovery and use of biogas as energy by adapting manure management practices to collect biogas. The biogas can be used as a fuel source to generate electricity for on-farm use or for sale to the electrical grid, or for heating or cooling needs. The biologically stabilized byproducts of anaerobic digestion can be used in a number of ways, depending on local needs and resources. Successful byproduct applications include use as a crop fertilizer, bedding, and as aquaculture supplements. A typical biogas system consists of the following components:

1. Manure collection
2. Anaerobic digester
3. Effluent storage
4. Gas handling
5. Gas use.

Livestock facilities use manure management systems to collect and store manure because of sanitary, environmental, and farm operational considerations. Manure is collected and stored either as liquids, slurries, semi-solids, or solids. Manure is excreted with a solids content of 8 to 25 percent, depending upon animal type. It can be diluted by various process waters or thickened by air drying or by adding bedding materials. This manure is typically “flushed” from where it is excreted, using fresh or recycled water. The manure and flush water can be pumped to treatment

and storage tanks, ponds, lagoons, or other suitable structures before land application. Liquid manure systems may be adapted for biogas production and energy recovery in “warm” climates.

Benefits of biogas technology:

Most confined livestock operations handle manure as liquids, slurries, semi-solids, or solids that are stored in lagoons, concrete basins, tanks, and other containment structures. These structures are typically designed to comply with local and state environmental regulations and are a necessary cost of production. Biogas technology can be a cost-effective, environment and neighborhood friendly addition to existing manure management strategies. Biogas technologies anaerobically digest manure, resulting in biogas and a liquefied, low-odor effluent. By managing the anaerobic digestion of manure, biogas technologies significantly reduce Biochemical Oxygen Demand (BOD), and pathogen levels; remove most noxious odors; and convert most of the organic nitrogen to plant available inorganic nitrogen.

Reduced odors:

Biogas systems reduce offensive odors from overloaded or improperly managed manure storage facilities. These odors impair air quality and may be a nuisance to nearby communities. Biogas systems reduce these offensive odors because the volatile organic acids, the odor causing compounds, are consumed by biogas producing bacteria.

High quality fertilizer:

In the process of an aerobic digestion, the organic nitrogen in the manure is largely converted to ammonium. Ammonium is the primary constituent of commercial fertilizer, which is readily available and utilized by plants.

Reduced surface and groundwater contamination:

Digester effluent is a more uniform and predictable product than untreated manure. The higher ammonium content allows better crop utilization and the physical properties allow easier land application. Properly applied, digester effluent reduces the likelihood of surface or groundwater pollution.

Vermicompost:

Vermicompost (also called worm compost, vermicast, worm castings, worm humus or worm manure) is the end-product of the breakdown of organic matter by some species of earthworm. Vermicompost is a nutrient-rich, natural fertilizer and soil conditioner. The process of producing vermicompost is called vermicomposting. The earthworm species (or composting worms) most often used are Red Wigglers (*Eisenia foetida*) or Red Earthworms (*Lumbricus rubellus*). These species are only rarely found in soil and are adapted to the special conditions in rotting vegetation, compost and manure piles.

Worm composting:

Vermicomposting is a process that relies on earthworms and microorganisms to help stabilize active organic materials and convert them to a valuable soil amendment and source of plant nutrients. Earthworms will consume most organic materials, including animal manure, agricultural crop residues, organic byproducts from industries, yard trimmings, food preparation scraps and leftovers, scrap paper, and sewage sludge.

Of the more than 4,000 species of earthworms, only half a dozen are used for vermicomposting worldwide. The earthworm species most frequently used for vermicomposting is *Eisenia fetida*, which is commonly called Red Wiggler.

How to choose a vermicomposting system?

A variety of methods may be used to process large volumes of organic residuals with earthworms, ranging from land and labor-intensive techniques to fully automated high-tech systems. Types of systems include windrows, beds, bins, and automated raised bioreactors. Choosing which vermicomposting system to use will depend upon:

- Amount of feedstock to be processed
- Funding available
- Site and space restrictions
- Climate and weather
- Facilities and equipment on hand
- Availability of low-cost labor

Advantages of vermicompost:

Earthworm casts are covered with mucus from their intestinal tract; this layer provides a readily available carbon source for soil microbes and leads to a flush of microbial activity in fresh casts. Vermicompost improves soil structure, reduces erosion, and improves and stabilizes soil pH. In addition, vermicompost increases moisture infiltration in soils and improves its moisture holding capacity. Plant growth is significantly increased by vermicompost, whether it is used as a soil additive, a vermicompost tea, or as a component of horticultural soilless container media. Vermicompost causes seeds to germinate more quickly, seedlings to grow faster, leaves grow bigger, and more flowers, fruits or vegetables are produced.

Conclusion:

To address the critical environmental problem faced in livestock waste management. The above mentioned technologies may help in significant reduction of green house gas emissions due to the following three components: 1. Avoidance of methane emissions from dumping livestock waste in the particular pits/particular place for composting/ biogas or vermicomposting. 2. Reduction of contamination of water table and soil by eliminating potential breeding grounds for bacteria. 3. Provisions of supply of renewable energy for household and agriculture.

Coping with Climate Variability through ITK Practices for Resilience in Livestock Rearing

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Climate variability refers to shorter term (daily, seasonal, annual, inter-annual, several years) variations in climate, including the fluctuations associated with El Nino (*dry*) or La Nina (*wet*) events.

Climate variability also refers to the climatic parameter of a region varying from its long-term mean. Every year in a specific time period, the climate of a location is different. Some years have below average rainfall, some have average or above average rainfall.

Climate change scenario:

India Meteorological Department (IMD) maintains a well distributed network of more than 500 stations in the country for more than a century. The salient findings of the IMD studies (IMD Annual Climate Summary, 2009; Tyagi and Goswami, 2009; Attri, 2006) are summarized as under:

Temperature:

Analysis of data for the period 1901-2009 suggests that annual mean temperature for the country as a whole has risen by 0.56°C. It may be mentioned that annual mean temperature has been generally above normal (normal based on period, 1961-1990). This warming is primarily due to rise in maximum temperature across the country. However, since 1990, minimum temperature is steadily rising and rate of its rise is slightly more than that of maximum temperature (IMD Annual Climate Summary, 2009). Warming trend over globe of the order of 0.74°C has been reported by IPCC (2007).

Spatial pattern of trends in the mean annual temperature show significant positive (increasing) trend over most parts of the country except over parts of Rajasthan, Gujarat and Bihar, where significant negative (decreasing) trends were observed (IMD Annual Climate Summary, 2009).

Season wise, maximum rise in mean temperature was observed during the Post-monsoon season (0.77°C) followed by winter season (0.70°C), Pre-monsoon season (0.64°C)

and Monsoon season (0.33°C). During the winter season, since 1991, rise in minimum temperature is appreciably higher than that of maximum temperature over northern plains. This may be due to pollution leading to frequent occurrences of fog.

Trends in maximum temperature:

The all-India maximum temperature shows an increase in temperature by 0.71°C per 100 Year.

Trends in minimum temperature:

Like the maximum temperature, mean annual minimum temperature has also significantly increased by 0.27°C per 100 years during the period 1901-2007. The spatial changes in minimum temperatures when observed are decreasing in most parts of Western Ghats, increasing in most parts of the Himalayan region and certain parts of the North-Eastern region. The warming stems mainly from winter and post-monsoon temperatures.

Precipitation trends:

During the winter season, rainfall is decreasing in almost all the subdivisions except for the sub-divisions Himachal Pradesh, Jharkhand and Nagaland, Manipur, Mizoram & Tripura. Rainfall is decreasing over most parts of the central India during the pre-monsoon season. However during the post-monsoon season, rainfall is increasing for almost all the sub-divisions.

Extreme rainfall events:

A large amount of the variability of rainfall is related to the occurrence of extreme rainfall events. The extreme rainfall series at stations over the west coast north of 12°N and at some stations to the east of the Western Ghats over the central parts of the Peninsula showed a significant increasing trend at 95% level of confidence. Stations over the southern Peninsula and over the lower Ganga valley have been found to exhibit a decreasing trend at the same level of significance .Various studies on extreme rainfall over India have found the occurrences of 40 cm or more rainfall along the west and east cost of India, Gangetic West Bengal and north eastern parts of India. Country's highest observed one day point rainfall (156.3 cm) and also world's highest 2-day point rainfall (249.3cm) occurred in Cherrapunji of northeast India in the year 1995 (IMD, 2006).

Significant increasing trend was observed in the frequency of heavy rainfall events over the west coast (Sinha Ray and Srivastava, 2000). Most of the extreme rainfall indices

have shown significant positive trends over the west coast and north-western parts of Peninsula. However, two hill stations considered (Shimla and Mahabaleshwar) have shown decreasing trend in some of the extreme rainfall indices (Joshi and Rajeevan, 2006). Increase in heavy and very heavy rainfall events and decrease in low and moderate rainfall events in India have been reported by Goswami *et al.* (2006). Rao *et al.* (2010) have assessed the role of Southern Tropical Indian Ocean warming on unusual central Indian drought of summer monsoon – 2008.

Importance of resilience in climate variability:

Climate change impacts on agriculture and livestock are being witnessed all over the world, but the developing countries like India, are much more vulnerable as, a large section of the population depends on agriculture for livelihood. The warming trend in India over the past hundred years was estimated to be 0.60°C. It has been estimated that yield fluctuations of many crops may be aggravated further due to climate change impacts. There is numerous evidence about the negative impacts of climate change and variability due to increased temperature, increasing water scarcity and reduction in number of rainy days. There has been a significant rise in the frequency of extreme weather events in recent years affecting the productivity and income at the farm level. The livestock productivity is also at stake. It is therefore important that all efforts are geared up to enhance the resilience of the farms and livestock in our country through dissemination-adoption of various coping strategies and mechanisms so that the production and productivity levels of the farm and livestock are maintained even in challenging climatic conditions.

What does “Resilience” mean?

The word resilient is derived from the Latin word resilire, meaning to jump back, recoil. So, we might recoil after sticking our foot in frigid water and, importantly, recover composure.

