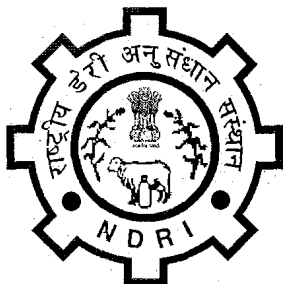


**EFFECT OF ENVIRONMENT ON HORMONES,  
BLOOD METABOLITES, MILK PRODUCTION AND  
COMPOSITION UNDER TWO SETS OF  
MANAGEMENT IN COWS AND BUFFALOES**



**THESIS SUBMITTED TO THE  
NATIONAL DAIRY RESEARCH INSTITUTE  
(DEEMED UNIVERSITY)  
KARNAL (HARYANA)**

**IN PARTIAL FULFILMENT OF THE REQUIREMENT  
FOR THE AWARD OF THE DEGREE OF**

**DOCTOR OF PHILOSOPHY  
IN  
DAIRYING  
(ANIMAL PHYSIOLOGY)**

**BY  
ANJALI AGGARWAL**

**DIVISION OF DAIRY CATTLE PHYSIOLOGY  
NATIONAL DAIRY RESEARCH INSTITUTE  
(I. C. A. R.)**

**KARNAL - 132001 (HARYANA), INDIA**

**2004**

**Regn. No. 1090103**

**DEDICATED**

**TO**

**MY HUSBAND**

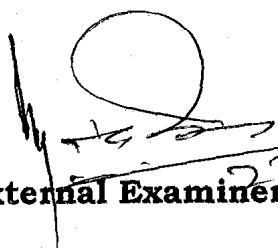
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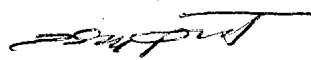
  
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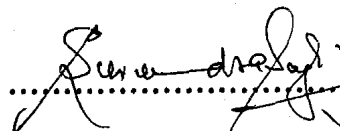
  
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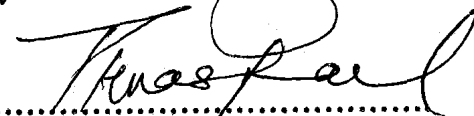
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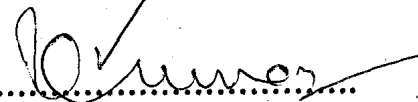
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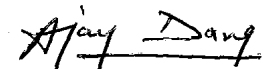
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## CERTIFICATE

This is to certify that thesis entitled “ **EFFECT OF ENVIRONMENT ON HORMONES, BLOOD METABOLITES, MILK PRODUCTION AND COMPOSITION UNDER TWO SETS OF MANAGEMENT IN COWS AND BUFFALOES**” submitted by Mrs. Anjali Aggarwal towards the partial fulfillment of the award of the degree of **DOCTOR OF PHILOSOPHY** in **ANIMAL PHYSIOLOGY** of the **NATIONAL DAIRY RESEARCH INSTITUTE** (Deemed University), Karnal (Haryana), India, is a bonafide research work carried out by her under my supervision and no part of the thesis has been submitted for any other degree or diploma.

Dated: 30<sup>th</sup> July, 2004.



(MAHENDRA SINGH)

Major Advisor and Chairman  
(GUIDE)

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*Dated : 30<sup>th</sup> July, 2004*

  
*Anjali Aggarwal*

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## LIST OF ABBREVIATIONS

ad lib	=	<i>ad libitum</i>
@	=	At the rate of
%	=	Percentage
A.M.	=	Ante Meridien (before noon)
ANOVA	=	Analysis of Variance
Av.	=	Average
°C	=	Degree Celsius
D <sub>b</sub>	=	Dry bulb
d.f.	=	Degree of freedom
°F	=	Degree Fahrenheit
g	=	Gram
HR	=	Heart rate
KF	=	Karan Fries
Kg	=	Kilogram
Km	=	Kilometer
μmol/litre	=	Micromole per litre
Max. temp.	=	Maximum temperature
mg	=	Milligram
min.	=	Minute
Min. temp.	=	Minimum temperature
ml	=	Millilitre
MP	=	Milk production
MSS	=	Mean Sum of Squares
NEFA	=	Non esterified fatty acid
ng	=	Nanogram
P.M.	=	Post Meridien (Afternoon)
RH (%)	=	Relative humidity expressed in percentage
RIA	=	Radioimmunoassay

RT	=	Rectal temperature
RR	=	Respiration rate
S.E.	=	Standard error
SCC	=	Somatic cell count
ST	=	Skin temperature
T <sub>3</sub>	=	Triiodothyronine
T <sub>4</sub>	=	Thyroxine
THI	=	Temperature Humidity Index
μl	=	Microlitre
μU/ml	=	Microunits per millilitre
Vs	=	Versus
Viz.	=	Namely
W <sub>b</sub>	=	Wet bulb

## ABSTRACT

To find out the effect of modified management through mister system during summer and in-house keeping of animals in winter, experiments were conducted on lactating crossbred KF cows and Murrah buffaloes. During hot-dry season, twelve lactating cows were divided in 2 groups of 6 each. Group-I cows (control) were maintained as per routine management practices in loose housing system while group-II (experimental) cows were provided with mist from 11.00 A.M. to 4.00 P.M. During hot-humid season, 12 cows were divided in 2 groups of 6 each and the experiment was carried out as stated during hot-dry season. During winter season, 6 cows (control) were kept in a loose housing paddock while group-II cows (experimental) were kept inside the houses having cemented floor and paddy straw bedding.

Experiments on lactating Murrah buffaloes during early lactation were conducted during 3 seasons. In hot-dry season, 6 Murrah buffaloes were kept under shower system from 11.00 A.M. to 4.00 P.M., while another 6 buffaloes were allowed to wallow in a water pond for the same duration. During hot-humid season, the experiment was repeated with 12 no. of animals as stated above during hot-dry season. During winter season, 6 Murrah buffaloes were kept inside the houses (experimental) while another group (control) was maintained under loose housing system.

From animals of all the experiments conducted in cows and buffaloes, blood samples were collected at an interval of 3 days upto 30 days and the sampling days were defined as periods. In blood samples, plasma glucose, NEFA and urea and hormones viz. plasma  $T_4$ ,  $T_3$ , insulin and cortisol were estimated. Milk samples were also collected on days as defined for blood sampling and milk samples were analyzed for fat, protein, lactose, NEFA, urea and SCC. Physiological responses during different experiments were recorded at 8.00 A.M. and 4.00 P.M. on day of blood sampling.

During hot-dry and hot-humid seasons, availability of mist and fan decreased cortisol ( $P < 0.01$ ) and increased insulin levels ( $P < 0.01$ ). Plasma  $T_4$  level was not influenced during hot-dry and hot-humid season, but  $T_3$  decreased ( $P < 0.01$ ) during hot-dry season. The values of physiological responses - RT, RR, HR and ST were significantly lower ( $P < 0.01$ ) during evening. Plasma

glucose significantly increased ( $P<0.01$ ) and levels of NEFA and urea decreased ( $P<0.01$ ) due to more feed intake ( $P<0.05$ ) by experimental animals in both seasons. Milk yield increased during hot-dry and hot-humid seasons in experimental cows by 10.23 and 8.03%, respectively. The values of fat, protein, lactose, urea and NEFA were influenced ( $P<0.01$ ) by mist and fan during both seasons. However, SCC declined ( $P<0.01$ ) only during hot-humid season. During winter, experimental cows produced 7.41% more milk due to more feed intake ( $P<0.05$ ). Further, plasma  $T_4$  and glucose increased ( $P<0.01$ ) and  $T_3$ , plasma NEFA and urea decreased ( $P<0.01$ ) in experimental cows, however, insulin and cortisol levels were not affected. Physiological responses significantly varied between the groups. In wallowing buffaloes, plasma  $T_4$  and insulin was more ( $P<0.01$ ) and cortisol level was less ( $P<0.01$ ) in comparison to buffaloes subjected to shower system. Wallowing resulted in increased milk production (13.45%;  $P<0.01$ ), feed intake ( $P<0.05$ ) and decreased water intake ( $P<0.05$ ), but SCC of milk was not affected. Milk composition varied ( $P<0.01$ ) between groups in comparison to shower group. Wallowing during hot-humid season resulted in more feed intake ( $P<0.05$ ), increased glucose levels ( $P<0.01$ ) and milk production ( $P<0.01$ ) and a decline in urea concentration ( $P<0.01$ ). The changes in milk composition between the groups were significant\* ( $P<0.01$ ), however, there was no effect on SCC of milk. During winter season, buffaloes kept inside the houses had more  $T_4$  and insulin ( $P<0.01$ ) without any effect on  $T_3$  and cortisol. Further, buffaloes kept inside the houses produced more milk (9.44%;  $P<0.01$ ) without any additional feed or water intake and SCC of milk was similar in both the groups. Milk composition between the groups also varied ( $P<0.01$ ) and the values of physiological responses (RT, RR, HR and ST) were more ( $P<0.01$ ) in the evening than morning. It was concluded that modified management by providing mister system during hot-dry and hot-humid seasons significantly improved milk production performance and was economical. Further, wallowing was found to be more advantageous than showers to augment milk production by improving the physiological responses during extreme hot-dry and hot-humid seasons. Keeping of animals inside the houses during winter season resulted in more milk production from cows and buffaloes.

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## सारांश

इस अध्ययन में पशु-प्रबंधन में मिस्ट (फुव्वारे) तथा पंखों का प्रभाव गायों के दुग्ध उत्पादन पर और भैसों को फुव्वारे एवं तालाब में रखने से हुए दुग्ध उत्पादन पर प्रभाव का अध्ययन किया गया। इसके अलावा सर्दियों में गायों और भैसों को घर में और खुले आवास में रखकर दुग्ध उत्पादन और अन्य पैरामीटर पर प्रभाव देखा गया। ग्रीष्म शुष्क (मई-जून) मौसम में 6 गायों को फुव्वारों व पंखों के नीचे दिन में 11 से 4 बजे तक रखा गया और दूसरे समूह की गायों को खुले आवास में बिना फुव्वारों और पंखों के नीचे रखा गया। शुष्क-आर्द्रता (अगस्त-सितम्बर) वाले मौसम में इसी प्रकार 6 गायों को खुले आवास में पंखों और फुव्वारों के नीचे रखा गया और दूसरे समूह की गायों को खुले आवास में रखा गया। शीत काल में 30 दिन के अध्ययन के दौरान 6 गायों को खुले आवास में एवं 6 गायों को पक्के घर में जिसका फर्श पुआल से ढका था, में रखा गया। इसी प्रकार 30 दिनों तक दुधारू भैसों को भी उपरोक्त लिखित 3 मौसम में अध्ययन किया गया। ग्रीष्म शुष्क में 6 भैसों को फुव्वारों के नीचे दिन में 11 से 4 बजे तक रखा गया और दूसरे समूह की भैसों को इसी दौरान तालाब में रखा गया। ग्रीष्म-आर्द्रता के प्रभाव को जानने के लिये इसी प्रकार का प्रयोग किया गया। शीत काल में 6 भैसों को घर के अंदर पुआल से ढके फर्श में रखा गया।

उपरोक्त लिखित सभी प्रयोगों के पशुओं से रक्त के नमूने 3 दिन के अंतराल (पीरियड) से 30 दिन तक एकत्रित किए गए। रक्त प्लाज्मा में हारमोन थाईरोक्सिन (टी-4), ट्राइआइडोथाईरोनिन (टी-3), इंसुलिन तथा कौरटिसोल, उपापचय (मैटाबोलाइट्स) जैसे कि ग्लूकोज, नेफा (NEFA) तथा यूरिया मापे गए। अध्ययन के दौरान सभी पशुओं से दूध के नमूने रक्त लेने वाले दिन ही लिए गए और इसमें वसा, प्रोटीन, लैक्टोज, नेफा, यूरिया और सोमैटिक सैल काउन्ट (एस.सी.सी) मापे गए। पशुओं की शारीरिक क्रियाएँ जैसे कि शरीर का तापमान, श्वास गति, हृदय गति तथा त्वचा का तापमान भी मापा गया। ग्रीष्म शुष्क तथा ग्रीष्म-आर्द्रता वाले मौसम में फुव्वारों और पंखों में रखने से कौरटिसोल की मात्रा कम ( $P < 0.01$ ) हो गई और इंसुलिन की मात्रा में बढ़ोतरी ( $P < 0.01$ ) पाई गई। लेकिन टी-4 हारमोन की सान्द्रता दोनों ही मौसमों के दौरान अप्रभावित रही। टी-3 हारमोन ग्रीष्म शुष्क मौसम में कम ( $P < 0.01$ ) पाया गया। शारीरिक क्रियाओं की गति व दर फुव्वारों में रखे पशुओं में कम हो गई थी ( $P < 0.01$ )। रक्त में ग्लूकोज की मात्रा में बढ़ोतरी ( $P < 0.01$ ) पाई गई और नेफा और यूरिया की सान्द्रता ( $P < 0.01$ ) पशुओं के ज्यादा चारा खाने ( $P < 0.01$ ) से दोनों ही मौसम में कम पायी गयी। इसके अलावा दोनों मौसमों में प्रयोगात्मक पशुओं को फुव्वारों तथा पंखों में रखने से दूध में क्रमशः 10.23 और 8.03 प्रतिशत की बढ़ोतरी पाई गई। इसके साथ ही वसा, प्रोटीन, लैक्टोज, नेफा और यूरिया ( $P < 0.01$ ) की मात्रा भी प्रभावित हुई। लेकिन आश्चर्यजनक रूप से एस.सी.सी. की संख्या में कमी ( $P < 0.01$ ) ग्रीष्म आर्द्रता वाले मौसम में पाई गई। शीत काल में प्रयोगात्मक पशुओं के दूध में दूसरे समूह की अपेक्षा 7.41 प्रतिशत की बढ़ोतरी ज्यादा चारा खाने ( $P < 0.05$ ) से हुई। प्लाज्मा टी-4 और ग्लूकोज का स्तर ज्यादा ( $P < 0.01$ ) पाया गया और टी-3, नेफा एवं यूरिया ( $P < 0.01$ ) की मात्रा में कमी पाई गई। लेकिन इंसुलिन और कौरटिसोल

हारमोन का स्तर प्रभावित नहीं हुआ। इसके साथ ही शारीरिक क्रियाएँ भी महत्वपूर्ण रूप से दोनों समूहों के पशुओं में प्रभावित हुईं।

भैसों को तालाब में रखने से टी-4 और इंसुलिन हारमोन बढ़े ( $P < 0.01$ ) र कौरटिसोल का स्तर फुव्वारों में रखी गई भैसों की अपेक्षा कम पाया गया। तालाब में रखने भैसों का दूध दूसरे समूह की अपेक्षा 13.45 प्रतिशत ( $P < 0.01$ ) बढ़ा और भैसों ने ज्यादा चा खाया तथा कम पानी पीया ( $P < 0.01$ )। दोनों समूह की भैसों के दुग्ध संगठन में परिवर्तन महत्वपूर्ण रहा ( $P < 0.01$ ) लेकिन एस.सी.सी. की संख्या पर कोई प्रभाव नहीं पाया गया ग्रीष्म-आर्द्रता वाले मौसम में भी भैसों को तालाब में रखने के फलस्वरूप ज्यादा चारा खा ( $P < 0.05$ ) से उनका ग्लूकोज का रक्त स्तर बढ़ा ( $P < 0.01$ ) और दुग्ध उत्पादन में बढ़ोतरी ( $P < 0.01$ ) पाई गई। इसके साथ ही रक्त में नेफा और यूरिया कम ( $P < 0.01$ ) पाए गए लेकिन एस.सी.सी. की संख्या दोनों समूहों में समान रही। शीतकाल के दौरान भैसों को घर के अंदर रखने से टी-4 और इंसुलिन की मात्रा में बढ़ोतरी ( $P < 0.01$ ) पाई गई लेकिन टी-3 और कौरटिसोल प्रभावित नहीं हुए। घर के अंदर रखने से भैसों ने 9.44 प्रतिशत ज्यादा दूध दिया और ज्यादा चारा और पानी ग्रहण किया। इस मौसम में भी दोनों समूहों में एस.सी.सी. की संख्या में बदलाव नहीं पाया गया। शारीरिक क्रियाओं की गति व दर सायंकाल में प्रातः काल की अपेक्षा ( $P < 0.01$ ) अधिक रही। इस अध्ययन से यह निष्कर्ष निकलता है कि ग्रीष्म शुष्क तथा ग्रीष्म आर्द्रता वाले मौसम में फुव्वारों और पंखों में पशुओं को रखने से दुग्ध उत्पादन बढ़ाया जा सकता है जो कि आर्थिक रूप से लाभदायक भी है। इसके अलावा तालाब में भैसों को रखने से फुव्वारों की अपेक्षा ज्यादा आराम मिलता है और दूध में बढ़ोतरी होती है। शीतकाल में भी घर के अंदर रखने से पशुओं की शारीरिक क्रियाओं को सामान्य रखते हुए दुग्ध उत्पादन को बढ़ाया जा सकता है।

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# CHAPTER 1

## INTRODUCTION

# 1. INTRODUCTION

India has acquired the distinction of being the largest milk producing country in the world with a total milk production of 84.1 million tons (Economic Survey, 2002). Buffaloes contribute more than half of the total milk production in our country. The milk production of dairy animals is greatly influenced by the ambient temperature, which above or below the critical levels has a direct adverse effect on physiological processes, and milk production may decrease from 3 to 10% in lactating animals (Collier *et al.*, 1982). Such effects of environment are more pronounced during early lactation of 60 days (McDowell *et al.*, 1976). During this period, high temperature restricts feed intake and the availability of inadequate nutrients for milk synthesis causes a rapid mobilization of body reserves and high losses in body weight (Armstrong, 1994). Conversely, cold temperature stimulate feed intake but less feed energy is available for productive processes, which also affects milk production performance of dairy animals (Young, 1978). During hot-dry and hot-humid seasons, high ambient temperature in conjunction with high humidity adversely affects the animal productivity (McDowell, 1972). The ambient temperature below 8°C during winter decrease milk production owing to more energy requirements to maintain body temperature and milk production. If energy requirement is not adequate the milk productivity is reduced from 2 to 2.5 kg per animal depending upon production potential of the crossbred cows (Singh and Mehla, 1999), however, high yielding cows register more decline. The adverse effect of low or high temperatures are mediated through stimulation of water or appetite centers through nervous stimuli (McDowell *et al.*, 1976).

Physiological responses like rectal temperature, respiration rate and heart rate are markedly changed (Flamenbaum *et al.*, 1986; Omar *et al.*, 1996; Rossi *et al.*, 1998; Prasanpanich *et al.*, 2002). These responses

have been used as a measure of dairy cow comfort and adaptability to an adverse environment or as sensitive physiological measures of environmental modification (Roman- Ponce *et al.*, 1977). In addition the hormonal profile of lactating animals also changes (Johnson, 1974; Habeeb, 1987). Plasma cortisol concentrations have been used as physiological marker of stress. During heat acclimation, there is reduction of plasma cortisol that help the animal in reducing the heat production (Yousef and Johnson, 1967; Stott and Wiersma, 1971). The metabolic hormones such as, T<sub>3</sub>, T<sub>4</sub> and insulin can be used to indicate the metabolic changes in relation to altered feed intake during different seasons. Insulin secretion and action probably change with the decrease in milk production following exposure of dairy animals to environmental stress (Sartin *et al.*, 1985). Thyroid gland is one of the most important correlates of body responses to environment (Aceves *et al.*, 1987).

During the past, attempts have been made in housing and management technologies through which adverse effects of environment on dairy animals can be minimized if not completely eliminated. Despite the availability of information on the effects of environment, however, dairy farmers still have a real problem in selection of appropriate management. The use of water as a cooling agent, through direct showers/mister system on the animal's skin has been reported as excellent technique for reducing heat stress (Lin *et al.*, 1996; Frazzi *et al.*, 1997; Rossi *et al.*, 1998; Lee- ShanNan *et al.*, 1999; Anjuli and Singh, 2003). But to what extent does the management of animals need to be altered? A proper answer requires a rational evaluation of the adverse effects of extreme high or low ambient temperature on animal production and the benefits to be derived from alteration through improving management.

Studies are needed to examine variability in heat tolerance of high producing animals and what possibilities may exist for the intensive

selection programs with these animals. Increased milk somatic cell counts and a high incidence of clinical mastitis in dairy cows occur during hot summer months. Reduction of thermal stress results in a lowered frequency of clinical mastitis than for cows exposed to their natural environment (Roman-Ponce *et al.*, 1981). Research in the areas of increased incidence of clinical mastitis during summer months is needed. Management practices such as sprinkling dairy animals with mister may tend to move more pathogenic organisms to the udder area and may increase incidence of mastitis (Roman-Ponce *et al.*, 1977), this also deserves further investigation. The sequential sprinkling and ventilation cooling system increases milk production during the summer by 1 to 5 kg/day in cows (Spain *et al.*, 1998; Lee-ShanNan *et al.*, 1999) and in buffaloes (Verma and Hussain, 1988; Chauhan, 2004). Cooling may directly influence milk secretion from the mammary gland, but its primary effect is to restore the feed intake (Verma and Hussain, 1988; Flamenbaum *et al.*, 1995; Lee-ShanNan *et al.*, 1999) which can be marked by energy metabolites like blood glucose and NEFA.

The animals could be maintained within a temperature range which is the comfort zone for crossbred cows (65 to 75 °F; Banerjee, 2000). The buffaloes certainly differ in response of the ambient temperature ranges. At high ambient temperature, buffaloes dissipate excess heat effectively through wallowing (Chikamune, 1986) though the innate thermoregulatory mechanism in buffaloes is inadequate compared to cattle (Benzamin, 1982; Thahar *et al.*, 1982). In addition, the black skin of buffalo absorb more radiant heat and thereby more stress (Benzamin, 1982). The available information reveals that scarce attempts have been made to modify the microclimate of buffaloes and information on interrelationship of hormones, metabolites and feed intake in relation to high or low ambient temperatures with or without mister/shower system and wallowing or in-house shelter vs. loose housing is not

available. The present study was therefore undertaken with the following objectives:

1. To determine the circulatory levels of hormones viz.; T<sub>4</sub>, T<sub>3</sub>, insulin and cortisol, blood metabolites (glucose, NEFA and urea) and physiological responses (rectal temperature, respiration rate and heart rate) during extreme hot-dry, hot-humid and cold (winter) seasons under two sets of management
2. To estimate the changes in body weight, milk production and composition (fat, protein, lactose) and somatic cell counts during seasons as in objective 1 and
3. To correlate the data of hormones, metabolites, milk production and composition during different seasons of the study

# **CHAPTER 2**

## **REVIEW OF LITERATURE**

## 2. REVIEW OF LITERATURE

India is a tropical country and in the study zone of north extreme climatic conditions prevail. In summer the atmospheric temperature rises as high as 46°C during daytime and 30°C during the night. The effects of heat rises are further pronounced by increase in day length (13-14 hours). Dairy animals are homeotherms (maintain constant body temperature) and therefore, when the environmental temperature rises or falls abnormally, the animals are subjected to stress.