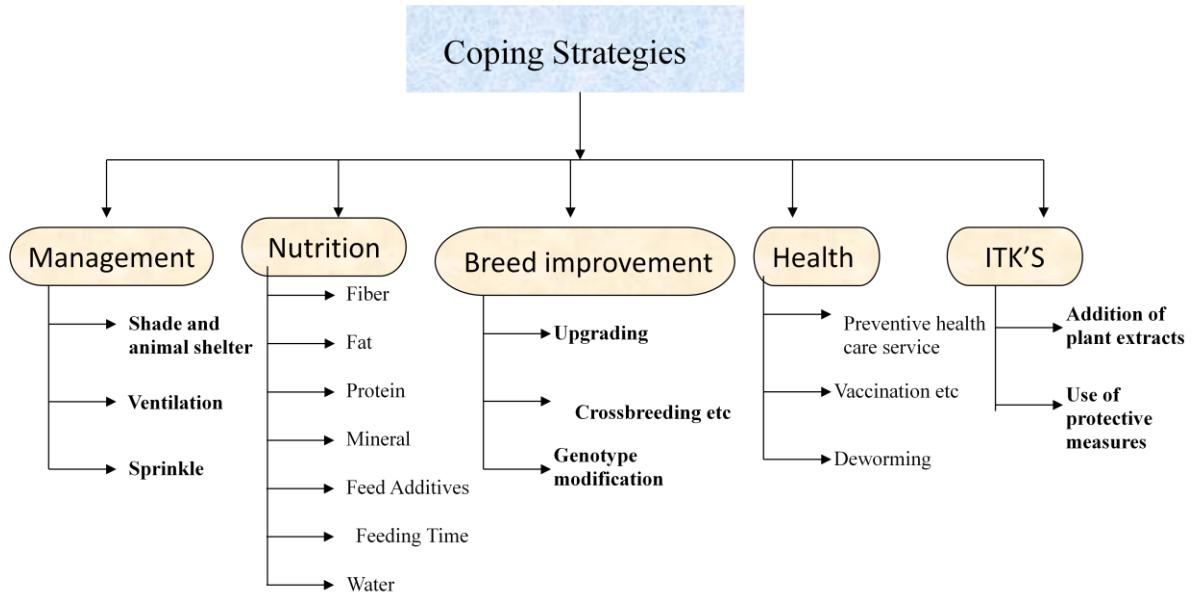
According to the Merriam-Webster’s Collegiate Dictionary, resilient means:

- a) capable of withstanding shock without permanent deformation or rupture; and
- b) tending to recover from or adjust easily to misfortune or change.

Climate change adaptation and the building of adaptive capacity are promoted as essential for future sustainable and equitable development, particularly for places and livelihoods that are sensitive to climate variability and climate change. In order to understand what adaptation options are needed and possible, it is important to identify the climatic variables to which the

adaptations relate and to consider the role of non-climatic factors that influence the sensitivity of rural livelihoods to climate change.

Coping strategies oriented towards resilient dairy farming technologies:



Emphasis on ITK:

The term indigenous technical knowledge refers to unique, traditional, local knowledge existing within and developed around specific conditions of women and men indigenous to a particular geographic area.

Indigenous knowledge (IK) is the participant's knowledge of their temporal and social space. Indigenous knowledge as such refers not only to knowledge of indigenous people, but to that of any other defined community.

It is the basis for local-level decision making in agriculture, health care, food preparation, education, natural resource management and a host of other activities in rural communities.

Indigenous technical knowledge (ITK) is the information gained over a period of time and passed on from generation to generation by word of mouth. ITK is the sum total of knowledge and practices which are based on accumulated experiences of people in dealing with situations and problems in various aspects of life. Such knowledge and practices are special to a particular culture. ITK in animal husbandry is a treasured source of local wisdom. It is comparatively easy to document the ITK but very difficult to validate by adopting appropriate scientific methods. Despite its advantages in terms of cost effectiveness and local availability of resources many a time ITK falls short of evidence based on scientific testing.

The ITK needs to be documented, validated and the appropriate technologies or practices could be taken up for wider adoption. ITK is specifically concerned with actual application of the thinking of the local people in various operations of agriculture and allied areas.

Belief: change in behaviour of insects, animals and vegetation indicating a forthcoming event without any scientific rational but may be true in happening.

Innovation: outside the area of ITK, but scientifically based development of practices using the locally available resources to solve specific problems.

Role of organizations in promoting ITKs:

Appreciable work was done by the Institute of Innovation Foundation under the guidance of Prof. Anil Gupta. Efforts have been made to document, validate and refine the ITK in animal husbandry by giving proper recognition to the source of ITK. These ITK is being circulated widely through the publication “Honey Bee”. Similar attempt was made by ICAR to document and validate the ITK across the country and published it as a reference book on ITK.

Indigenous knowledge system (IKS): delineates a cognitive structure in which theories and perceptions of nature and culture are conceptualized. Thus it includes definitions, classifications and concepts of the physical, natural, social, economic and ideational environments. The dynamics of the IKS takes place on two different levels, the cognitive and the empirical. On the empirical level, IKS are visible in institutions, artefacts and allied technologies.

Importance of ITK in climate change:

- 1) Indigenous knowledge, although new to climate science, has been long recognized as a key source of information and insight into domains such as agro forestry, traditional medicine, biodiversity conservation, customary resource management, impact assessment, and natural disaster preparedness and response. Indigenous people and rural populations are keen observers of their natural environment.
- 2) Indigenous observations and interpretations of meteorological phenomena are at a micro level, have considerable temporal depth and highlight elements that may be marginal or even new to scientists. They focus on elements of significance for local livelihoods, security and well-being, and are thus essential for adaptation.

- 3) Indigenous peoples' observations contribute importantly to advancing climate science, by ensuring that, assessments of climate change impacts and policies for climate change adaptation are meaningful and applicable at the local level.
- 4) Indigenous responses to climate variation typically involve changes in livelihood practices and other socioeconomic adjustments. Strategies such as engaging in multiple livelihood activities and maintaining a diversity of plant varieties and animal races provide a low-risk buffer in uncertain weather environments. The ability to access multiple resources and rely on different modalities of land use contributes to their capacities to manage for local-level climate change.
- 5) Traditional systems of governance and social networks improve the ability to collectively manage diversity and share resources, while dissipating shocks and reinforcing innovative capacities.

Some ITKs Practiced by livestock farmers for coping with climate variability impact:

S. No.	Climate challenges	Problems/ Diseases/Disorder	ITK	Location	Perceived benefits
1	Extreme weather conditions	Breeding problems: Delayed Heat	Paste of two number of coconut (Cocos nucifera) and 70gm of Toon (Cederalla toona) is given with neem oil for 3 days. Juice of Agave sisalana (kattalai) is given for 1 week and then water soaked Bengal gram (Cicer arietinum) is given for 3 days. Mixture of leaves of Ficus bengalensis (banyan tree), Ficus religiosa (Peepal tree) mango and neem is given for 5 days.	Tamilnadu	Animal shows heat symptoms
		Low conception rate	Tribulus terrestris (Nerinja), moringa flowers, Adalsa (Adhatoda vasica) with 300 ml of neem oil is given in the night and next morning hot water soaked cloth is pressed in the vagina (to avoid the	Tamilnadu	Induces heat in animals and enhances growth
				Tamilnadu	
				Tamilnadu	Increases chances of conception

			wastage of sperm). Glyricidia (g. Maculate) is given to animals to increase lactation.	Tamilnadu	Increase lactation
2	Extreme temperatures	Low work efficiency of draft animals	Dried flowers of Madhuca latifolia (Illupai) is given to improve work efficiency of the bullock.	Tamilnadu	Improves work efficiency of the bullock
			Prosopis julifera fruits with gur and rice flour are given to improve digestibility.	Tamilnadu	Improves digestibility
			Tamarind seeds are roasted and outer coats are removed; these are soaked, ground and fed to the animals.	Tamilnadu	Improves lactation
			Seeds of subabul (<i>Leucaena leucocephala</i>) are fed to animals.	Tamilnadu	Improves milk secretion
			Extracts of Omum (<i>Trachyspermum ammi</i>), black cumin (<i>Nigella sativa</i>), pepper (<i>Piper nigrum</i>), gingelly (<i>sesamum indicum</i>) and cardamom (<i>Elettaria cardamomum</i>) are given.	Tamilnadu	Cures digestibility problems
4	Heat Stress	To improve digestibility	Leaves of mint (<i>Mentha viridis</i>), peepal and omum, salt and bitter gourd (<i>Momordica charantia</i>) are given.	Tamilnadu	Indigestion is cured
			Asafoetida (<i>Ferula asafoetida</i>) ground with gur is given to animals as balls.	Tamilnadu	
			Pepper, gur and betel leaf (<i>Piper betle</i>) powder is given to animals.	Tamilnadu	
5		Indigestion	Betel leaf and pepper ground and mixed with	Tamilnadu	Induces appetite

			coconut oil is given.		
6	Lack of appetite		Powder of sweet flag (Acorus calamus), coriander (coriandrum sativum) ,pepper ,cardamom and county gur is given to animals for	Tamilnadu	Cures indigestion, diarrhoea and general debilitation
			Agave sisalana is peeled and kept in the roof during night and given to animals in morning.	Tamilnadu	Enhances appetite
7	Diarrhoea		Paste of Harjora-pirandai (Cissus quadrangularis), salt, tamarind fruit, chillies is put in a cloth and tied around the mouth for 2 hrs.	Tamilnadu	
			3 gm of thorn apple (Datura stramonium) seeds, 20gm arecanut and 500 gm white clay mixed with rice gruel is given twice a day.	Tamilnadu	
			Leaves of kolinji (Tephrosia purpurea) mixed with carved coconut, cumin and dried chillies are fed to animals.	Tamilnadu	
			Cotton seeds and 4-5 leaves of Portia tree (Thespesia populnea) boiled in water is given to animals.	Tamilnadu	Cures Diarrhoea
8	Intestinal worms		Orilai thamarai (Hybanthus enneaspermus), curd from buffalo and cumin are mixed and given to animals.	Tamilnadu	
			Powder of subabul seeds mixed with water is given to goats.	Tamilnadu	
			Leaves, flowers and bark of neem along with 100gm	Tamilnadu	Acts as dewormer

9		Lice and Fleas	<p>of cucumber (<i>cucumis sativus</i>) seeds are given for 3 days.</p> <p>Powder of sweet flag (<i>Acorus calamus</i>) and Tulsi (<i>Ocimum sanctum</i>) leaf juice is smeared over the body.</p> <p>Powdered naphthalene balls are applied all over the body and washed with water.</p> <p>Jaggery, garlic and ginger paste is given to animals.</p>	Tamilnadu	Kills the ectoparasites
10	Winter stress	Constipation	<p>200 gm castor seeds ground and mixed with 250 ml water are given.</p> <p>200 gm of gur dissolved in water is given.</p> <p>Turmeric paste made into arecanut size is given.</p> <p>Leaf powder of <i>Datura stramonium</i> mixed with honey is given.</p>	Tamilnadu	Cures constipation and guards against ulcers.
11		Breathing diseases	<p><i>Acalypha indica</i> (<i>Kuppaimeni</i>) mixed with sacred basil (<i>Ocimum sanctum</i>) leaf juice is given.</p> <p>Paste of turmeric (50 gm),ajwan (100 gm) and rock salt (200 gm) is applied on tongue.</p>	Tamilnadu	Cough is cured.
12	All Weather conditions	Animal shelters	Made up of locally available materials such as wood, coconut or palm leaves, paddy straw etc.These sheds appear primitive but , they are economical and sustainable	All over India.	

13.	Insect repellent		as the so called scientific recommendations about modern cattle sheds are often too expensive for small farmers condition. Cotton (Gossypium barbarese) plant acts as mosquito repellent.	All over India.	Repels mosquitoes
14	Milking practice		Many dairy farmers in Indian villages milk the first few stripplings on the floor, logic being that the fore milk has maximum bacterial load which can reduce the keeping quality of milk if mixed with the clean milk. Feeding of cotton seed, cotton seed cake and oil to milk producing animals was traditionally practised by the farmers in Gujarat.	All over India Gujarat	

Rapid rural appraisal (RRA):

An RRA can be defined as a systematic but semi-structured study, carried out in the field by a multi-disciplinary team over a short period ranging from three days to three weeks, based on information collected in advance from published and/or unpublished sources, direct observations and interviews as to generate working hypotheses for subsequent action (Chambers, 1985; Ison and Ampt, 1992).