Severe weather conditions drastically affect milk production. The most remarkable reaction to heat exposure is the depression in feed consumption and the changes in physiological reactions. Thus fewer substances become available for enzymatic activities, hormone synthesis and heat production, which minimizes thermal load (Kamal, 1975). Exposure to severe heat suppresses the production of hormone releasing factors from the hypothalamic center causing a decrease in pituitary hormonal secretion (Johnson, 1974), insulin and possibly thyroxine (Habeeb, 1987). The metabolic pathways slow down, causing drastic impairment of protein utilization due to the shortage of energy substrates, hormones and enzymes, and a significant decrease in dry matter intake, apparent digestibility, volatile fatty acid production (Niles *et al.*, 1980). Under these conditions, the protein synthesis becomes unable to counteract the protein catabolism which leads to a negative nitrogen balance. This destruction in protein tissue is due to increase in glucocorticoid hormones responsible for protein catabolism.

Since temperature, humidity, air velocity, thermal radiation and other climatic factors comprising the microclimate are involved in the heat balance, the milk production and health of animals can be directly related to modification of such factors in adverse climate. The temperature of the ambient or surrounding air about an animal's body is extremely important to its comfort and general functioning of

physiological processes (Kamal, 1975). A rise in humidity of the air interferes with heat loss and increases the stress on the animal. Air movements tend to reduce heat dissipation by increasing the flow of heat from the environment through the skin into the body of the animal. The air movement in fact reduces the insulation of the surrounding air and may also reduce that of the hair themselves. The provision of a favorable microclimate must be matched by good housing, feeding, management and medical care. A review based on available literature is presented below.

## **2.1 Microclimatic Alterations in Shelter Management:**

Microclimate is the climatic conditions directly surrounding the animal. Any microclimatic modification method will necessarily involve a change in the amount of animal heat exchanged by one of the four modes of heat transfer involved in energy exchange between the animal and its climate. In hot climates, the energy exchange can result in a net heat gain if excess heat cannot be dissipated by evaporation of moisture from the skin or respiratory tract. The only alternative is to favorably alter one or more microclimatic factors. Several methods have been successfully used to modify the microclimate. The basic modification for protecting animals from heat stress during the day is the simple shade (or radiation shield). Efficient cooling systems for dairy cattle have been devised based on the latent heat of water evaporated from the cow itself or from the air surrounding the cow. Cooling devices such as zone cooling (Roussel and Beatty, 1970), air conditioning (Thatcher *et al.*, 1974), evaporative cooling (Roman-Ponce *et al.*, 1977) and various shade structures have been used with different success rates to ameliorate detrimental effects of heat stress.

Asbestos roofed loose house produced a moderate microclimate than open (Satya Pal *et al.*, 1973). Berman and Meltzer (1973) observed that in open sided shelter during winter and summer season, at a

maintenance level, a dry cow maintained homeothermy up to 24°C without sweating and up to 40°C at 50% of maximum sweating capacity. For each additional 10 kg daily FCM yield, the ambient temperature at which the animal might maintain homeothermy was reduced by approximately 4°C. Ideal barn temperature for tropics could be 10-27°C (Williamson and Payne, 1974).

Efforts have been made to use air conditioning for dairy cows to improve milk production and reproductive performance (Thatcher *et al.*, 1974). Problems associated with the system included dust filtration, high ammonia concentrations and cost of operation. Evaporative cooling reduces air temperature through the removal of heat required to evaporate water.

Stott and Wiersma (1974) reported that under evaporative cooling system, peak air temperature decreased by 10-12°C than ambient temperature under conventional shade system.  $D_b$  (°C), black globe temperature (°C), dew point temperature (°C) and wind speed (km/hr) in shade and no shade structures were 27.5 and 27.9, 28.4 and 36.7, 23.7 and 23.7 and 7.6 and 7.2, respectively. Black globe temperature was the climatological response that differed among treatments (Roman-Ponce *et al.*, 1977).

Jadhav (1979) reported that minimum temperature, maximum temperature and relative humidity in loose housing during winter varied from 2.5 to 17°C, 14 to 25.8°C and 52 to 100% respectively, while in close housing, the respective values were 9.5 to 18°C, 13.4 to 26°C and 64 to 100%. Various workers reported higher maximum temperature in loose housing and higher humidity percent in hot seasons in loose house than in shed whereas in cold season humidity was similar (Karki, 1981; Mehla, 1982; Singh, 1982) or lower (Razdan, 1965).

Fuquay *et al.* (1979) reported that overhead insulation (In), insulation combined with roof sprinkling (InS) and roof sprinkling (RS) resulted in lower average daily temperature. Although shade structures

reduce the direct solar radiation reaching the animal during the day, they have little effect on ambient temperature and humidity under the shaded area (Buffington *et al.*, 1981).

Valuiskava *et al.* (1981) found that in 4 row brick cowshed unheated and ventilated by natural air flow, microclimate was much better than in other cow sheds of herd (average temperature 9 vs. 7°C, RH 82 vs. 87%, air flow 0.21 vs. 0.25 m/s).

Soepard Jo *et al.* (1982) found that barn microclimate was affected by the housing system especially by relative humidity and air temperature. Conditions inside animal houses were affected to a considerable extent by the meteorological factors of external environment, the construction itself and the animals housed in them (Padmanabhamurty, 1983). When indoor temperature/humidity was compared, it was indicated that it was preferable to keep cows indoor during the day. Central stall being the best because radiant heat load was lower in the center compared to the sides of the barn (Kawanishi and Nagashima, 1983). In individual stalls the maximum and minimum temperatures were 24.5 and 4°C, respectively (Sharma, 1983).

In a comparison between shade with loose housing, it was found that THI in open and loose house was similar giving an indication that thermal load on animal under two conditions may not be varying in hot-humid summer (Bempong, 1983).

Snedelchev *et al.* (1985) compared the naturally ventilated system-loose housing and tethered stall over one calendar year. Temperature variation was more in loose housing. Relative humidity generally exceeded established norms. Plotted theoretical regression curves indicated that internal temperature of dairy cattle housing exceeded 25°C when external temperature was >30°C and fell below 5°C when external temperature was below -10°C.

Singh *et al.* (1985) compared the microclimate variables inside a brick-walled, asbestos-roofed shed and in a loose house system. The levels inside shed and loose house of minimum temperature were 2.5-3.5°C and 1.5-2.5°C higher, the daily maximum temperature was 0.5-9.8°C lower and 0.5-1.6°C higher and the vapor pressure were 3 and 5 mm Hg higher, respectively.

Igono *et al.* (1985) investigated the benefits of spray cooling with 24 Holsteins in mid lactation, which were assigned randomly to two groups of 12 each and maintained under loose housing system. Spray nozzles were installed in the walkways and under the manger shade for the spray treatment group. Maximum temperature and temperature humidity index during the spray study were 27°C and 73.9.

In shaded cubicles and in cubicle with spray and fans, Igono *et al.* (1987) observed that dry bulb temperature averaged 22 and 31°C at 04.00 and 16.00 hr while black globe temperature was 22 and 43°C, respectively. Graves *et al.* (1988) recommended keeping cows in barns with large sidewall and roof opening, shade with fans and water sprinkling system.

Taylor *et al.* (1988) found that effectiveness of evaporative cooling depends on the capacity of the air to take up moisture. Consequently, it was found to be more effective in hot-dry climates (Flamenbaum *et al.*, 1986) than in hot-humid climates.

Thiagarajan and Thomas (1991) reported that housing for cattle in hot-humid tropics should be minimal to allow maximum ventilation and air movement. Ryan *et al.* (1992) compared two cooling systems, and found that evaporative cooling (EC) was better than spray and fan system (SF). Both cooling systems were dependent on EC, each used a different method for removing the heat load from the cows. Both cooling systems were dependent on fans to increase air movement over the surface of the

animals. However, the EC system precooled air by evaporation before it reached hair coat and respiratory system of the cow.

Turushev *et al.* (1992) compared the effect of ventilation system (BSKHI system) over traditional ventilation system on cowshed microclimate. Average monthly temperature in both sheds was within normal limits, but a much wider variation in temperatures was recorded in traditional shed system. Higher level of moisture, ammonia and bacteria were also recorded in the air in traditional shed system.

Armstrong (1994) observed beneficial effects of cooling through shades, ventilation, sprays and fans. Loose sand bedding had an overall positive effect on cow comfort (Niles and Bucklin, 1994). Petersen *et al.* (1985) while comparing stall barn and a free stall barn and a deep-bedded pack barn, found that atmosphere in free stall barn had highest CO<sub>2</sub> content.

Patel *et al.* (1995) studied different housing systems of concrete shed, thatched roof shed and shelters for buffalo heifers and reported that minimum temperature was significantly affected by the housing conditions. But maximum temperature, relative humidity and THI did not differ significantly among housing conditions.

With the advent of modern gadgets, a new cooling system was designed which detects a cow, spray a metered amount of water on the back of each cow, minimizing any over spray that might wet the bedding have been reported by Hillman *et al.* (2000). An ultrasonic transceiver is used to detect the presence of a cow and a single spray nozzle above the cow is used for wetting.

Prasanpanich *et al.* (2002) conducted studies in cows and one group was kept outside without shade and the other group was housed indoors in an open-sided barn. At 14.00 hr D<sub>b</sub> temperature was 29.5°C outdoors and 29.0°C indoors. Black globe temperature was 36.2 and

29.5°C, RH was 76% outdoors and 72% indoors and THI was 79.1 and 78.3 in outdoor and indoor, respectively.

## **2.2 Hormones:**

During prolonged heat exposure, the hypothalamic hormone releasing factors are suppressed which consequently affects the release of pituitary and other hormones (Johnson, 1974).

### **2.2.1 Cortisol levels during summer and winter seasons:**

Plasma cortisol concentrations have been used as a physiological marker of stress. During heat acclimation there is reduction of plasma cortisol that help in reducing the heat production of animals (Yousef and Johnson, 1967; Stott and Wiersma, 1971). An initial increase in cortisol occurs due to acute heat stress followed by a decline after prolonged exposure in cows (Stott and Robinson, 1970; Alvarez and Johnson, 1973). Therefore, the animals adjust physiologically to elevated heat loads by decreasing adrenal corticoid output (Stott and Wiersma, 1971).

Christison and Johnson (1972) observed that moderate heat stress (35°C) increased plasma cortisol levels from 30 to 37 µg/litre, the levels continued to rise for 2 hours after onset of heat exposure and plateaued around 43 µg/litre 2-4 hour after exposure. After 7-10 weeks of mild heat stress, plasma cortisol levels decreased to 25 µg/litre. Abilay *et al.* (1975) reported in Guernsey heifers that cortisol significantly increased to 9.1 ng/ml at 37.5°C from 5.5 ng/ml at 18.2°C (55% RH). Lee *et al.* (1976) reported that the lower levels of plasma corticoids in the hot temperate season suggests that animals were able to adjust physiologically to elevated heat loads by decreasing adrenal corticoid output. Ingraham *et al.* (1979) found plasma corticoid levels in shaded vs. non-shaded cows to be 15.1 and 11.9 ng/ml, respectively. Neuwirth *et al.* (1979) observed that male calves (3-4 weeks old) responded with increased cortisol level at 32.2°C and 60% RH.

Roman-Ponce *et al.* (1981) reported higher cortisol concentration in no shade compared to cows under shade (13.04 vs. 8.72 ng/ml). Wise *et al.* (1988) found that cortisol decreased to 3.0 ng/ml from 6.2 ng/ml in heat stressed Holstein cows (control, 41.7°C, protected, 26.0°C). According to them higher concentration's of this catabolic hormone may have been produced by heat stressed cows to maintain milk production. Glucocorticoids affect protein and lipid metabolism. In liver they have got anabolic action but in skeleton muscles and adipose tissue they have got catabolic activity. Further, they organize the function of the sympathoadrenal system, which helps in managing stress conditions (Hadley, 1988).

Kamal *et al.* (1989) reported no significant effect of cooling by water spraying at 22°C for 2-min. (5 times) periods on cortisol levels in Friesian calves.

Cortisol secretion is regulated by hormonal interactions among the hypothalamus, the pituitary and the adrenal gland. Neural stimuli originate from brain in response to stress. Physical stress can include exercise, cold exposure, burns etc. (Orth *et al.*, 1992).

El-Nouty *et al.* (1989) found lower plasma cortisol levels in buffaloes compared to Holstein cows.

Sarma and Ludri (1989) reported that lactation had no effect on plasma cortisol levels. However, Kumar *et al.* (1993) found low plasma cortisol levels during early lactation phase,  $0.98 \pm 0.15$  ng/ml as compared to mid and late lactation values of  $1.16 \pm 0.13$  and  $1.56 \pm 0.19$  ng/ml, respectively.

Lee *et al.* (1976) studied the effect of temperature+season on cortical function in bovines and it was reported that under cool, intermediate and hot conditions the corticoid levels were 42.3, 36.3 and 22.8 ng/ml respectively.

### **2.2.2 Insulin levels during summer and winter seasons:**

Insulin secretion is inhibited during lactation (Sartin *et al.*, 1985). Moreover insulin secretion of dairy cattle has been reported to differ depending on the physiological state (Sartin *et al.*, 1985) under normal ambient temperatures. Insulin secretion and action probably change with the decrease in milk production following exposure of dairy cows to hot environment.

Chiesa *et al.* (1991) reported that plasma insulin level reaches peak concentrations only after the seventh month of lactation when milk yield decreased and concluded that insulin is mainly influenced by milk production.

Flamenbaum *et al.* (1995) studied the effect of cooling by sprinklers and ventilators in cows and reported insulin levels in cooled and uncooled cows as 10.0 mU/L and 13.2 mU/L, respectively. However, in lactating cows, plasma insulin concentration does not correlate with the utilization of glucose by the mammary gland (McGuire *et al.*, 1995), and the whole body utilization of glucose mostly occurs through non insulin-dependent pathways (Rose *et al.*, 1997).

Jindal and Ludri (1990) conducted studies in 12 lactating animals comprising of six Karan Swiss (KS) cows and six Murrah buffaloes in second and third lactation. At the start of experiment cows and buffaloes had completed on an average 51 and 53 days, respectively in lactation. Insulin concentration during different fortnights in buffaloes varied within narrow limits, whereas in cows the changes were significant. The mean plasma insulin concentration in cows and buffaloes were 10.58 and 9.54  $\mu$ U/ml.

Some researchers have reported increments in insulin concentration in cold exposed heifers (Scott and Christopherson, 1993).

Itoh *et al.* (1997) studied the effect of cold and heat exposure in Holstein heifers on changes in plasma concentration of insulin.

Temperatures were 20°C (thermoneutral), 0°C (cold) and 30°C (hot). Plasma insulin concentration was significantly decreased on day 2, 3, 6, 10 and 16 of heat exposure compared with the thermoneutral environment, and it also decreased on 3<sup>rd</sup> day of cold exposure. There was no significant difference between the respective values for the cold and heat exposure. The decrease in insulin was caused by protein catabolism during heat exposure. Initial response to cold exposure in heifers was related to enhanced sympatho-adrenomedullary activity, which has been shown to inhibit insulin secretion.

### **2.2.3 Thyroidal hormonal levels during summer and winter seasons:**

Thyroid gland is one of the most important correlates of the body responses, to environment. Heat production and body temperature regulation have its effective control through thyroid. There exists seasonal variation in thyroid gland activity, which is related to ambient temperature and humidity conditions (Aceves *et al.*, 1987).

Gomila *et al.* (1976) conducted study on 10 lactating cows and paired them by breed and stage of lactation and assigned randomly into equal groups to study the effect of zone cooling on thyroid activity. They found no significant difference in plasma thyroxine levels even though the means of two groups differed by 2.1 µg/ml probably due to large variation among individual.

Ingraham *et al.* (1979) reported negative correlation of thyroid hormone with THI. The effective thyroxine ratio was 0.915 and 0.918 in shaded and non-shaded cows, respectively. Valtorta *et al.* (1980) reported a decline in plasma T<sub>4</sub> and T<sub>3</sub> concentration at 30°C heat exposure in lactating dairy cows.

Khurana (1983) studied T<sub>4</sub> and T<sub>3</sub> levels in crossbred cows during hot-dry, hot-humid and winter seasons. T<sub>4</sub> levels were 39.10, 41.44 and 42.73 ng/ml during hot-dry, hot-humid and winter seasons, respectively.

Corresponding values of T<sub>3</sub> during the three seasons were 0.99, 0.37 and 1.23 ng/ml.

Scott *et al.* (1983) reported that under simulated diurnal environmental temperatures maximal plasma thyroxine levels were found in the weaning period and concluded that night cooling may be most effective method of alleviating the thermoregulatory limitations of a hot climate on optimal performance of cattle under these conditions.

Aceves *et al.* (1985) studied serum levels of T<sub>4</sub>, T<sub>3</sub> of lactating Holstein Friesian cows at moderately hot (28°C; RH 60%), comfortable (22°C; RH 40%) and at hot conditions (34°C; RH 40%). During moderately hot or comfortable conditions, cows in early lactation exhibited significantly low levels of T<sub>4</sub> and T<sub>3</sub>. During very hot, T<sub>3</sub> concentrations were at their highest during the study for all animals. Thus, during early lactation, circulating levels of T<sub>4</sub>, T<sub>3</sub> resemble the so called 'euthyroid sick syndrome' and that this pattern disappears when animals are subjected to conditions like to produce heat stress suggesting thereby a major role of thyroid hormone metabolism in regulating homeorhetic responses for the maintenance of high priority functions.

Broucek *et al.* (1985) reported that exposure of 5 lactating cows for 72 h at 33°C and RH of 40 to 60% decrease thyroxine levels. Within the thermoneutral zone (18°C; RH 60% to 28°C; RH 40%), lactating animals had significantly lower blood serum T<sub>4</sub> and T<sub>3</sub> than dry animals. Relative increases in T<sub>3</sub> from comfortable to cold or hot environments were significantly higher for lactating than for dry animals (Aceves *et al.*, 1987). They suggested that heat-acclimation in dairy cattle does not depress thyroid gland activity and that adaptive thermoregulatory mechanisms involve peripheral mono-deiodinative pathways of thyroid hormones.

Leftcourt *et al.* (1988) reported T<sub>4</sub> levels to be 69 ng/ml at 20°C and at 33°C, the T<sub>4</sub> levels were decreased to 46 ng/ml due to heat stress.

In a study by Aboulnaga *et al.* (1989), one group of cows was kept outdoors at 22- 35°C with RH 40 to 77%. In cows sprinkled with tap water 5 times daily for 15 days, plasma thyroxine concentration was significantly increased. El-Nouty *et al.* (1989) reported that high yielders generally had low plasma thyroxine and higher plasma triiodothyronine than the low yielders.

Abdel-Samee and Ibrahim (1992) reported that tap water sprinkling of heat stressed lactating Friesian and Holstein cows results in a significant increase in plasma T<sub>3</sub> levels.

Liang-SunYi *et al.* (1996) determined plasma T<sub>4</sub> and T<sub>3</sub> in dairy cattle after administration of (unspecified) heat-stress alleviators. The mean daily cow-house temperature was 30.7±1.33°C and the relative humidity was 75.6%. The T<sub>4</sub> and T<sub>3</sub> levels in the test groups increased by 13.5% and 7.7%, respectively. It was concluded that changes in hormone concentrations should be correlated with the increase in milk yield. Summer season associated decrease in T<sub>3</sub> levels were reported earlier by Marai *et al.* (1997).

Khurana (1983) studied T<sub>4</sub> and T<sub>3</sub> levels in buffaloes during hot dry, hot humid and winter seasons. T<sub>4</sub> levels were 39.10, 37.06 and 45.47 ng/ml during hot dry, hot humid and winter seasons, respectively. Corresponding values of T<sub>3</sub> were 0.87, 0.70 and 1.37 ng/ml. Hassan and El-Nouty (1985) found an increase in thyroid gland activity on exposing the buffaloes to cold conditions. The magnitude of the reduction in thyroid gland activity in summer heat was higher in buffalo than in cows.

Galhotra *et al.* (1988) reported that mean serum T<sub>4</sub> levels in high, medium and low yielding Murrah buffaloes ranged from 16.9 to 50.0, 14.7 to 39.5 and 23.5 to 47.12 ng/ml, respectively.

Duggal *et al.* (1989) reported that thyroxine levels in buffaloes during early lactation was 80.25 ng/ml at 1<sup>st</sup> week postpartum which then declined to 62.85 ng/ml during 2<sup>nd</sup> week and attained a value of 69.50 ng/ml by the end of first month of lactation.

Kumar *et al.* (1991) reported increasing trend in plasma T<sub>3</sub> level and decline in plasma T<sub>4</sub> level from first week to ninth week after parturition in Murrah buffaloes. Further, Kumar *et al.* (1993) found in Murrah buffaloes that the average plasma T<sub>3</sub> varied from 1.35 to 9.99 ng/ml and attained the peak during third and fourth month of lactation.

Broucek *et al.* (1987) studied the effect of extremely low temperature in experimental dairy cows kept in an open house and control cows in an insulated cowshed. The daily temperatures averaged -4.6°C and 2.2°C inside the experimental barn and the insulated shed respectively. Plasma thyroxine level was non-significantly higher in experimental cows than in controls.

### **2.3 Plasma Glucose Levels During Summer and Winter Seasons:**

Blood glucose as the precursor of lactose, is the most important substrate required to maintain milk production since ruminants depend on gluconeogenesis for their glucose requirements. Moreover, energy balance of the animal is also responsible for the changes in the levels (Blum *et al.*, 1973).

Zahor-Honory (1973) reported that glucose content in lactating cows was lowest during peak (44.1-48.1 mg%) and increased (56.3-63 mg%) during mid-lactation. However during lactation glucose was non-significantly different in low and high yielding groups despite the fact that substantially less glucose was required for milk production. Further, concentration of glucose was 18 percent higher in high yielding group in dry state as compared to during early lactation (Hart *et al.*, 1979).

Vasilatos and Wangsness (1981) reported higher plasma glucose levels at 90 days postpartum than at 30 days postpartum. In view of this, plasma concentration of glucose may not reflect actual utilization or availability and these measurements alone do not preclude changes in glucose metabolism. Jindal (1988) reported overall average concentration of blood glucose in cows as 49.83 mg/100ml respectively. In a study by Aboulnaga *et al.* (1989) one group of cows was kept outdoors at 22- 35°C and RH 40 to 77%, and other group of cows were sprinkled with tap water 5 times daily for 15 days. Cooling of heat stressed cows increased greatly their plasma glucose concentration. Abeni *et al.* (1993) found in Holstein Friesian cows that at THI value of > 71, there was significant decrease in glucose and increase in urea levels.