RRAs can be used to:

- quickly identify, explore and diagnose rural systems and problems
- identify and prioritize the climate variability problems and challenges
- document the local adaptation strategies to overcome the problems
- assess the satisfaction of the farming community towards ITKs practiced.

Types of RRA:

Based on the objective, RRAs are categorised into, (i) Exploratory RRAs, (ii) Topical RRAs, (iii) Participatory Rural Appraisals or (PRAs) and (iv) Monitoring RRAs

PRA- participatory rural appraisal (PRA):

It comprises a set of techniques aimed at shared learning between local people and outsiders. The term itself is misleading because more and more PRA is being used not only in rural settings, and for project appraisal, but throughout the project cycle, as well as for research studies. Indeed, the term PRA is one of many labels for similar participatory assessment approaches, the methodologies of which overlap considerably. It is probably more useful to consider the key principles behind PRA and its associated techniques, rather than the name per se, when assessing its appropriateness to a particular situation.

There are *five key principles* that form the basis of any PRA activity no matter what the objectives or setting:

- **Participation:** PRA relies heavily on participation by the communities, as the method is designed to enable local people to be involved, not only as sources of information, but as partners with the PRA team in gathering and analyzing the information.
- **Flexibility:** The combination of techniques that is appropriate in a particular development context will be determined by such variables as the size and skill mix of the PRA team, the time and resources available, and the topic and location of the work.
- **Teamwork:** Generally, a PRA is best conducted by a local team (speaking the local languages) with a few outsiders present, a significant representation of women, and a mix of sector specialists and social scientists, according to the topic.
- **Optimal Ignorance:** To be efficient in terms of both time and money, PRA work intends to gather just enough information to make the necessary recommendations and decisions.
- **Systematic:** As PRA-generated data is seldom conducive to statistical analysis (given its largely qualitative nature and relatively small sample size), alternative ways have been developed to ensure the validity and reliability of the findings. These include sampling based on approximate stratification of the community by geographic location or relative wealth, and cross-checking, that is using a number of techniques to investigate views on a single topic (including through a final community meeting to discuss the findings and correct inconsistencies).

PLA- (Participatory Learning and Action):

Participatory approaches offer a creative approach to investigating issues of concern to poor people, and to planning, implementing, and evaluating development activities. They challenge prevailing biases and preconceptions about people's knowledge. The methods used range from visualisation, to interviewing and group work. The common theme is the promotion of interactive learning, shared knowledge, and flexible, yet structured analysis.

Common principles of PLA:

- Outsiders do not have all the answers.
- Local people have a rich knowledge base and experience of making a living in a complex environment (e.g. 10 different crops and 4 species of livestock all being farmed on 2 acres of land supporting a family of 6).
- People realised that local communities are much more likely to come up with appropriate solutions to problems than outsiders.
- Within PLA the role of outsiders is more of facilitating a process, where by local people identify, prioritise and analyse their own problems, and develop their own solutions.
- Outsiders can also play a useful role in facilitating links between communities and other organisations with technical expertise or funding. Outsiders can also offer their own skills and expertise but should not impose these on local people

Scouting:

Constraints in scouting for ITK:

There are many constraints that researchers face while scouting for ITK. The lack of information on constraints could puzzle researchers on what prevents them from finding and hence documenting ITK. Hence, some experiences of the constraints in scouting for and documentation are given below;

1. Difficulty in finding persons (local people) who possess ITK.
2. Availability of IK in a village is limited by the extent of modernization in it and its proximity to an urban area.
3. Local people might be prejudiced against officials dealing with land records or income tax and hence might be wary of ITK researchers.

4. Researches may be unable to understand the dialectical nomenclature used by local people to describe plant species.
5. The local language used by resource persons to describe ITK can at times be incomprehensible to researchers, and hence, it can be difficult to document ITK.
6. Researchers at times do not have adequate information on the strategies required to scout ITK.
7. Many local people refuse to divulge ITK, and hence it is difficult to scout for it.
8. Some local people find it difficult to understand the concept of ITK as perceived by researchers.

However, once such constraints have been experienced, a few strategies emerge as a response.

Strategies to facilitate scouting for ITK:

Strategies to make searching for ITK easy are suggested here. These strategies were found to create an environment conducive to scouting for ITK. They are:

1. Conducting peripatetic group meetings in order to promote awareness and to motivate local people to share ITK often works well. Appropriate counselling ensures that local people do not get ensnared by vested interests.
2. Using extension teaching methods and aids helps educate local people about the Importance of ITK and hence documentation.
3. Arranging scouting competitions for local people (women, farmers, and children etc) and selecting winners for awards for aiding scouting of ITK.
4. Informing local people well in advance regarding the rewards/incentives for outstanding innovators could motivate local people to share ITK.
5. Holding public ceremonies to honour innovators by a reward/award could be useful. This could inspire local people to share ITK.
6. Informing local people about publishing documented innovations of local people in newsletters/journals acknowledging the identity of the innovator(s) and popularizing their innovations.

7. Communicating to the community the possibility of obtaining intellectual property rights for ITK and equitably sharing the benefits accrued through commercialization with the resource person /community holding the knowledge.
8. Conducting location specific peripatetic group meetings, demonstrations, and farm and home visits in order to address local people's problems and priorities (e.g. insects, disease) could oblige local people to reciprocate by sharing their ITK.

Validation of ITK's:

Importance: Documentation of ITKs for resilience in livestock farming is not sufficient, until the ITKs are valid. Establishing the validity of the ITKs, should be performed by the scientific community.

Method:

Validation of ITKs can be done through *QuIK* (*Quantification of Indigenous Knowledge*) method by some identified persons who are experienced in particular ITK(s), using the method (QuIK) developed by *Anne K de Villiers in 1996*. The basic premise of this method is that farmers know and understand the environment in which they farm and that answers to many questions can be found in the collective experience of the farming community and doing informal experiments over years. It can be used to unpack the practices of successful farmers, so that information can be disseminated to a wider group of farmers. QuIK methodology represents a rapid and relatively cheap way to elicit indigenous technical knowledge.

Farmers who were experienced in the particular ITK taken for validation are chosen. In QuIK, matrix ranking is combined with an interview schedule to elicit numerical data from experienced farmers. The matrix was designed through preliminary discussions with farmers and was then obtained as part of a systematic process to obtain quantitative data. The experienced respondents were asked to weigh the ITK(s) in comparison with modern veterinary drugs for its performance on different criteria and effectiveness (How many animals are cured?, cost effectiveness, quickness in healing, ease in preparation, side effects and availability). The respondents were asked to put required numbers of pieces of stone out of 10 in each block of matrix. Unlike others, in case of side effects, the greater value of stones indicates fewer side effects. The same matrix was used to interview a number of farmers and the data from each farmer were treated as an independent result. Then the data were put in the statistical analysis (a standard analysis of Variance, ANOVA).

Methodology for validation of ITKs:

1. Documenting the ITKs' from different sources.
2. Presentation before the scientists, farmers (preferably aged, traditional and experienced) in a scientific fora / workshop for establishing its rationality.
3. Classifying the ITKs' based on its domain. (e.g. ITKs based on climate change etc.)
4. Assigning the most significant ITKs' to expert groups (consisting of scientist groups and farmers groups for establishing the rationality).
5. The ITKs' which were validated by expert groups are authenticated and selected for publishing.

Limitations of ITK:

A number of literature have argued that the ITK has its limitations and it is not in itself capable of addressing all the issues related to sustainable socio-economic development and poverty alleviation. Some researchers argued that ITK needed to be formalized, since it is essentially of a fragmentary and provisional nature. It is in this formalization phase that problems with respect to the application of ITK most likely to arise. This type of knowledge is still not as well known as the coded and circulated objective language and the printed products of scientific discourse.

Others reported that the collection of ITK from diverse indigenous sources is often a laborious, time-consuming and costly process. They have noted that proper storage and management must be ensured, if the ITK is to be made available and accessible for quick analysis and manipulation to all those who need it, including the planners and decision makers.

Protection for ITK in Indian Acts:

Some of the India's new laws have implications for ITK and bio-resources. The following are given below.

1. The Geographical Indications of Goods-Registration and Protection Act 1999.
2. The protection of Plant Varieties and Farmer's Rights Act 2001.
3. The Biological Diversity Act, 2002.

Conclusion:

To increase the resilience capacity, that is to cope with the changing climatic scenarios and maintain levels of farm and livestock production, the livestock owners need to be made aware and trained to adopt various coping strategies, including specially, the traditional validated wisdom, the ITKs. Therefore, ITKs emerge as the thrust areas for socio-technical empowerment of the rural community with changing climate scenarios.

ICT Enabled Extension Approaches for Capacity Building of Dairy Farmers against Climate Change

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Climate Change is one of the most seriously talked global issue in several fora. The Intergovernmental Panel on Climate Change (IPCC) in its fourth assessment report (2007) indicated the vulnerability of developing countries to extreme climatic events due to their over-dependence on climate sensitive fields like agriculture, livestock and allied sectors. Agriculture releases to the atmosphere significant amounts of CO₂, CH₄, and N₂O (Paustian *et al.*, 2004). CO₂ is released largely from microbial decay or burning of plant litter and soil organic matter. CH₄ is produced when organic materials decompose in oxygen-deprived conditions, notably from fermentative digestion by ruminant livestock, from stored manures, and from rice grown under flooded conditions (Mosier *et al.*, 1998). Changes in atmospheric temperature and precipitation pattern increase the likelihood of short-run crop failures and long-run production decline. Several climate impact studies indicate a probability of 10-40% loss in crop production with the increase of temperature during 2080-2100.

Despite highest livestock population possessed by India, this contributes about 17% of India's GHG emission in C_O₂ equivalent to the total GHG emission by the agriculture sector. Earlier researchers revealed that an estimated annual loss of milk production due to direct climate stress on livestock is about 1.8 million tonnes, that is nearly 2% of the total milk production in the country(value Rs.2661.62 crores). Subsequently the annual loss in milk production due to adverse climate effect in 2020 is likely to increase about 3.4 million tonnes milk costing more than Rs.5000 crores at current prices.

Information and communication technology (ICT) have the potential to contribute to the sustainable development of agriculture and livestock in the country. Though agriculture and livestock enterprises are the last to embrace ICT tools for Research, Education, Extension and Development, the rate of reach is much faster Inspite of the fact that farmers are by and large semi-literate, mostly the ICT language happens to be in English and the connectivity is poor at block levels in rural sector.

Climate change- Different perceptions:

Perception is the process by which we receive information or stimuli from our environment and transform it into psychological awareness (Ban and Hawkins, 2000). Saarinen (1976) talks about perception as an extremely complex concept and confines social perception-which is concerned with the effects of social and cultural factors on cognitive structuring of our physical and structural environment. As an earlier stated definition indicates that the cognitive behaviour of an individual will differ in different situation based on their factual condition, need analysis and existing problems. So the different stakeholders will vary with the climate adverse effect like:

- i. Farmers: The farming community mainly consist of smallholder to commercial dairy farmers based on their herd size and eventually they stated positively that the changes in the climate parameters like temperature, rainfall, humidity, wind pattern and ground water level is gradually changing last 2-3 decades and in turn it is affecting the animal behaviour and milk production.
- ii. Extension functionaries: Extension agents also have the conceptual fact related to the changing weather conditions with respect to farming situations. However they need the proper sensitization on climate-resilient farming practices and cope up strategies esp. in the extreme summer & winter seasons.