Flamenbaum *et al.* (1995) studied in Holstein cows the effect of cooling by sprinklers and ventilators during summer (June to Oct.) and reported that glucose levels in cooled and uncooled cows were 3.17 and 3.29 mmol/L. Itoh *et al.* (1997) found that plasma glucose significantly decreased after day 3 of heat exposure (30°C) in Holstein heifers. Glucose levels decreased significantly to 69.5 mg/dL under hot conditions (28°C, RH 60%) than at thermoneutral conditions (18°C, RH 60%; Itoh *et al.*, 1998). Chla'dek and Machal (2002) found positive correlation between glucose and milk yield.

Jindal (1988) reported overall average concentration of blood glucose in buffaloes as 47.47 mg/100ml respectively. Yash Pal (1996) reported that glucose levels were more in buffaloes than in cows during early lactation due to differences in milk production. The glucose levels in cows and buffaloes ranged between 37.40 to 42.20 mg/100ml and between 39.88 to 44.30 mg/100ml, respectively. Sharma *et al.* (2002) reported blood glucose of normally lactating cows and buffaloes to be 50 mg%.

Itoh *et al.* (1997) found an increase in plasma glucose concentration in cold exposed (0°C) cows. Plasma glucose concentration's were different between the hot (79.4 mg/dl) and cold (90.5 mg/dl) environments.

## **2.4 Plasma and Milk NEFA Levels During Summer and Winter Seasons:**

NEFA is one of the most sensitive metabolites to environmental stress. The increased NEFA concentration during early lactation in cows suggest mobilization of free fatty acids (NEFA) from adipose tissue due to negative energy balance to meet energy requirements (De Boer *et al.*, 1985).

Plasma NEFA concentrations were in the range of 100 to 2000 µeq/litre in cows and were low in low producing cows (Radloff *et al.*, 1966). Sidhu and Emery (1972) reported increased level of NEFA in lactating cows as compared to non-lactating cows. However, Vasilatos and Wangsness (1981) found no significant differences in free fatty acid (FFA) concentrations between lactation periods in high yielding dairy cows. During early lactation, NEFA concentration increase sharply immediately before feeding and fell back to their normal levels within 1 hour after feeding in high yielding cows (Kunz *et al.*, 1985). However, magnitude of rise in NEFA concentration depends on the degree of energy deficiencies. Flamenbaum *et al.* (1995) observed that cooling of animals does not influence NEFA level and the levels were 0.40 and 0.41 mmol/L in cooled and uncooled cows.

Jindal (1988) reported average plasma level of NEFA in lactating buffaloes to be 0.209 mmol/litre. FFA were significantly ( $P < 0.01$ ) lower in mid and late lactation as compared to early lactation in both cows and buffaloes (Setia *et al.*, 1992).

Yash Pal (1996) reported plasma levels of NEFA to be around 534 to 299 µ mol/l up to day 19 of lactation and declined gradually till day 54.

of lactation in buffaloes to 256  $\mu$  mol/l. Bahga and Gangwar (1992) reported that season of calving also influence blood levels of free fatty acids. NEFA levels were significantly higher in animals parturated in summer compared to those parturated in winter during 6 to 57 days of lactation. Highest values were obtained on day 8 postpartum in both summer and winter seasons (55.38 and 33.81 mg/100ml) which declined consistently with number of days in both the seasons.

Setia *et al.* (1992) reported that plasma free fatty acid concentration in Murrah buffalo during early lactation was 47.38 mg/l. Nangia and Garg (1992) observed increased NEFA concentration in cooler environment in buffaloes.

Itoh *et al.* (1997) reported that plasma NEFA concentration decrease following heat exposure at 30°C in lactating cows and increase after onset of cold exposure (0°C) as compared with thermoneutral zone (20°C). The concentration of plasma NEFA was higher in cold (201.0  $\mu$  eq/l) than in hot (97.6  $\mu$  eq/l) environment in lactating cows.

## **2.5 Plasma and Milk Urea Levels During Summer and Winter Seasons:**

Urea in milk can be derived from the end product of digestion and amino acid catabolism (DePeters and Cant, 1992). Milk urea concentration has been found positively correlated with milk yield (Carlsson *et al.* 1995), whereas, a negative relationship has also been established (Broderick and Clayton 1997). A trend for high milk urea level at the beginning of the lactation was reported by Rajcevic *et al.* (1993).

Improved management through cooling cows also influence urea levels. Fuquay *et al.* (1979) found urea nitrogen levels to be higher in control group (12 mg/100 ml) than in animals which were cooled by inspired air and the cows kept under insulation with roof sprinkling (9 mg/100 ml). Wolfschoon and Klostermeyer, (1981) studied urea

concentration in individual milk samples from 17 Simmental and 15 German Black-and White cows and the mean urea contents were 16.8 and 11.6 mg/100 g. There were significant correlations between breed, stage of lactation and season and the urea content of milk.

Abdel-Samee and Ibrahim (1992) found no significant change in urea levels when the heat stressed lactating Friesian and Holstein cows were cooled by use of tap water sprinklers. Zadnik *et al.* (1993) reported significant positive correlation between urea and protein concentrations and a negative correlation between urea and fat in samples of bulk tank milk. Carlsson and Pehrson (1993) reported significantly higher concentration of urea when the cows were grazing than when they were housed. In herds with very low milk urea concentration while they were housed (<2.4 mmol/l), the mean increase during the grazing season was 2.0 mmol/l whereas, in herds with very high urea concentration while they were housed (>6.0 mmol/l), there was a slight decline in urea concentration during the grazing season.

Flamenbaum *et al.* (1995) reported that urea concentration in cooled cows to be 6.48 mmol/L, while in uncooled cows the urea level was 7.0 mmol/L. Urea concentrations in cow milk differ widely. Milk urea is affected by feed intake and composition. It reflects the balance between dietary carbohydrate and protein and can therefore serve as a feed efficiency indicator. It is also affected by lactation stage and number and body weight. Milk urea is correlated with blood urea and varies over the 24- hr period (Roth *et al.*, 1996).

Zieminski and Juszcak (1997) reported that milk of high and low production herds contained 16.52 vs. 25.55 mg/100ml urea. The highest urea level in summer season was observed in afternoon milked milk (by 6 mg/100 ml higher than in the morning milk). Similarly daily differences were in winter season. The lowest urea content was found in late summer and winter seasons, while in spring they exceeded 30 mg/100

ml. High urea and low protein contents in milk of high yielding cows indicated deficiency of energy at surplus of crude protein in diet.

Whitaker (1998) reported low blood or milk urea levels during early lactation and suggested that more attention to protein balance before calving is needed. Sato (1998) found that milk urea and blood urea were correlated. Milk protein levels were lower in milk samples with significantly lower or higher milk urea. Milk protein levels were also lower in cattle with lower plasma glucose levels. Milk fat levels were not significantly correlated with milk urea or plasma metabolite levels.

Wittwer *et al.* (1999) determined urea in milk and blood samples. They reported that mean urea concentration in milk was highly correlated with blood urea concentration. In milk the urea concentration ranged between 1.5 to 11.6 mmol/l with the highest value found during summer. Hwang *et al.* (2000) reported the reference values for milk protein and milk urea nitrogen (MUN) concentrations to evaluate the feeding status of dairy cattle-

Milk protein %	Low MUN <11 mg/dl	Optimal MUN 11-17 mg/dl	High MUN >17 mg/dl
<3.0	Protein and energy deficiency	Protein in balance and energy deficiency	Protein surplus and energy deficiency
>= 3.0	Protein deficiency and energy in balance or slightly surplus	Protein and energy in balance	Protein surplus and energy in balance or slightly surplus

Spicer *et al.* (2000) reported that milk urea nitrogen was significantly increased between weeks 1 and 3 of lactation. After 3 weeks of lactation milk urea nitrogen did not change and varied between 20 and 22 mg/dl. They concluded that week of lactation should be considered when interpreting milk urea nitrogen values.

The high milk urea during summer months have been reported (Carlsson *et al.*, 1995; Godden *et al.*, 2001). Milk urea was associated with month and season and the concentration was highest from July-September and generally lower in 1<sup>st</sup> lactation cows. Milk urea was lowest during the 1<sup>st</sup> 60 days of lactation, higher between 60 and 150 days in milk, and lower after approximately 150 days in milk (Godden *et al.*, 2001). They reported a negative association between milk urea and both milk fat and total protein percentages.

Johnson and Young (2003) observed that Holstein cows with milk protein percent of >3.2% had lower milk urea compared with cows having milk protein <3.2% for milk yields from 27.3 to 54.5 kg/d and lower than cows having a milk protein <3.0% for milk yield of 54.5 to 63.6 kg/d.

Bhavadasan *et al.* (1982) reported average urea concentration in 20 milk samples of cows and buffaloes as 31.9±9.3 (range, 18.7- 48.4) and 17.3±3.9 (12.0- 26.6) mg/100 ml. Contrary to this, Khan *et al.* (1986) reported higher urea level in buffalo milk (30.4 mg/100 ml). Higher levels of serum urea were reported in summer and spring than in autumn and winter (P<0.05) in Nili Ravi dairy buffaloes (Qureshi *et al.*, 1999). Kavitha *et al.* (2001) reported that the urea levels in buffalo milk varied from 16.83 to 33.70 ng/ml.

Foltys *et al.* (1994) reported effect of season on urea levels and the content of urea were significantly different during winter (11.83 mg/100 ml) and during summer season (20.65 mg/100 ml). Rajcevic *et al.* (1993) reported that content of urea in milk increase in summer (5.24±0.57 mmol/l) than in winter (3.57±0.53 mmol/l).

Hwang *et al.* (2000) reported that milk urea levels in cool and warm seasons were 15.15 and 19.18 mg/dl, respectively.

## **2.6 Physiological Responses:**

Cattle manifest physiological responses in the way of increased body temperature, heart and respiration, when they are kept in hot

environment (Bianca, 1965). These responses have been used as a measure of dairy cow comfort and adaptability to an adverse environment or as sensitive physiological measure of environmental modification (Roman-Ponce *et al.*, 1977).

### **2.6.1 Physiological responses during summer:**

Seath and Miller (1947) reported that increased respiration rate is the first reaction when animals are exposed to environmental temperature above the thermoneutral zone.

Mullick and Kehar (1952) reported higher values for rectal temperature, respiration rate and pulse rate with rise in ambient temperature. Ragab *et al.* (1953) reported beneficial effect of shade on cattle and buffaloes for lowering down the body temperature during summer conditions. Ambient temperature ranging from 20°C to 40°C resulted in an increased rectal temperature (Appleman and Delouche, 1958). Respiration rate continues to rise linearly with increased environmental temperature until it reaches a certain temperature where the rate of increase in respiration rate slows down (Bianca, 1961). Slightest variation in air temperature affects greatly rectal temperature, respiration rate and pulse rate (Rao and Mullick, 1965). A significant correlation exists between rectal temperature and ambient temperature (Pandey and Rao, 1969).

Various breeds of cattle exhibited a diurnal rhythm. Mean morning temperature (38.62°C) and late afternoon (39.19°C) differed significantly (Amakiri and Funsho, 1979). Canton *et al.* (1982) found no effective reduction in rectal temperature with cooled inspired air treatment but a well-designed shade structure was economical than it. Rectal temperatures of Holstein Friesian and Red and White Friesian cows unprotected from sun were higher at 0900 hr and 1500 hr than the cows kept in full or partial shade but there was no significant difference between the partial and fully shaded groups (Cardoso *et al.*, 1983).

Sastry and Georgie (1985) found loose house environment to be better than the barn or shed during night of hot or hot humid conditions.

Lall *et al.* (1987) reported values of physiological reactions during morning were lower than evening during all the seasons. They further reported an increase in physiological responses with rise of ambient temperature and relative humidity. Sastry and Georgie (1988) reported lower heat load in hotter climates in barn type houses than the loose house for lactating cows.

Patel and Dave (1988) reported that rectal temperature and respiration rate during hot-humid season had significant positive correlations with maximum ambient temperature and THI. Evaporative cooling reduced respiration rate and body temperature faster as compared to shade (Taylor *et al.*, 1988) and the stress was lowest in buildings in which temperature fluctuations were lowest (Frazzi, 1989).

In addition to the ambient temperature, wind velocity also affect respiration rate, heart rate and rectal temperature negatively and significantly whereas black globe thermometer readings have significant influence on the respiration rate (Thiagarajan and Thomas, 1991).

Itoh *et al.* (1997) observed a significant increase in respiration rate during heat exposure (88 breaths/min.) at 30°C than at thermoneutral temperature (31 breaths/min.) at 20°C in Holstein heifers.

Prasanpanich *et al.* (2002) found higher values of rectal temperature (40.4 vs. 39.0°C) and respiration rate (87.9 vs. 62.9 breaths per min.) in cows kept outdoors without shade and housed indoors in an open sided barn.

### **2.6.2 Effect of ventilation/water sprinkling on physiological responses during summer:**

Use of water sprinkling was first reported by Seath and Miller (1947) in cows that were exposed to solar radiation from 1200 to 1400 h

and then wetted by a hand sprinkler. The decrease in rectal temperature was greater than in non-sprinkled cows.

Rectal temperature of cows kept under shade was 102.5 and 104.4°F at 6.30 A.M. and 2.30 P.M., respectively however, in sprinkled group of cows the respective values were 101.5 and 102.9°F. Respiration rate was 79 in control group and 58 in sprinkled group of cows at 6.30 A.M. In the afternoon these values were 109 and 90/min., respectively (Morrison *et al.*, 1973).

Gomila *et al.* (1976) found that zone cooling in dairy cows resulted in decline in rectal temperature by 0.31°C and respiration rate by 10.58/min.

Igono *et al.* (1985) observed that rectal temperature following milking was less for spray-cooled cows than control cows (38.8 vs. 39.1°C). Thus, cooling cows by sprinkling and forced ventilation for 15, 30 and 45 min. resulted decrements in body temperature of 0.6, 0.7 and 1.0°C, respectively. When the cows were cooled 5 times per day for 30 min., temperatures were maintained within 38.2 to 38.9°C during the day, which were significantly lower than those not cooled (Flamenbaum *et al.*, 1986).

In an experiment by Huang *et al.* (1986) Holstein Friesian cows (8) were provided with electric fans which operated when the air temperature reached 22°C and sprinklers, which operated (7.5 litres/cow at 30-50 min. intervals) when temperature reached 27°C. Compared with controls ventilated/sprinkled cows had lower average temperature (39.0 vs. 39.6°C) and respiration rate (60.9 vs. 78.5/min.)

A fine mist injected into the air stream from fans mounted in a shade roof provided cooling for the cattle and resulted in respiration rate 16% less in cooled cows (Armstrong *et al.*, 1985). In another study by Armstrong *et al.* (1988) mean milk production was higher in cows cooled

by spray and fans (18.3 kg) or evaporatively cooled under a shade (20.6 kg) as compared to control group cows (15.0 kg).

The effect of mist particles sprayed over the cow's body and evaporated almost immediately by a fan was studied over 3 years in lactating Holstein cows by Aii *et al.* (1989). Body temperature was reduced by 0.9 – 1°C (P<0.05) and respiration rate by 22-25 counts/min. (P<0.01).

Abdel-Samee and Ibrahim (1992) found decreased rectal temperature and respiration rate (P<0.05) by cooling the Friesian and Holstein cows using water sprinklers during summer.

Mishra *et al.* (1995) reported a correlation coefficient of 0.7069 between ambient temperature and pulse rate and of – 0.5640 between relative humidity and pulse rate in crossbred heifers. The effects of bathing the animals and keeping them under shed in summer result in decrease in physiological responses.

Flamenbaum *et al.* (1995) reported that cooling of cows by sequential sprinkling and forced ventilation results in decrease in rectal temperature from 39.7°C to 38.9°C. Omar *et al.* (1996) reported significant decline in rectal temperature (0.7°C), respiration rate (18.7 breaths/min.) and heart rate (8.3 pulses/min.) by cooling the cows with sprinklers and ventilators.

Marai *et al.* (1997) found a decrease in body temperature and respiration rate of HF cows by alleviating heat stress by treatments (i) diuretics + *ad lib* drinking water or (ii) ammonium acetate + spraying with tap water for 5 min. 7 times in during summer. Rossi *et al.* (1998) and Lee-ShanNan *et al.* (1999) also reported a significant decline in rectal temperature and respiration rate in cows given ventilators and sprinklers cooling or automated system of cooling.

By sustaining body temperature close to normal range 38.4 to 38.6°C, Singh and Mehla (1999) observed that the average daily milk

productivity of cooled crossbred cows increase by 2.66 and 2.26 kg in hot-dry and hot-humid season, respectively.

Radadia *et al.* (1980) conducted an experiment in lactating buffaloes and found that the buffaloes exhibited better reductions in respiratory and cardiac rates and body temperature if they were given cooled drinking water between midday than if they were showered with water or if these two treatments were combined. Untreated animals had increased rates.

Gangwar (1985) reported a decrease in rectal temperature of buffaloes provided with showers (1<sup>o</sup>F) and wallowing (2-3<sup>o</sup>F). The respiration rate also decreased by showers (2/min.) and by wallowing (3-4/min.).

Sethi *et al.* (1994) kept the lactating Murrah buffaloes (ambient temperature ranged from 26 to 45<sup>o</sup>C) under 4 shelter types: covered shed with sides closed (group 1), shed and given 2 showers at 1100 h and 1500 h (group 2), tied under shade from 0800 h to 1700 h (group 3) and exposed to direct sunrays and given 2 showers at the above timings (group 4). Group 2 and 3 were more comfortable as was evident from their physiological response. Group 3 exhibited least adverse change in body parameters.

Vijay *et al.* (1996) studied the effect of cooling the buffaloes by splashing water and found significantly higher RR and RT in control group than the treatment group.

Verma *et al.* (2000) reported in buffaloes that rectal temperature displayed a significant linear ( $P < 0.01$ ) increase (up to 38.5<sup>o</sup>C) during summer. Respiration rate showed highly significant positive correlation with ambient temperatures, being highest ( $32.75 \pm 0.23$ /min) during summer evenings.

### **2.6.3 Physiological responses during winter:**

During winter in the morning pulse rate in crossbred and buffalo cows was higher inside the barn than that of animals in loose housing. However, there was no difference in pulse rate in the afternoon between the two group of cows (Thomas *et al.*, 1978).

Itoh *et al.* (1997) observed that on exposure to cold (0°C) the respiration rate was 9 breaths/min. but rectal temperature and heart rate were not affected.

### **2.7 Skin Temperature Changes During Summer and Winter Seasons:**

Brody (1948) gave a series of formulae relating rate of heat transfer by radiation, conduction and convection to differences in skin and environmental temperatures. Other variables being equal, heat loss by radiation and convection increases as the temperature of the skin rises above that of environment. The rate of evaporation of moisture on the skin also increases as the skin temperature rises.

The higher mean skin temperature of the Zebus in comparison with the Jerseys at air temperature above 95°F would be advantageous to them, as long as they were below those for the core of the animals so that heat would not be conducted from the skin to the core. The lower skin temperatures of the Zebu at air temperature below 75°F would again be advantageous, as heat losses would be minimized when heat conservation was important to the animals (Allen, 1962). Cutaneous evaporation is more closely controlled by skin temperature than by rectal temperature (Taneja, 1959).

Schleger and Turner (1965) reported mean skin temperature in Brahman cross cows as 97.3°F at ( $D_b/W_b$ ; 92°C/63°C) and 96.8°C at ( $D_b/W_b$ ; 80°C/50°C).

Joshi *et al.* (1968) reported mean skin temperature of Haryana cattle under test room conditions of cool (18.5°C  $D_b$ , 13.0°C  $W_b$ ) or hot-

humid (40.5°C D<sub>b</sub>, 35.0°C W<sub>b</sub>). The skin temperatures under cool conditions during summer, monsoon and winter were 29.30, 29.11 and 29.27 °C, whereas under hot-humid conditions the respective values were 39.42, 38.98 and 38.48°C. Omar *et al.* (1996) found that cooling by sequential sprinkling and forced ventilation during summer reduced skin temperature by 3.5 and 3.7°C for white and black skin in lactating Friesian cows.

At high ambient temperature, buffaloes dissipate excess heat effectively through wallowing (Chikamune, 1986). The innate thermoregulatory mechanism in buffaloes is inadequate compared to cattle (Benzamin, 1982; Thahar *et al.*, 1982). In addition, the black skin of buffalo absorb more radiant heat and thereby more stress (Benzamin, 1982). In buffaloes skin temperature increased with the rise of intensity of solar radiation and skin temperature was highly correlated with rectal temperature (Das *et al.*, 1997; Anjuli and Upadhyay, 1998).

Chauhan *et al.* (1998) found higher skin temperature in buffaloes kept in closed shed with deep litter system as compared to open shed during extreme cold environment. Further, skin temperature (41.2 vs. 38.2°C) was higher in group outdoors without shade than the cows housed indoors in an open sided barn (Prasanpanich *et al.*, 2002).

## **2.8 Milk Production:**

Optimum milk production usually is obtained within the comfort zone. Even moderate increases in temperature above comfort zone result in measurable decline in milk production. Temperature below comfort zone also has adverse effects on milk production. Reduction in feed intake is largely responsible for the decreased milk production in heat exposed lactating animals.

High yielding cows are more vulnerable to heat stress (Silanikove *et al.*, 1997) and the effect of heat stress is more during early lactation (McDowell *et al.*, 1975; Sharma *et al.*, 1988; Barash *et al.*, 2001). This

would be expected because cows during the first trimester of lactation are either in a negative or near negative energy balance, and decreases in energy intake due to low feed intake would reduce milk production. Cooling can alleviate heat stress and can improve thermal balance and productive performance in dairy cows.

### **2.8.1 Milk production under different management during summer:**

Microclimatic variations had greater effect on milk yield in cow sheds with one open side than the closed cowsheds (Angelksovski *et al.*, 1966). Lower lactation yield, higher fat and butter fat yield was reported by Martin (1970) in loose housed than in closed housed cows.

Zook *et al.* (1975) reported no effect of summer modification in a free stall shelter in dairy cattle on milk yield, fat and protein content.

Ingraham *et al.* (1979) found that milk yield was higher (18.5 vs. 14.5 kg/day) in shaded than in non-shaded cows.

Soepard Jo *et al.* (1982) reported no effect of closed and open barn housing on milk yield of cows, while Wiersma and Armstrong (1985) found modest improvement in milk yield from animals kept under the shaded manger. It was concluded that 50% shade was sufficient and the modification was also economic. Further, composition of feed had about 60% influence on overall milk yield, 20% was due to general health of animals and remaining 20% due to the conditions inside housing (Kuba and Onisko, 1986).

Ludri and Singh (1987) reported that milk production of Karan Fries cows can be sustained by providing *ad lib* green fodder and water during extreme summer. Further, the composition of milk also remained unaffected.

Vermeulen (1988) reported that daily milk yield increased by 10.6% by provision of shade. High environmental temperature had little effect on 122 or 305 day milk yield. The effect in early lactation being less

than in late lactation and a lower milk yield at beginning of following lactation. However, during hot-humid climate the weekly average milk yield was higher but the average fat and protein content was lower in unshaded than shaded cows (Thiagarajan and Thomas, 1991).