Conventional extension approaches:

The conventional extension approaches are popular in developing countries including India which informs, stimulates and guides such aspects of the system as its structure, its leadership, its program, its resources and its linkages. Axinn (1988) identified eight different approaches to extension work. The approaches are briefly summarized below.

- (a) The general agricultural extension approach
- (b) The commodity specialized approach
- (c) The training and visit approach
- (d) The farming systems development approach
- (e) The participatory agricultural extension approach
- (f) The project approach
- (g) The cost sharing approach

(h) The educational institution approach

But over the years, a number of models and methods have been used to enhance the effectiveness of extension services and advisory to the clientele system. Perhaps the conventional mass media methods have lack in

- The amount of information that can be transmitted is limited;
- Radio and television reception is poor in some areas and the target group may not own advanced gadgets and the electricity problem often occurs in rural areas.
- It is difficult to evaluate the impact since there is no immediate feedback in the conventional systems of technology transfer;
- Production of both programs and printed materials is costly and requires special skills.

These approaches are not able to deliver the technologies as the expectation of farming community. The existing extension functionaries in line departments are not matching with the growing rural population in the villages which has resulted in low uptake of transfer of technologies and delivering of untimely information. In addition, most of the time they are not giving need based farm advices than general or blanket. Further, following are the problems faced by the end user or farmer

- Lack of timely advisory support to the farming community
- Lack of instant technical information support to field extension officials
- Poor weather and disease complex advisory services
- Lack of market information support services

The role of ICT:

Since civilization, people have been cultivating crops, rearing livestock, and fish for sustaining their life, they have sought information from one enterprise to another. Updated and relevant information allows the farmers to cope with and even benefit from the dynamism of farming situation. Providing knowledge to farmers can be challenging, because the highly localized nature of agriculture means that information must be tailored specifically to distinct conditions. Though Extension is playing crucial role for dissemination the reliable information through conventional mass media for effective solutions to address both the long and short-term challenges in agriculture and livestock, but the technology adoption rate is low at filed level.(i.e. till date A.I. adoption rate is 25-35%) ICT is one of the prominent solutions,

and has recently unleashed incredible potential to improve agriculture in developing countries specifically.

An ICT is any device, tool, or application that permits the exchange or collection of data through interaction or transmission. The ability of ICTs to bring refreshed momentum to agriculture appears even more compelling in light of rising investments in agricultural research.

The potential of ICT to contribute to poverty reduction lies in its power to give poor women and men access to improved information and communications. ICT, broadly, allows for a reduction in transactions costs, improved communications with markets and within the supply chain, and improved information about new opportunities. ICTs can also improve the internal information systems of enterprises. More specifically, ICTs can:

- Provide reliable access to markets (local, regional and international) through increased use of affordable communications (phone, fax, scan, email).
- Improve contact with suppliers, and transport links to and from markets (e.g., through databases of enterprises, products, and suppliers).
- Exhibit choices regarding prices of raw materials and finished goods, enabling better prices for enterprises when dealing with traders.
- Inform about locally and internationally available non-financial business development services (BDS) (e.g., training schemes, business skills, and marketing).
- Link direct or intermediated access to BDS (e.g., training packages, advice on better practice).
- Provide access to legal information and financial services, and access to financial services (e.g., via micro-finance institutions – MFIs).

ICT applications for livestock management:

Livestock sector is an input & information-intensive one and new approaches and technical innovations are required to cope with these challenges and to enhance the livelihoods of the farming community. So the technical and scientific advancement will enhance the livestock productivity in better manner through scientific adoption. The following pertinent information will address the animal rearing in modern era through ICT interventions.

1. Dairy Production (Feeding & Management- MIS for Progeny testing, HERDMAN software)

2. Animal Health (Geo informatics in Disease surveillance, EWS, GIS, GPS & RS)

3. Disease Diagnosis

- Image Intensifier TV system
- Ultrasound
- Computerised Tomography (CT)
- MRI
- Nuclear Scintigraphy
- Digital Subtraction Angiography
- Laparoscopy
- Endoscopy
- Pulse Oximetry

4. Vet. Hospital management software

- Appointment scheduling
- Client screen
- Patient screen
- Visit screen
- Electronic medical Record
- Finance screen
- Inventory control
- Remainder & Reports

5. e-Livestock (Unique ID, Wireless Sensor Network etc..)

Use of ICT in agriculture & successive initiatives:

India has the second largest number of extension workers in the world providing continuous knowledge support to this number of extension workers. However, 24 hours service all the seven days is possible only through e-learning strategies to the farming community. Some successful public ICT initiatives in agriculture are e-choupal (ITC), aAQUA which stands for almost All Questions Answered, Warana Wired Village, Infosys' ICT initiatives for

empowering Indian farmers, Agropedia, RKMP (Rice Knowledge Management Portal), InDG (India Gateway), Kisan Kerala, Agrisnet, Agmarknet, Dacnet, Agricoop, Intradac, seednet, Metrological Information by Ingen Technologies, Honey-Bee knowledge network. MSSRF, Ikisan portal, Uttamkrishi, Mahindra kisanmitra, Indiaagronet, Krishi world, Agriwatch, Agriculture information were some of the private initiative for agricultural development.

Though above mentioned initiatives have been providing services to the agriculture sector and the following will be has the potential to deliver services to the livestock farming esp. dairy stakeholders.

1. Automatic weather station (AWS):

AWS is a network at block level establishment for dense weather observation and it gathers the network data through GSM-GPRS communication and providing them on real time to the farmers. Mainly it is computer cluster for running regional climate models for developing medium range weather forecast.

2. India development gateway (InDG):

An initiative on knowledge management through ICT:

India Development Gateway (InDG) is a national level initiative dedicated to empowerment, provides credible information, products and services in local languages that respond to the real and strategic needs of the rural people. As the rural landscape in India is set to take the advantage of the flourishing ICT initiatives pioneered by various institutions, more specifically the Common Service Centers (CSCs), InDG initiative offers the much required content and services in local languages that makes the difference in the lives of the rural people.

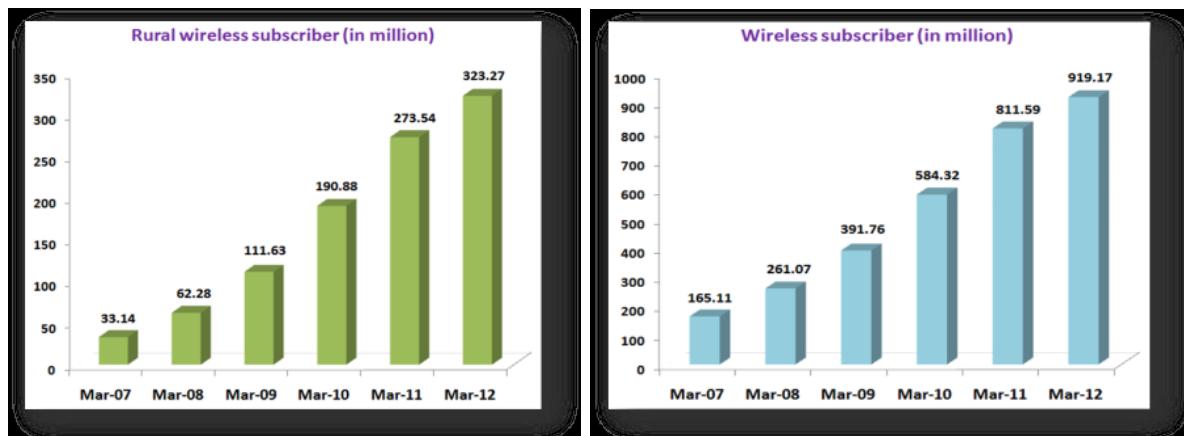
3. Community radio station for community development:

Community Radio is owned and managed by the community and the community members who participate in planning, production and dissemination of content. This makes it an ideal platform for generating and delivering information suited to the educational, developmental and cultural needs of the community it serves. Community radio stations are expected to produce at least 50% of their programmes locally, as far as possible in the local language or dialect. Information could be related to agriculture, animal husbandry, horticulture, water

resource management, weather, market information; health or education, government schemes, etc.

4. Mobiles for technology dissemination:

Access to technological information is one of the most important enablers for the farming community to improve productivity sustainably. Indian telecommunication revolution that too wireless connectivity made it possible to reach to unreachable located consumers through mServices. During the present decade, India has seen an exponential growth in the telecom particularly in wireless. Rural teledensity has just reached the two digit level whereas the urban teledensity is moving towards the three digit level. Mobile- penetrations among the 700 million rural population are put at about 16-18 percent and growing rapidly (TRAI, 2009).



Source: Telephone Regulatory Authority of India – 2012

The following projects have been successfully implemented by public and private players for the welfare of the farming communities.

S.No	Project Name	Partners (with service option)
1	aAqua Mini	Agrocom (on GSM,CDMA)
2	mKrishi	TCS-Qualcomm (on GSM)
3	Fisher Friend	TATA Tele-Qualcomm-MSSRF + Astute Systems Technologies (CDMA)
4	Reuters Market Light (RML)	Reuter-MSAMB-India Post (on GSM, CDMA)
5	IFFCO Kisan	IFFCO-Airtel (on GSM)

	Sanchar (IKSL)	
6	Life Tools	Nokia+ Idea+ RML (on GSM)
7	CERES	CERES+ Reliance (on CDMA)
8	KISSAN Kerala	PAN India based service
9	Sanchar Shakti	Universal Service Obligation Fund of Telecommunication Department

5. Agropedia –ICAR initiative:

Content availability and its intelligent organization continue to be a serious challenge in agriculture. This initiative has been taken by IIT-K and ICAR to make a database for content and subsequently given attempt for information storage and retrieval. This prevents offer of meaningful and efficient advisory and allied services to farmers and other stakeholders. Agropedia is an attempt to infuse semantic and social networking technologies into agriculture information management to alleviate this problem.