Milk yield of cows kept in shady conditions were on an average 5.2% higher than those of cows kept without shade (Korsun, 1993). A negative correlation between milk yield and temperature humidity index was observed (Hsu and Lin, 1994).

### **2.8.2 Effect of ventilation/water sprinkling on milk production during summer:**

Roussel and Beatty (1970) found that daily milk yield of the zone-cooled cows was 19% above that of control cows. The air supply to the cows in the zone cooling was 8.4°C cooler during midday and 3.0°C cooler during midnight, but an increase of 1.34 kg (9.3%) in 4% FCM yield was reported by Thatcher *et al.* (1974) when the cows were kept in an environmentally controlled structure.

Zone cooling was beneficial in relieving some of the depression in milk yield due to high temperature of summer days. There was a significant ( $P < 0.01$ ) increase in milk yield by 1.29 kg/day in lactating dairy cows (Gomila *et al.*, 1976).

Folman *et al.* (1979) observed no significant change in control and forced ventilation group of cows during summer. During hot season cooling with sprinklers was effective even with water temperature of 35°C (Ansell, 1981). Armstrong *et al.* (1985) observed the effect of fine mist injected into the air stream from fans mounted in a shade roof. Control group was placed in corral with conventional shade with 4.6 sq meters shaded space per cow and the other group was in corral with equivalent space under shade with evaporative cooling system during May-September. Milk production was 28.1 kg/day for control and 30.5 kg/day for cooled cows.

Igono *et al.* (1985) conducted a study using sprinklers equipped with water meters installed in the walk ways behind the free stalls and under the shade and feed bunk for cooling the Holstein cows in midlactation during summer. There was an increase in milk yield by 0.70 kg/day than the control cows.

Armstrong *et al.* (1986) reported no significant difference in milk fat content, somatic cell count or 1<sup>st</sup> lactation milk yield among groups of 20 Holstein-Friesian cows kept in an open corral with (i) a conventional shade structure or (ii) evaporative cooling or in tie stalls with (iii) evaporative cooling or (iv) refrigerated cooling. Milk yield was significantly higher in cooled open corral in multiparous cows.

Huang *et al.* (1986) found beneficial effects of ventilation and sprinklers and milk yield was higher (15.0 vs. 14.6 kg/day) in Holstein-Friesian cows. Cows sprayed and provided with fans produced more milk yield (2 kg per day) with lower somatic cell count as compared to controls i.e. shaded cubicles without spray and fans (Igono *et al.*, 1987). Such increase in milk yield by 0.66- 1.90 kg ( $P < 0.01$ ) was also reported by Aii *et al.* (1988) in Holstein cows when sprayed with mist particles over the cow's body and evaporated by fan, the break-even point being an increase of 0.81 kg. Improvement in milk yield by 11.6% with use of sprinklers and fan evaporative cooling system (Strickland *et al.*, 1989).

Aboulnaga *et al.* (1989) found an increase in milk yield by 13.3% in cooled cows by cooling one group of cows sprinkling with tap water 5 times daily for 15 days and the other group being kept outdoors at 22-35°C and RH 40 to 77%. Thus, heat relieving by combination of shades, feed and water offered under shade, ventilation and sprinkler and fan cooling can improve cow's comfort and increased milk production of cows in hot humid climates (Bucklin *et al.*, 1991).

Ryan *et al.* (1992) observed that milk production was higher in cows (0.98 kg/day/cow) kept under the evaporative cooling system as

compared with spray and fans. A significant increase in milk yield was observed by Abdel-Samee and Ibrahim (1992) provided with water sprinklers during summer in cows.

Armstrong (1994) reported increase in milk production by cooling the cows by spray and fans. In high yielding cows (>38.5 kg/d), increase in milk production was 4.0 kg at 40°C. In medium producing cows (29.5-38.5 kg/d) increase in milk production was 3.5 kg/d and in low producing cows (<29.5 kg/d) the increase in milk production was 3.2 kg/d. Flamenbaum *et al.* (1995) reported an increase in milk production by 1.9 kg/d in cows cooled by sprinklers and ventilation during summer (June to October).

The use of ventilators in the housing area resulted in increased ( $P<0.05$ ) milk yield (Calamari *et al.*, 1995). Lin *et al.* (1996) studied the effect of different cooling systems on lactating Holsteins during summer. The cows were in four groups viz. (A) Limited time in the barn with fans (B) In the barn with fans only (C) In the barn with fans and direct spray and (D) In the barn with fans and mister system. They reported 13% increase in feed intake in group C and D and 15.3% increase in milk production.

Omar *et al.* (1996) reported an increase of 15% in milk yield of Friesian cows during summer (July- October) by provision of ventilation and sprinkling. The sprinkling treatment lasted 30 seconds, followed by forced ventilation for 4.5 min. repeated cycles. The cows were cooled in 3 cycles of 15 min. rest period between 10.00 and 14.00 h daily.

Frazzi *et al.* (1997) studied the effect of ventilation with or without sprinkling in Holstein cows and observed improvement of physiological parameters by aeration and water sprinkling. Air speed in control group ranged between 0.1 and 0.2 m/s and in ventilated and sprinkled group it ranged between 0.8 and 1.5 m/s.

Spain *et al.* (1998) studied the effect of fan cooling in 12 mid-lactation Holstein cows. The temperature was raised to 28.5°C from 18°C by 3.5°C/d, fan cooling being introduced on 4<sup>th</sup> day. They observed a decrease in body temperature but milk production and feed intake remained at lower level.

Lee-ShanNan *et al.* (1999) reported an increase by 13% in milk production by cooling between 06:00 and 22:00 h using an automated system in cows. A significant increase in milk production was also reported by Thompson *et al.* (1999) in high producing cooled cows using sprinklers.

Bakniet *et al.* (2000) found that an increase by 1°C environmental temperature caused reduction in milk yield of cows by 0.18 kg /d.

Gangwar (1985) conducted an experiment on three groups of buffaloes viz. control, showers and wallowing during hot dry (Av. temp. 39°C, May- June) and during hot humid (Av. temp. 35°C, RH, 91%, July-September) seasons and found an significant increase in milk production in wallowing group.

Yadav and Gupta (1985) reported that average milk production of lactating buffaloes in three housing conditions (i) conventional tie-barn (ii) open and shed (iii) loose house was 7.57, 7.77 and 8.67 kg, respectively.

Verma and Hussain (1988) conducted experiments in three groups of Murrah buffaloes, control, water application for 15 (group 2) and 20 (group 3) minutes before milking. Milk production in the three groups was 4.27, 5.26 and 6.06 kg/d in control, group 2 and group 3, respectively.

In a study by Chauhan *et al.* (1998) one group of buffaloes was kept under open shed and the other group in a closed shed with deep litter system during extreme cold environment. The milk yield remained unaffected during a 60 days of trial. Chauhan (2004) reported that when the buffaloes were kept 3-4 hrs during day time in a wallowing tank or

were given 3-4 times water splashing during extreme hot conditions, an improvement in milk production occurs.

### **2.8.3 Milk production during winter:**

Brandsma *et al.* (1971) compared the milk production of cows housed in (i) cow kennels (ii) cubicle type cowsheds or (iii) traditional byres. The milk production was not significantly different in all the groups in normal winters.

Flitz-Pries *et al.* (1978) observed that daily 4 percent FCM yield in winter was higher in deep litter housing than loose housing cubicles without bedding.

Andreae (1973) observed the milk production performance per cow of matched groups of 20-25 cows kept in (i) tie up type (ii) cubicle type cowsheds during winter and reported milk yield values of 19.0 and 16.6 kg/day, respectively.

Marai *et al.* (1997) studied the effects of heat stress and cold stress in Friesian Holstein cows 80-130 days postpartum. Experiment (i) was the effect of protection during winter by covering with jute sheets, (ii) methods to alleviate heat stress by giving diuretics + *ad lib* drinking water or ammonium acetate + spraying with tap water for 5 min. (7 times). No effect on milk yield was found by winter protection, however during summer milk yield increased in (ii).

### **2.9 Milk composition during summer:**

Kitchen (1981) reported the values of fat, protein and lactose in normal cow milk as 3.45, 3.61 and 4.5-5.3 %. Roman-Ponce *et al.* (1981) studied the effect of keeping the cows in shade under subtropical climate. The shade structure had dimension of 9.1 x 24.4 m with an insulated metal gable roof and floor of reinforced concrete. They reported no change in fat and protein% of milk of cows kept under shade and without shade.

Foltys *et al.* (1994) reported fat and lactose content of milk between 3.89- 5.15 g and 4.43- 4.78 g per 100 g milk. However, fat and lactose content decreased during summer.

Itoh *et al.* (1998) studied the effect of exposing the lactating cows to hot environments at 28°C. They observed a decrease in milk fat and protein production by heat exposure than at thermoneutral temperature (18°C). Protein percent decreased significantly to 3.01% on heat exposure from 3.22% at thermoneutral environment. Fat and lactose during heat exposure and at thermoneutral environment were 3.37 and 3.31% and 4.78 and 4.97%, respectively. Mena-Guerreno *et al.* (1998) reported lower protein percentage in milk during the hottest months in cows.

The effect of environmental temperature and relative humidity of air on protein and lactose content of milk was studied (Baknik, <sup>*et al.*</sup> 2000) on a farm with 210 black and white cows. During the trial, average environmental temperature varied from 13.5-27.0°C, relative humidity of air from 52.3-87.9% and THI from 57.9 to 75.6. The protein content of milk ranged from 3.19 to 3.42% and lactose content from 4.56 to 4.77%. It was established that 1°C higher environmental temperature caused reduction in protein content by 0.1 g/kg and lactose by 0.07 g/kg milk (Baknik, <sup>*et al.*</sup> 2000).

In studies by Sebastian *et al.* (1972) and Sud and Singh (1974), values of fat, protein and lactose varied from 6.5 to 10.5, 3.3 to 4.24 and 4.6 to 5.6 percent.

### **2.9.2 Effect of ventilation/water sprinkling on milk composition:**

Decrease in milk fat concentration during heat stress has been related to alteration in rumen fermentation. Factors such as milk yield and stage of lactation interact with high temperatures and may determine different responses in cows reared under hot environments. There are conflicting reports on milk fat percentage changes in hot

environment. However, milk fat content decreased when cows were exposed to severe heat stress but milk fat and total solids were not altered by zone cooling the cows (Roussel and Beatty, 1970).

Flamenbaum *et al.* (1995) conducted a study by cooling HF cows using sprinklers and ventilators. Milk fat was 2.32% in cooled cows in comparison to 2.59% in uncooled ones. Cooling significantly increased protein production as a result of increase in feed intake and hence in protein supply from heat stress relief, or from the prevention of protein synthesis in the mammary gland. Protein % in cooled vs. uncooled cow milk was 3.01 vs. 2.88%, respectively. Another factor that may be directly involved in milk synthesis is mammary blood flow, for example a reduction of amino acid transport to the udder may affect synthesis of milk protein. A slight decline in blood flow in midlactation cows has been reported (Lough *et al.*, 1990).