6. vKVK: voice Krishi Vigyan Kendra:

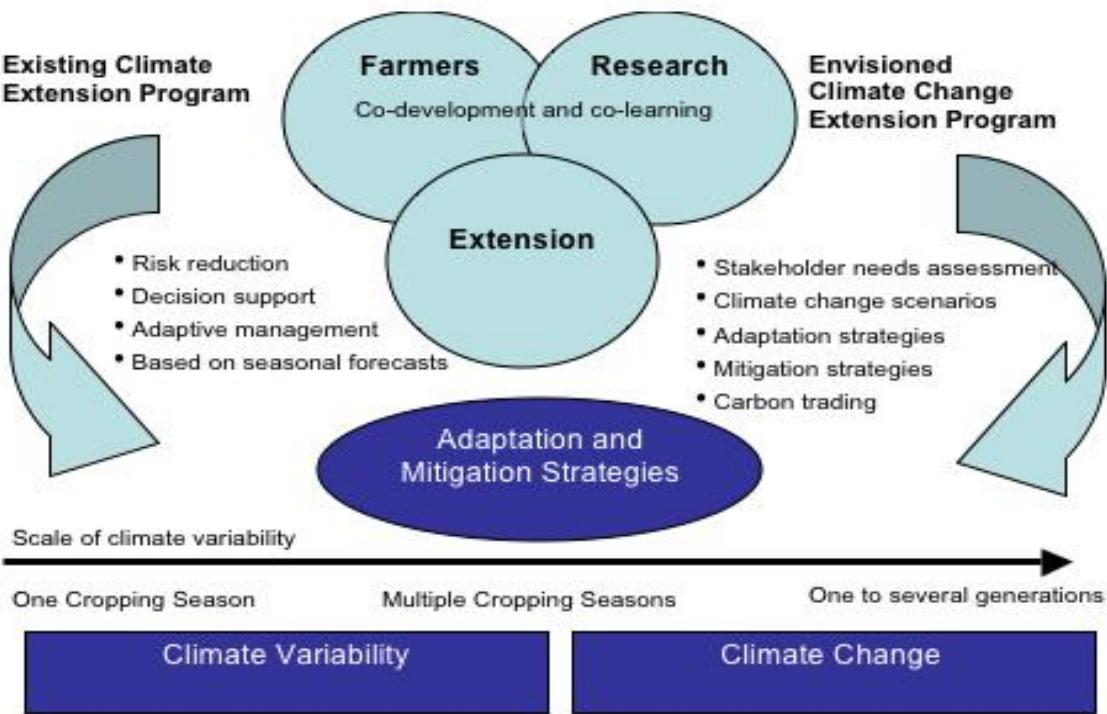
A voice KVK (vKVK) is a set of advisors (KVK experts) and peers (lead smallholder farmers) connected through mobile and internet technologies. In the vKVK, the interaction between the two parties can be entirely electronic. Sub-systems are developed to address the needs of vKVK scientists, farmers and the middle ware to deliver advisory services, alerts and Q & A services over SMS, voice and Web. Mainly E to F (Expert to Farmer), F to E (Farmer to Expert) and E to E (Expert to Expert) services have been tested and operationalized in voice mode.

Application of ICT to address climate change issues:

There is much scope for technological developments to reduce GHG emissions in the agricultural and livestock sector. For example, increases in crop yields and animal productivity will reduce emissions per unit of production. Such increases in crop and animal productivity will be implemented through improved management and husbandry techniques, such as better management, genetically modified crops, improved cultivars, fertilizer recommendation systems, precision agriculture, improved animal breeds, shelter management, improved animal nutrition, dietary additives and growth promoters, improved animal fertility, bio-energy crops, anaerobic slurry digestion manure disposal mechanisms and methane capture systems. And also in these line ICT could deliver promptly

1. Awareness creation about climate resilient dairy husbandry practices.
2. Sensitization of line dept.officials and field functionaries about the adverse climate effect and adaptation practices.
3. Preparedness towards the external climate impact and implementation of appropriate measures.
4. Evolving the risk aversion strategies with multi-stakeholders.
5. Framing the cope-up methods for resilient practices and reduce the vulnerability.
6. Awareness on carbon trading and conservation agriculture.

The following Climate-Extension-Program framework will also depict the climate impact program at field level.



Source: Clyde W. Fraisse *et. al.* (2009)

Conclusion:

Keeping above view, there have been experiments in technology dissemination using ICT but instant and needy information can reach and revolutionize the resource poor small farmers on real time basis. These interventions in agriculture and animal husbandry enable the farmers to get the information at right time at their door steps. Mostly farmers are prepared to utilize the potential of the ICT for their development provided that the adequate infrastructure

and better connectivity. Timely and actionable information from trusted sources, locally relevant, storable and referenceable and access of experts may enhance the effectiveness of ICT services. The best example is that Video calling facility may further enhance the quality of communication at field level.

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Application of *iWorx LabScribe2* in Animal Physiological Data Monitoring

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Introduction:

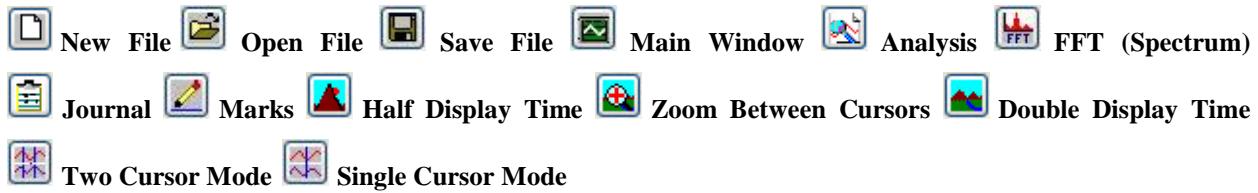
iWorx LabScribe2 is data recording and analysis software in which we can analyze carbon dioxide, oxygen, oxygen saturation, respiration rate, ECG, heart rate, skin temperature, air-flow and lung volume (STPD) etc. Large amount of data can be recorded with *iWorx LabScribe2* software.

Equipment required:

- PC or Mac Computer
- IWX/214 data acquisition unit
- SP-304 Spirometer with long flowhead tubing
- A-FH-1000 Flow head
- Animal face mask
- 5 Liter Mixing Chamber
- GA-300 CO₂/O₂ gas analyzer with filter
- PHRM-100 Heart rate monitor
- 3 Liter Calibration Syringe
- IWX/214 Recorder,
- ECG Electrodes

Various tool bars:

In *iWorx LabScribe2* various tool bars use for various function. The functions of *iWorx LabScribe2* can be accessed via the icons in the toolbar, which is directly beneath the menu headings on the main window display. These tool bars and their functions are described following:



Experiment protocol:

Start the software:

1. Click on the *iWorx LabScribe2* shortcut on the computer's desktop to open the program.
2. On the *iWorx LabScribe2* main window, pull down the settings menu and select load group (IWX/214).
3. Click on the settings menu, again. Select the Aerobic Fitness-GA300-LS2 settings file in Human Exercise-GA300.
4. For your information, the settings used to configure the *LabScribe2* software and IWX/214 units for this experiment are programmed on the preferences dialog window, which can be viewed by selecting preferences from the edit menu on the *LabScribe2* main window.
5. After loaded, settings file click the experiment button on the toolbar to open any documents like: Appendix, Background, Labs and Setup (opens automatically).

A. Flow head calibration before experiment:

1. Click on *LabScribe2* than *iWorx/214*
2. Load setting file select GA300 Spirometry Calibration
3. Click on edit prefances
4. Select A4 channel bar and click OK
5. Go to display time and manage time 10-30 sec and click ok
6. Pull the plunger on the 3L calibration syringe outside
7. Click the record button
8. Wait for at least 10 seconds of recording
9. Push the plunger in all the way until it stops. Pull the plunger out all the way until it stops
10. Repeat the above procedure for 5-10 time
11. Wait 5 seconds after the final stroke, then click Stop
12. On the expired air volume channel, click on STPD Vol. mixing chamber

13. Click on add function
14. Open the spirometer calibration dialog box

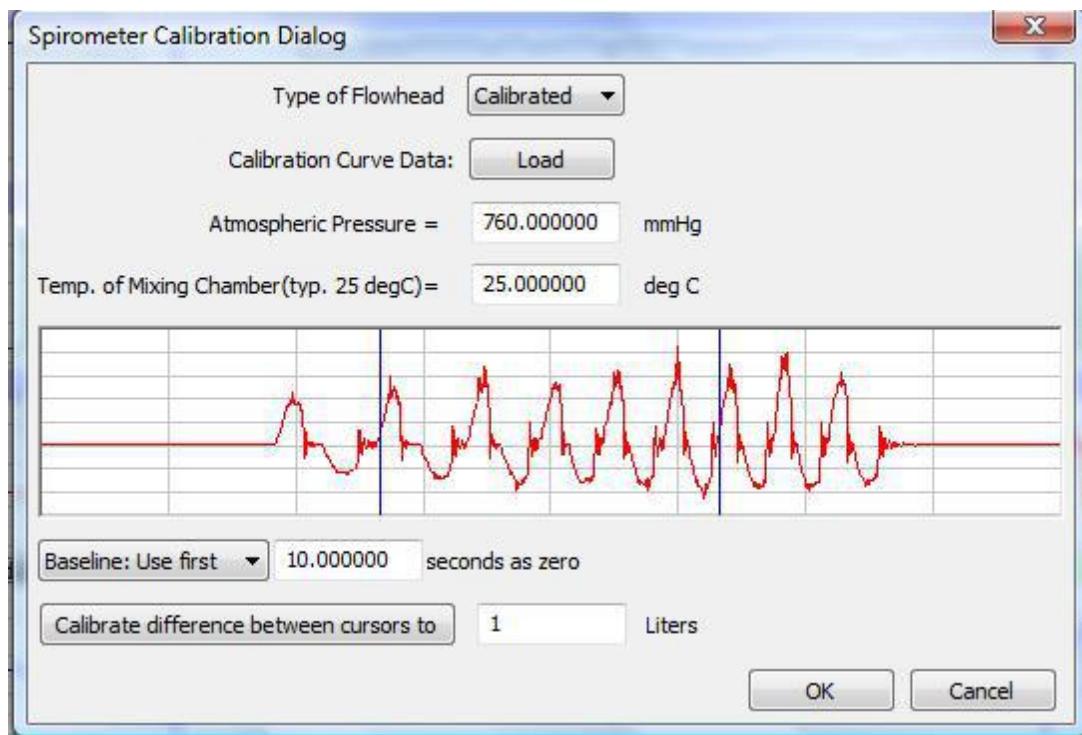


Figure: Spirometer Calibration dialog window

15. Enter these values:
 - a. Type of flow head- Calibrated
 - b. Click Load a new window will open so the *.iwxfcfd file that was previously generated can be loaded into your settings. Choose the file and click Open.
 - c. Atmospheric Pressure - enter your local atmospheric pressure.
 - d. Temperature of Mixing Chamber (typically 25 deg C)
 - e. Baseline use first 10 seconds as zero.
 - f. Calibrate difference between cursors to 3 Liters.
16. Move the left hand cursor to the flat line at the beginning of the calibration recording.
17. Move the right hand cursor to the flat line at the end of the calibration recording.
18. Click OK and select save as in the file menu

B. Calibrating the GA-300 Gas Analyzer and Calibrate the O2 and CO2 Channels:

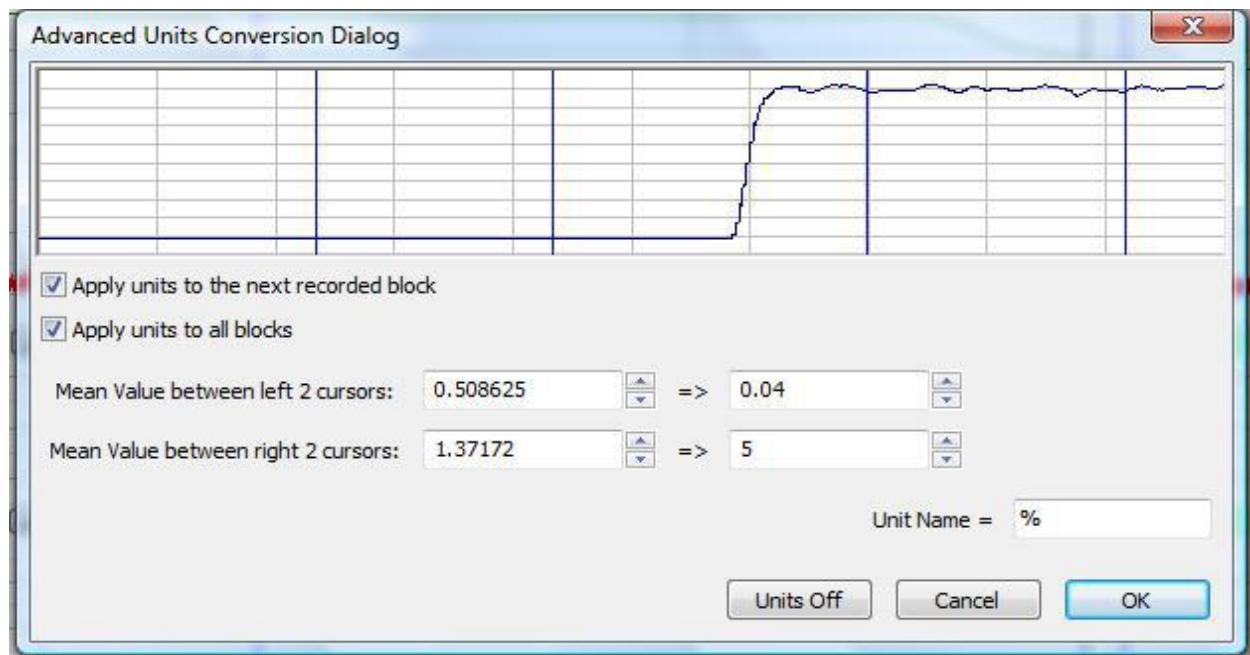
Note: Warm up the GA-300 for at least 30 minutes prior to use.

1. Clamp and secure any gas cylinders that will be used to provide gas samples near the GA-300 gas analyzer.
2. Place the gas sampling tubing away from the users to prevent the sampling of exhaled air. Allow room air to be pumped through the gas analyzer for about 10 seconds before recording the outputs of the sensors.
3. Type “Room Air” in the Mark box to the right of the Mark button.
4. Click on the Record button. The recording should scroll across the screen.
5. While recording, press the Enter key on the keyboard to mark the recording for the room air gas sample.
6. Measure the oxygen and carbon dioxide sample of gas mixture containing known concentrations of oxygen and carbon dioxide.
7. Open the secondary valve on the regulator of the cylinder providing the gas sample.
8. Type known gas in the mark box.
9. While continuing to record with the sample gas flowing into the GA-300, press the enter key on the keyboard to mark the recording for the known gas sample.
10. Once the recordings of the gas concentrations reach a steady level, record for another ten seconds.
11. This can take up to 30 seconds or so.
12. Click the stop button.
13. Select save as in the file menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder). Designate the file type as *.iwxdata. Click on the Save button to save the data file.

Convert the units on gas concentration channels:

1. Use the Display Time icons to adjust the display time. The required data can also be selected by: Placing the cursors on either side of data required.
2. Click the double cursor icon. Place one cursor on the section of data recorded of room air and the second cursor on the section of data recorded when the known gas was recorded.
3. Click on the arrow to the left of the carbon dioxide channel to open the channel menu. Select units and select advanced, next click to apply units to all blocks.

4. Click on and move the cursors so that they are in position such that: the first cursors are on the area where room air was recorded. The second cursors are on the area where the known gas sample was recorded.



- Room air concentration of CO₂ = 0.04
 - The second gas concentration will be the known one from the gas cylinder. Generally a 5% CO₂ concentration is recommended.
 - Enter the name of the units, %,
 - Click the OK button
5. Repeat steps on the expired O₂ concentration (%) channel.
- Room air = 20.9
 - Second gas concentration will be the known one from the gas cylinder. Generally a 12%
 - O₂ concentration is recommended.
 - Click on the Save button.

Metabolic parameter calculation:

Values for VO₂, VCO₂, RER, TV and other parameters from the segments of the test can be calculated automatically by using the metabolic calculation window.

To use the metabolic calculation window, pull down the advance menu and select metabolic. Select mixing chamber: Offline calculation from the submenu to open the metabolic calculation dialog window.

1. on the left side of metabolic calculation window: pull down the CO₂, O₂, volume, heart rate, and energy channel menus to select the channels on which the CO₂ and O₂ concentrations, lung volumes, heart rates, and workload were recorded.
2. Enter the time (in secs) in the Average box to 0 select the time length of each segment.
3. Click on calculate button on the left side of metabolic calculation and calculate the average value of each parameter for the recorded data and to plot the selected parameter against each other in the plot panel.

Measurement of oxygen saturation heart rate and ECG:

Oxygen saturation:

1. Click on the *LabScribe2*
2. On the main window, pull down the settings menu and select load group.
3. Pull down the settings menu, again. Select the O₂ saturation-exercise-LS2 settings file.
4. After a short time, *LabScribe2* will appear on the computer screen as configured by the O₂ saturation-exercise-LS2 settings.
5. Put up O₂ saturation sensor on animal tail.
6. Click on the record button and then on the auto scale button

ECG Recording:

1. Click on the *LabScribe2*
2. Pull down the settings menu again. Select the ECG-PulseOx-LS2 settings file from human circulation.
3. Click on the record button.
4. Click on the auto scale button
5. Click stop to halt recording, recording file should look like the figure.

Data Analysis:

- a. Click on analysis window icon in the tool bar
- b. Click on add function button

- c. Adjacent ECG/Pulse cycles can also be selected by: placing the cursors on either side of four complete ECG/Pulse cycles.



Figure: ECG, pulse, oxygen saturation level, and heart rate displayed on the analysis window

- d. Look at the function table that is above the uppermost channel displayed in the analysis window.
- e. The names of the mathematical functions used in the analysis, T2-T1, Max, Min.
- f. Once the cursors are placed in the correct positions for determining the oxygen saturation levels and heart rates on the ECG/Pulse cycles, the values of the parameters can be recorded in the online notebook of *LabScribe2* by typing their names and values directly into the journal, or on a separate data table.
- g. The functions in the channel pull-down menus of the analysis window can also be used to enter the names and values of the parameters from the recording to the journal. To use these functions:
- h. Transfer the values for the saturation levels, rates, and intervals to the Journal using the Add Ch. Data to journal function in the ECG channel pull-down menu.
- i. Click on the save button in the file menu.

Importance of Climograph, Psychrometrics and Temperature Humidity Index

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Psychrometrics of moist air is a basic understanding of the environment related to domestic animals. The production of the domestic animals is severely affected by adverse climatic conditions during extreme winter and extreme summer especially in hot and humid environment in tropical countries. Moisture removal from livestock buildings is a problem of environmental control in cold climate however, in hot environment additional water may be introduced by spray/ mist coolers.

The psychrometric chart shows graphically the parameters relating to water moisture in air. Air comprises approximately 78% nitrogen, 21% oxygen, and 1% other gasses but air is never dry, even in a desert. Two-thirds of the earth's surface is covered with water and this, along with other surface water and rain, maintain low water vapor pressure to be suspended in the air making up part of the 1% of other gases. The psychrometric chart indicates the properties of this water vapor through various climatic elements i.e. dry and wet bulb temperatures, relative humidity, Dew Point temperature, enthalpy, specific volume and moisture content. A psychrometric chart is valid at a certain pressure of air since pressure of air is related to the height above (or below) mean sea level. The chart provided by ASRAE is valid at mean sea level (29.921" of Hg or 760 mm of Hg); therefore, necessary correction factor must apply for different altitudes. By determining any two factors, other five parameters can be found from the Ashrae chart. The study of psychrometrics is useful, as the living beings feel comfortable over a narrow range of temperature and humidity. The amount of heating or cooling required for a certain space of animal shed can be calculated from moisture content and flow of air these values.

Measurement of air parameters for psychometrics:

Dry and wet bulb temperature:

To measure dry bulb air temperature (DB) one can make use of thermometer, thermocouple, electronic meter, Infrared thermometer etc. This temperature is shown on the horizontal axis of

the chart. Wet bulb temperature (WB) also known saturation temperature can be recorded through moist bulb (do'nt dip) of thermometer (Sling Psychrometer) measured in °F or in °C. When the moisture evaporates, it lowers the temperature recorded by the thermometer depending on the moisture content of air i.e. less moisture in the air will result in a faster rate of evaporation and therefore less value then dry bulb. When the air sample is saturated with water (i.e. 100% relative humidity), almost no water can evaporate from the moist tissue therefore the WB temperature will be the same as the DB temperature. This temperature is therefore also referred to as the saturation temperature. Diagonal lines on the chart indicate WB temperature.

Relative humidity (RH):

This is the ratio of the fraction of water vapor in the air to the fraction of saturated moist air at the same temperature and pressure. RH is dimensionless, and is usually expressed as percentage. 100% RH indicates the air is saturated and cannot hold any more moisture. Preferred values of comfort for people are between 25% and 70%. Lines of constant relative humidity are expressed as exponential lines on the psychrometric chart. The line at 100% is referred to as the saturation line.

Dew point (DP) temperature:

The temperature of the air at which a moist air sample reaches water vapor saturation is called as dew point. It is equivalent to a wet bulb temperature at 100% relative humidity. At this combination of temperature and humidity, further removal of heat results in water vapor condensing into liquid. In other words when a sample of air is cooled, its RH rises till 100% (saturated air). At saturation, the dew point temperature equals the wet bulb temperature, which also equals the dry bulb temperature, and the RH is 100%. This temperature is shown as horizontal lines on the Psychrometric chart.

Moisture content:

This is also known as the humidity ratio and is usually designated as W. It is the proportion of the mass of water vapor per unit mass of dry air. Humidity ratio is dimensionless, it is usually expressed as pounds of moisture per pound of dry air or grams of water per kilogram of dry air or as percentage. The moisture content is the vertical axis of the chart.

Enthalpy (total heat):

Enthalpy (usually designated as h) is the total amount of heat energy of the moist air and therefore includes the amount of heat of the dry air and the water vapor in the air. In the approximation of ideal gases, lines of constant enthalpy are parallel to lines of constant WB temperature. Thus, diagonal lines on the chart indicate the enthalpy. Enthalpy is measured in BTU per pound of dry air or Joules per kilogram of air.

Specific volume:

The inverse of density is Specific volume i.e. the volume per unit mass of the air sample. This is shown as diagonal lines on the chart. It is expressed in cubic feet per pound of dry air or cubic meters per kilogram of dry air.

Annual Climatology: New Delhi, India (Elev: 695 ft Lat: 28° 35' N Long: 77° 12'E)

Month	Daily Max	Daily Min	Mean	Month	Mean
January	70.0	45.1	57.6	January	0.8
February	75.6	50.2	62.9	February	0.6
March	86.0	59.7	72.9	March	0.6
April	97.2	70.7	83.9	April	0.3
May	103.3	78.6	91.0	May	0.7
June	102.7	82.9	92.8	June	2.2
July	95.2	79.9	87.5	July	9.1
August	91.9	78.6	85.3	August	10.2
September	93.0	75.9	84.5	September	5.0
October	91.2	67.1	79.2	October	1.4
November	82.9	55.0	69.0	November	0.2
December	73.4	46.8	60.1	December	0.3
Annual	88.5	65.9	77.2	Annual	31.4

Climograph:

A graph that shows the annual cycle of temperature and precipitation for a specific geographical location i.e. average monthly temperature on X-axis and average monthly relative humidity on Y-axis . Through this chart, one can classify the average climatic conditions in following types.

- 1) Hot and dry climate
- 2) Hot and humid climate
- 3) Cold and dry climate
- 4) Clod and humid climate

Comfort zones:

Living beings feel comfortable within small ranges of temperature and humidity. The ranges vary based on altitude, latitude and longitude. Animals are sensitive to humidity because water evaporates from the skin and this evaporation cools the body. At a high humidity, water evaporates from the skin slowly, so we feel warmer. On contrary at a low humidity, water evaporates faster and we feel cooler. The effect of perceived increase in temperature with increase in humidity is referred to as the Heat Index.

In a climograph one can interpret that during different months the climate of a particular place is within comfortable range or in discofortable range. Humidity (%) <25 and > 70 both are in discomfort levels i.e. dry or humid type of environment whereas temperature ($^{\circ}\text{C}$) <15 and >25 classify environment as cold and hot conditions. Therefore, hot dry and hot humid, cold dry and cold humid and thermal comfort range are being depicted by a climograph.

Temperature–humidity index (THI):

THI is a combination of temperature and humidity that is a measure of the degree of discomfort experienced by an individual in warm weather, originally called the discomfort index. The index is essentially an effective temperature based on air temperature and humidity; it equals 15 plus 0.4 times the sum of simultaneous readings of the dry- and wet-bulb temperatures. Thus, if the dry-bulb temperature is 90°F (32°C) and the wet-bulb temperature is 50°F (10°C), the discomfort index is 15+0.4 (140), or 71. Mostly living beings are quite comfortable when the index is below 70 and very uncomfortable when the index is above 80 to 85. In tropical countries the average daily values of the THI, exceeding 80-85, consistently occurs during summer i.e. July to

September. The majority of fundamental information on temperature effects on lactation is on cattle. The yield and composition of milk are affected by the temperature of the environment or the animal's body temperature. Environmental temperatures lower and higher than the comfort zone temperatures diminish yield and alter many of the components of milk. The temperature and the relative humidity in stable were recorded at each milking. The daily temperature-humidity index (THI) values were calculated using the equation by Kibler (1964):

$$\text{THI} = 1.8\text{Ta} - (1 - \text{RH}) (\text{Ta} - 14.3) + 32$$

Where,

Ta- measured ambient temperature in °C, RH- relative humidity as a fraction of the unit. According to the value of the temperature-humidity index (THI), $\text{THI} \leq 72$ (Comfortable) and $\text{THI} > 72$ (under Stress). For example, at two temperatures of 20 and 30°C calculated THI will be 67 and 82 showing that at RH of 75% 20 °C is comfortable and 30°C is stressful for a particular group of cows in an particular environment. Another widely used formula is

$$\text{THI} = 0.72 (\text{Tdb} + \text{Twb}) + 40.6 \text{ where Tdb and Twb are dry and wet bulb temperatures in } ^\circ\text{C}$$

If THI is < 72 No stress, 72-78 Mild stress and > 78 Severe stress.

Production impacts:

As per the NRC (1971) formula i.e. $\text{THI} = (1.8 \times \text{T} + 32) - [(0.55 - 0.0055 \times \text{RH}) \times (1.8 \times \text{T} - 26)]$

Where, T= Temperature in °C and RH= Relative Humidity in %. When THI reaches, 68 for Friesians and 75 for Jerseys (equivalent to 21 and 25.5°C respectively at 75% relative humidity) New Zealand cows begin to experience the effects of heat stress. This is seen by a reduction in feed intake and a drop of around 10g milk solids per day per unit increase in THI. For example, an increase in THI from 68-78 would equate to a 100g drop in milk solids/cow/day. Note that a drop in fat/protein percentage occurs before a drop in yield or milk solids is visible. At a temperature of 40°C and 70% RH , THI rises to about 96 expressing a danger level of extreme heat stress requires immediate relief from hot humid environment.

Sensible and latent heating and cooling:

Sensible heat is heat that is added or subtracted without a change in the state of the substance. Latent heat is the addition or subtraction of heat results in a change of state of the substance. Therefore, when there is a heating or cooling of the air, the moisture changes as follows:

1. If sensible heat is removed (no moisture condenses)
2. If sensible heat is added (the humidity ratio and dew point are constant)
3. If latent heat is removed (moisture is removed from the air)
4. If latent heat is added (moisture is added to the air, but the dry bulb temperature is constant)

Calculation for cooling an animal house:

A house is 4500 ft² and has 12 ft ceilings. For comfort, the homeowner specifies 0.3 changes of air per hour. The outside air temperature is 90°F dry bulb and 73.5° wet bulb. The air indoors is 75°F dry bulb 50% relative humidity. What is the amount of cooling required to provide the fresh air?

Answer: The total volume of the house is $4500 \times 12 = 54,000 \text{ ft}^3$. We need to change $54,000 \times 0.3 = 16,200 \text{ ft}^3/\text{hour}$ (which equates to 270 ft³/minute or cfm). From the psychrometric chart, the enthalpy of the incoming air is 37.0 BTU/lb and the specific volume is 14.2 ft³/lb. Therefore the energy of the incoming air is $16,200 \times 37 / 14.2 = 42,211 \text{ BTU/hour}$. Similarly, the enthalpy of the air indoors is $16,200 \times 28.1 / 13.7 = 33,228 \text{ BTU/hour}$. The heat difference is 8,984 BTU/h, or about 0.75 tons.

THI, Climographs and psychrometrics play a decisive role in manipulating the microenvironment within a comfortable range of temperature and relative humidity. In addition to this, the related values i.e. enthalpy, moisture, dew point and other significant parameters can also be estimated from the chart.

Recording of Meteorological Data and Physiological Parameters in Animals

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Nowadays, various instruments are available to record the meteorological parameters with high accuracy. For measuring the air and soil temperatures, different kinds of meteorological thermometers and thermographs are used. Psychrometer (wet and dry bulb hygrometer), Dew point probe, Relative humidity sensor (electrical impedance), are used to measure the humidity of air while atmospheric pressure is measured by Mercury Barometer, Aneroid barometers, aneroid barograph, electronic barometers. The intensity of solar radiation and the radiation from the earth's surface and atmosphere are measured with pyrheliometers, pyrgeometers, actinometers, pyranometers, pyranographs, albedometers and radiation balance meters; sunshine duration is recorded by campbell-stokes recorder. Wind speed and direction are measured with anemometers, anemographs, anemorumbometers, anemorumbographs and wind vane; visual range by Transmissometer. The amount and intensity of precipitate are determined by rain gauge and snow gauge. The reserves of water in snow cover are measured with Satellite measurements; dew is measured with a drosometer; evaporation with a atmometer and atmospheric electricity are measured by electrometers. Automatic metereological instruments are becoming increasingly important for measuring the meteorological parameters.

Automatic weather station:

An automatic weather station (AWS) is an automated version of the traditional weather station, either to save human labour or to enable measurements from remote areas. An AWS will typically consist of a weather-proof enclosure containing the data logger, rechargeable battery, telemetry (optional) and the meteorological sensors with an attached solar panel or wind turbine and mounted upon a mast. The specific configuration may vary due to the purpose of the system. The system may report in near real time *via* the Argos System and the Global Telecommunications System, or save the data for later recovery. In the past, automatic weather stations were often placed where electricity and communication lines were available. Nowadays, the solar panel, wind turbine and mobile phone technology have made it possible

to have wireless stations that are not connected to the electrical grid or telecommunications network.

Sensors: Most automatic weather stations have

- thermometer for measuring temperature
- Anemometer for measuring wind speed
- Wind vane for measuring wind direction
- Hygrometer for measuring humidity
- Barometer for measuring atmospheric pressure

Some stations can also have

- Present weather sensor and/or visibility sensor
- Celiometer for measuring cloud height
- Rain gauge for measuring liquid-equivalent precipitation
- Ultrasonic snow depth sensor for measuring depth of snow
- Pyranometer for measuring solar radiation

Data-logger:

The data-logger is the heart of the Automatic Weather Station. In high quality weather station, the data-logger is designed by the supplier to have the solution, which is perfect for meteorological client. Indeed, usually data-logger found in the market doesn't fit the requirement in terms of consumption, inputs, communication, protection against animals (ants, rats...), humidity, salty air, sand,... The main function of a data-logger is:

- Measures: the data-logger collects the information of each sensor and archives it.
- Calculation: the data-logger processes most of the meteorological data for the users (avg,min,max...).
- Data storage: the data-logger saves all the data either on its own memory or on uSD memory card.
- Power supply: the data-logger manages the power supply of the Automatic Weather Station such as solar panel.

- Communication: the data-logger does manage the communication protocol with the remote server. The different communication protocols are usual are GSM, GPRS, RTC, WIFI, uSD, RS232.

Enclosures:

Enclosures used with automatic weather stations are typically weather-proof fiberglass, ABS or stainless steel. With ABS being the cheapest, cast aluminium paint or stainless steel the most durable and fiberglass being a compromise.

Power supply:

The main power source for an automatic weather station depends on its usage. Many stations with lower power equipment usually use one or more solar panels connected in parallel with a regulator and one or more rechargeable batteries. As a rule of thumb, solar output is at its optimum for only 5 hours each day. As such, mounting angle and position are vital. In the Northern Hemisphere, the solar panel would be mounted facing south and vice versa for the Southern Hemisphere. The output from the solar panels may be supplemented by a wind turbine to provide power during periods of poor sunlight, or by direct connection to the local electrical grid. Most automated airport weather stations are connected to the commercial power grid due to the higher power needs of the ceilometer and present weather sensors, which are active sensors and emit energy directly into the environment.

Mast:

The standard mast heights used with automatic weather stations are 2, 3, 10 and 30 meters. Other sizes are available, but typically, these sizes have been used as standards for differing applications.

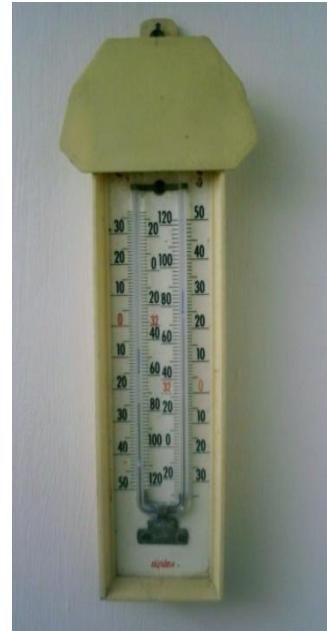
- The 2 meter (6.6 feet) mast is used for the measurement of parameters that affect a human subject. The mast height is referenced to head height
- The 3 meter (9.8 feet) mast is used for the measurement of parameters that affect crops (such as wheat, sugar cane etc.) The mast height is referenced to crop top.
- The 10-meter (32.8 feet) mast is used for the measurement of parameters without interference from objects such as trees, buildings or other obstructions. Typically the most important weather parameter measured at this height is wind speed and direction.
- The 30 meter (98.4 feet) mast is used for the measurement of parameters over stratified distances for the purposes of data modelling. A common application is to take

measurements of wind, humidity and temperature at 30, 10, and 2 meters. Other sensors are mounted around the 2-meter or lower height.

Commonly used meteorological equipments:

Maximum-Minimum thermometer:

It is used to record the extremes of temperature at a location. The maximum and minimum readings are recorded by two small steel markers, which are sprung into the capillary tube so that they can slide, but only if a force is applied to them, either by being pushed by the mercury or under the influence of an external magnet. Before a new maximum or minimum reading can be taken, the thermometer must be reset by moving the markers to the top of the mercury, usually by hand using a small magnet to slide them along the tube. Any change in temperature after that will push one of the markers along with it. (If the markers are not reset, they will register maxima and minima only if they exceed the values already encountered.)



Humidity:

Humidity is the amount of water vapour in the air. There are three main measurements of humidity: absolute, relative and specific. Absolute humidity is the water content of air. Relative humidity, expressed as a percent, measures the current absolute humidity *relative* to the maximum for that temperature. Specific humidity is a ratio of the water vapour content of the mixture to the total air content on a mass basis. The psychrometer consists of two thermometers of the same specifications, which are suspended side by side in the air. One of them measures the actual air (dry-bulb) temperature while the other, whose bulb is covered with a wet-bulb temperature (bulb wetted continuously by a muslin sleeve dipped in a water reservoir). Air passing over this wet bulb evaporates some water and wet bulb temperature depending upon the humidity of the air. The relative humidity can be found by noting the difference between the wet and dry bulb and the looking up the RH value in a set of tables.



Wind:

Wind is the flow of gases on a large scale. The direction from which the wind blow can be observed by looking at smoke from chimneys or at flags or church weather-vanes. Wind speed can be measured with cup anemometers.



Sunshine:

The Campbell-Stokes recorder (sometimes called a Stokes sphere) is a kind of sunshine recorder. The unit is designed to record the hours of bright sunshine, in this light will be focused from the sun onto a piece of card, on which, if the sun is bright enough, a burnt trace is left.



A Campbell-Stokes sunshine recorder

Rain and snow:

A rain gauge (also known as an雨量计, pluviometer, or an ombrometer) is a type of instrument used to measure the amount of liquid precipitate over a set period of time. Similarly, a snow gauge is a type of instrument used to measure the amount of solid precipitate over a set period of time.



Atmospheric pressure:

The simplest and cheapest device for measuring the atmospheric pressure is the aneroid barometer. To avoid the confusion of two units of measurement, it is better to prefer millibars only.



Recording of physiological parameters in animals:

The physiological parameters of animals like respiration rate , heart rate, rectal temperature and skin temperature can be recorded by flank movement, stethoscope, by inserting clinical thermometer in rectum and using a non-contact thermometer (RaytekR, model Raynger ST 2L) respectively.



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Measurement of Body Surface Area

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Generally two methods are used to determine the body surface area of large animals.

I) Indirect Method:

a) According to Rubner's law, which indicates the correlation among body weight and surface area using regression equation, where body surface area is proportional to the body weight raised to the power of 0.74.

$$\text{Surface area} = W^{0.74}$$

W = Body weight (kg)

b) Brody (1945)

$$A = 0.15W^{0.56}$$

A = Surface area (m^2)

W = Body weight (kg)

II) Direct method:

Surface integrator is used to measure the surface area of live animal.

This instrument was initially fabricated by Brody and later on modified by Mc Dowell and associates for bovines.

Surface integrator:

The instrument consists of two wheels. The distance between the two wheels of the integrator is fixed/adjustable. The recording wheel of the instrument is connected with revolving counter. The other wheel is in contact with white chalk in such a way that it leaves a white line on the skin, when the instruments move.

$$A = (\text{Circumference of wheel}) \times (\text{Distance between two wheel}) \times (\text{Number of revolutions})$$

A = Surface area in square meters

The circumference of wheel is in centimetres.

The surface area is measured on one side of the animal with the animal standing squarely on all four feet in the following manner.

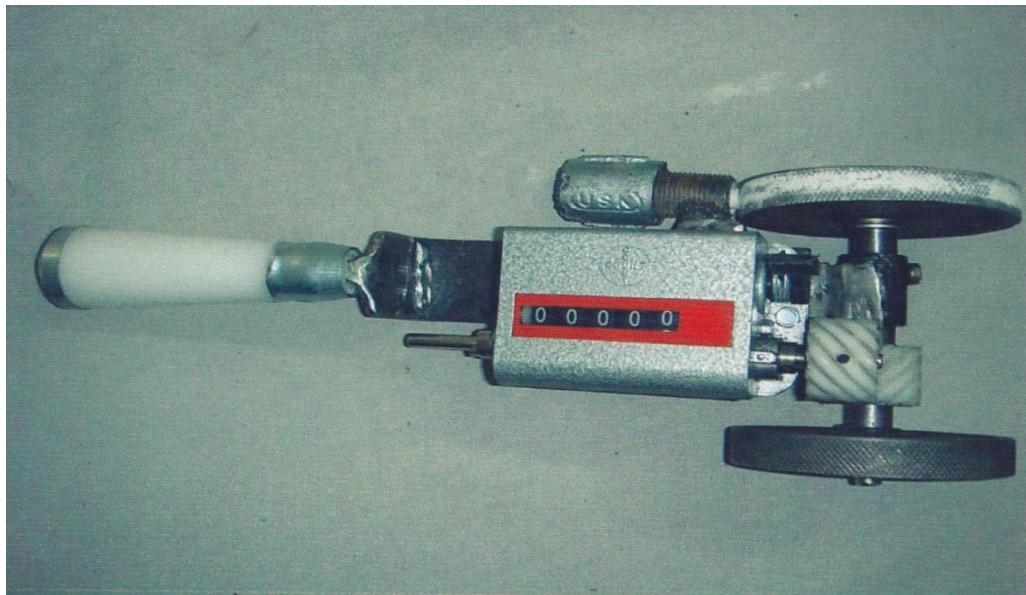


Photo 1. Surface Integrator

Measurement of surface area:

Trunk:

- After setting the counter at zero, start from the base of the tail and the integrator is pushed along the back with the plain wheel running along the centre line of the spinal cord.
- When reached to shoulder, the integrator is lifted off, and second run started for the centre line under tail with the plain wheel during the first run.
- This process is repeated until there remains only one step before the midline of the belly and chest is reached. The counter reading is recorded.
- The counter is now re-set at zero and a run made near the last strip, but since this strip will seldom have the same width as the distance between the wheels, the count must be reduced by multiplying it by the ratio between the width of the strip and distance between the wheels.
- The counter is re-set at zero and is run over the area between the shoulder and head in vertical strips from the lower midline to the centre of the spinal column. At the time of the direction to remove the wrinkles in the skin and the hand is held on the opposite side of the dewpal.



Photo 2. Measurement of surface area by Surface Integrator

Legs:

- Fore and rear legs are measured with the integrator by placing the feet wide apart and the integrator by placing the feet wide apart and the integrator run around the legs down to the vicinity of the knee joints.
- The lower legs are treated as cylinders, by taking the length from the lower mark of the integrator to the top of hoof with a measuring tape and the average of several measurement of circumference.

Area= circumference x length

Tail:

- The tail is measured from the base to the tip and the average circumference determined.

Area= circumference x length

Head:

- Length of the head is measured from the top of the poll to the mouth and the width determined from the base of one ear to another.

Area = $\frac{1}{2}$ width x length

Ear:

- The area is determined by measuring the outside from base to tip and the width at the center of the ear. The total area is calculated by treating the ear as a rectangle.

$$\text{Area} = \text{width} \times \text{length}$$

Total surface area of the animal is determined by the equation.

$$S=2(m+f+r+e)+t+h$$

Where as, S is the total surface area od the animal (m^2)

“m” is the area of trunk “e” is the area of ear

“f” is area of four leg “t” is the area of tail

“r” is area of hand leg “h” is the area of head

Heat Tolerance Indices for Cattle and Buffalo

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Heat tolerance: It is the ability of the animals to withstand heat when all other factors are constant. Zebu breeds of cattle are more heat tolerant than temperate breed of cattle.

Physical responses: Respiration rate, heart rate and rectal temperature are reliable indices for recording the heat tolerance of the animals. A change in respiration rate is a good measure for indicating heat tolerance. Deep body temperature is the most significant reaction to indicate heat stress. In an environment above the body temperature the only way to heat loss is evaporative cooling. When evaporative heat loss is insufficient homeothermy is disturbed and body temperature rises.

Heat tolerance indices:

To evaluate animals for their tolerance capacity the following test may be applied.

a) Iberia heat tolerance test (HTC):

This test is performed in open when the environmental temperature is between 85 to 95° F with bright sun shine. The experimental animals are exposed from 10.00 am to 3.00 pm and rectal temperature and respiration rate is recorded before and after exposure. This experiment is repeated for three days under identical atmospheric conditions.

$$\text{HTC} = \frac{\text{RT}}{101}$$

HTC= Heat Tolerance Coefficient

RT= Average rectal temperature of six reading.

101° F= Average temperature of Cattle.

Interpretation: If the calculated value of test is more nearer to 100 then the particular animal is more heat tolerant than others. When the two particular animals have the same heat tolerance coefficient, than the animal, which has higher respiration rate is less heat tolerant.

b) Gaalaa's heat tolerance test:

All the conditions for conducting the experiment are similar like Iberia heat tolerance test. The formula for calculating the heat tolerance coefficient is as follows

$$= 100 - \frac{RT - 101}{100}$$

Interpretation of results are also similar to Iberia heat tolerance test i.e. if the calculated values is more nearer to 100 than particular animals is more heat tolerant than other.

c) Benezra's coefficient of adaptability (BCA):

Test is based on the rectal temperature and respiration rate responses after exposure of animals for 7 hours continuously for 3 days.

$$BCA = \frac{RT}{38.33} + \frac{RR}{23}$$

Where,

RT= Rectal Temperature

RR= Respiration Rate/min.

38.33= Normal RT ($^{\circ}\text{C}$)

23= Normal RR/min.

Interpretation: A calculated value of 2 shows maximum adaptability and values over 2 indicate a state of lower adaptability.

d) Dairy search index (DSI):

In this rectal temperature and respiration rate and pulse rate is taken under consideration by giving appropriate weightage to each parameters (Thomas *et al.*) The animals are exposed for seven hours on a sunny clouds day...

$$\frac{0.5 X_1}{X} + \frac{0.03 Y_1}{Y} + \frac{0.2 Z_1}{Z}$$

Where X_1 , Y_1 and Z_1 are rectal temperature, pulse rate and respiration rate after exposure and X , Y and Z the same parameters before exposure respectively.

Interpretation: If the calculated value is more nearer to one than the animals is more heat tolerant than the animal are deviating more from one.