In buffaloes, Rao and Ranganathan (2000) reported the normal values of fat, protein and lactose as  $6.7 \pm 0.07$ ,  $3.65 \pm 0.30$  and  $5.66 \pm 0.09$  percent respectively. Gangwar (1985) studied the milk production in three groups of buffaloes viz. control, showers and wallowing during hot dry (Av. temp.  $39^{\circ}\text{C}$ , May- June) and during hot humid (Av. temp.  $35^{\circ}\text{C}$ , RH, 91%, July-September) seasons and found a significant increase in milk production in wallowing group. Milk fat % in control, showers and wallowing group was 7.59, 7.65 and 7.42% in hot-dry season and 7.97, 7.65 and 7.70% in hot-humid season, respectively. The values for milk lactose were 4.28, 4.44 and 4.58% in the three groups during hot-dry season. The corresponding values in hot-humid season were 4.40, 4.52 and 4.76%. Cooling by spraying water, wallowing and mud plaster on buffaloes for 30 days in summer (June-July) significantly improved physiological responses. Wallowing was found to be the best system for alleviating heat stress (Balakrishnan and Nagarcenkar, 1988).

Verma and Hussain (1988) conducted experiments in three groups of Murrah buffaloes. The treatments were control, water application for 15 (group 2) and 20 (group 3) minutes before milking. Fat was 7.29, 7.33 and 7.32 % in control, group 2 and group 3, respectively. Protein % increased in group 2 (4.16%) and in group 3 (4.17%) from value of 4.04% in control. Milk production in the three groups of buffaloes was 4.27, 5.26 and 6.06 kg/d in control, group 2 and group 3, respectively.

### **2.9.3 Milk composition during winter:**

Fat and protein are generally lowest during hot months and highest during cooler months. These changes are in part compounded by seasonal changes in forage type and availability. Fat percent, total solids and solid-not fat (SNF) are highest during winter. Seasonal variation in SNF is due primarily to variation in protein content of milk (Bauman and Davis, 1974; Larson, 1985).

In the study of Zadnik *et al.* (1995), the model of multiple analyses of variance revealed that milk composition (fat, protein, lactose, urea, somatic cell count) were significantly ( $P < 0.05$ ) influenced by the year, farm, season (winter-summer feed intake) and to a lesser extent by health status of the udder (SCC x 1000/ml).

Chauhan *et al.* (1998) found no change in milk composition in buffaloes kept under open shed and in a closed shed with deep litter system during extreme cold environment.

### **2.10 Somatic cell counts during summer and winter seasons:**

Somatic cell count (SCC) of milk is a quantitative index of inflammatory condition of udder. Several factors like season, geographical region, type of housing, use of teat disinfectants, management, type of feed and teat injury significantly influence SCC in cattle and buffaloes (Vecht *et al.* 1989; Schukken *et al.* 1992). Somatic cell counts are negatively correlated with fat, lactose and milk

production. The hot-humid season increases SCC in milk of dairy cows, mainly due to favorable environment for growth of bacteria. Highest SCC are reported just after calving which decreases up to days 31 to 60 of lactation and then increase slowly to the end of lactation (Sethar *et al.* 1979).

Kennedy *et al.* (1992) observed high SCC shortly after calving due to excessive shedding of epithelial cells as the mammary gland resumes or commences functioning. On an average SCC were recorded to be around  $1.0 \times 10^5$  cells/ml irrespective of time of milking and stage of lactation in buffaloes (Singh and Ludri, 2001). The normal values of SCC in Tharparker, Sahiwal, Karan Swiss and Karan Fries cows in N.D.R.I. herd were found to be  $1.26 \times 10^5$ ,  $1.31 \times 10^5$ ,  $1.54 \times 10^5$  and  $1.61 \times 10^5$  cells/ml, respectively. Milk yield was negatively correlated with SCC during different stages of lactation and parity (Singh and Ludri, 2001).

Increased milk SCC and a high incidence of clinical mastitis in dairy cows occur during hot summer months. Higher incidence of milk SCC's during summer months apparently is from development of clinical mastitis of infected cows from subclinical stage. Reduction of thermal stress by air-conditioning (Thatcher *et al.*, 1974) or shelter management (Roman-Ponce *et al.*, 1981) resulted in a lower frequency of clinical mastitis than for cows exposed to their natural environment. Apparently either heat stress impairs defence mechanisms or else cows in attempting to cool themselves are exposed to greater contact with pathogens (Roman-Ponce *et al.*, 1981).

Lee-ShanNan *et al.* (1999) reported that cooling by automated system reduced SCC of milk in cows and SCC in cooled cows remained within normal average value of  $3.5 \times 10^5$ /ml.

Jyotsna (2000) reported normal values of SCC during early lactation from  $0.54$  to  $0.75 \times 10^5$  cells/ml and use of oxytocin for let down of milk in doses of 2.5 I.U. (i.m.) increases SCC of milk in buffaloes.

## **2.11 Feed and Water Intake:**

Environmental temperature has direct effect on animal energy expenditure and voluntary feed intake. Animals maintain the body warmth by regular feed intake, but under thermal stress reduces feed intake to prevent hyperthermia (Brobeck, 1960). Heat stress is well known to depress feed intake in dairy cows. High temperature restricts feed intake causing a rapid utilization of body reserves and high losses in body weight. During heat stress reduced feed intake is related to the heat increment associated with feed intake (McDowell *et al.*, 1976). There is direct effect of heat stress on hypothalamic feed intake centers, increased gut fill due to reduced rate of passage and increased water intake, and the increase in respiration rate (McDowell *et al.*, 1976).

### **2.11.1 Feed intake changes during summer:**

Woodward (1918) observed that a decrease in atmospheric temperature increased the consumption of roughages more in cows in open than those kept inside the barns. Feed consumption decreased in cows when air temperature increased above 24°C but in animals kept under shade conditions feed intake increase by 9% than those without shade (Korsun, 1993).

Availability of shade associated increases in feed intake in cows than that of unshaded cows was reported by Muller *et al.* (1994). Further, at high ambient temperature (40°C) the feed intake is reduced by 20-40% compared to cows kept in thermoneutral environment (Armstrong, 1994).

Sharma and Singh (2001) found that feed intake was significantly higher in cows managed in loose housing system than closed housing or loose housing with shed.

### **2.11.2 Effect of ventilation/water sprinkling on feed intake:**

Morrison *et al.* (1973) reported significantly higher feed intake (13.64 lb vs. 16.01 lb) when the cattle were sprinkled during summer for

1 min. every 30 min. above temperatures of 27°C compared with cattle kept under shade only. Similar observations of higher consumption of forage and concentrates (17.1 vs. 17.4 kg/d) was found by Huang *et al.* (1986) in cows provided with electric fans which operated when air temperature reached 22°C and sprinklers which operated (7.5 litres/cow at 30-50 min intervals) when temperature reached 27°C.

The availability of manger misting improved dairy cow's appetite, success being greatest using misters angled such that bulk of the mist pattern was on a mid body point of the cow (Schultz and Morrison, 1987). Wiersma and Armstrong (1989) observed that in hot arid region, evaporatively cooled shade reduce the level of stress during rest periods and therefore shade and/or misters at feeding manger may be used to encourage cows to eat more at hotter part of the day. Daily cooling by splashing of water at 10 minutes interval during 12.30 to 14.30 hrs significantly increased dry matter intake and decreased water intake in Kankrej cows (Fulsoundar and Radadia, 1994).

Flamenbaum *et al.* (1995) studied the benefits of direct evaporative body cooling by sequential sprinkling and forced ventilation of high producing dairy cows exposed to a moderately hot and humid environment. They observed that cooled cows consumed 1.6 kg more DM/day and drank 9 litres less of water/day than uncooled cows.

Lee-ShanNan *et al.* (1999) found that cooling by automated system caused a 2.8 kg/day increase in feed intake of cooled cows than the control ones.

Verma and Hussain (1988) observed effect of water application in Murrah buffaloes. The treatments were control, water application for 15 (group 2) and 20 (group 3) minutes before milking. They found an increase by 12% in feed intake.

Vijay *et al.* (1996) studied the effect of cooling of buffaloes by water splashing and found significantly higher water intake in the control group.

### 2.11.3 Feed intake during winter:

D'yakov *et al.* (1967) reported that during winter and spring, feed consumption of cows per kg milk increased by 22.3% in loose houses than in stanchion barns while no difference was noted in the summer-autumn period. It was found that unsheltered animals consumed more dry matter and TDN than sheltered ones. If animals are experiencing cold stress under less protected conditions, they would increase the heat production to maintain homeothermy with a consequent increase in feed consumption (Thomas, 1969). Andreae (1973) concluded that cows utilized better their basal diets and concentrates when kept in tie up type cowsheds than in cubicle type during winter.

Feed intake during winter was reduced with a provision of wind breaks (Pontif *et al.*, 1974). Consumption of feed units/kg gain for loose barn, semi-loose barn, and paddocks as 4.96, 4.82, 4.59 during winter and 6.11, 5.85, 5.61 during spring and 9.71, 10.41, 9.30 during summer respectively (Rusev, 1976). Further, in a study better feed conversion efficiency was found in bedded loose housing system for cows (Konggaard, 1977) and also the cows with shelter tended to convert feed more efficiently (Leu *et al.*, 1977).

Young (1978) reported that feed intake in cows is stimulated by cold but the efficiency of use of dietary energy is reduced. Dry matter intake in crossbred cows was less in closed housing as compared to loose housing in winter season (Jadhav, 1979). Significantly higher feed consumption in heifers of 13-19 months was observed housed loose than in cubicles (Kovalcik and Broucek, 1982).

Wiersma and Armstrong (1989) found no significant increase in dry matter, TDN and net energy in dairy animals exposed to cold, though it was higher for cold exposed animals. Broucek *et al.* (1995) studied effect of low temperature on feed intake of Holstein cows, one group (trial group) was kept in an open free-stall barn and the control group in a

warm enclosed tie-stall barn. During the whole experiment feed intake was higher in dairy cows kept in the open barn, the average daily feed consumption in the animals from the open barn was 35.79 kg, which is 123.1% of the intake of the dairy cows kept in the warm barn. The feed consumption per 1 kg of milk in dairy cows from the open barn was higher by 16.8% compared with the control group from the warm barn.

Ahmed and Amin (1997) reported a decrease in feed intake by 0.24 and 0.06g/kg/hr, respectively for every 1°C increase in ambient temperature in Holstein Friesian and Boran cows. Dry matter intake per kg body weight was significantly different ( $P < 0.01$ ) between seasons, being highest during winter ( $3.63 \pm 0.03$ ) and lowest in the summer ( $2.83 \pm 0.03$ ) and rainy season ( $2.69 \pm 0.03$ ). Dry matter digestibility was also higher in winter as compared to other seasons (Verma *et al.*, 2000).

#### **2.11.4 Water intake:**

The restriction of water impair the milk synthesis of mammary gland during the summer. Water intake slightly decreases during cold stress and rapidly increases during heat stress due to increased water requirement for evaporative heat loss (Hafez, 1968; McDowell *et al.*, 1976). Maximizing water intake should facilitate heat loss to metabolism through increased water turnover (Fuquay, 1978). Although water intake is accepted to be a function of dry matter (Mullick *et al.*, 1952), when the ambient temperature rises above the zone of thermoregulation, it is no more a simple function of the dry matter, but of the ambient temperature also (Mishra *et al.* 1963). The importance of water intake in thermoregulation is evident from the finding that at 93.5°F the ratio of water intake to feed consumption shoots up suddenly (Mishra *et al.* 1963).

Roman-Ponce *et al.* (1981) reported a 19% greater water intake for lactating cows kept under no shade as compared to shade. Thiagarajan

and Thomas (1991) found that unshaded crossbred cows in hot-humid climate drank 21-33% more water.

Korsun (1993) observed that feed consumption decreased for all cows when air temperature were  $>24^{\circ}\text{C}$ , water consumption increased. Flamenbaum *et al.* (1995) reported decrease in water intake by 9 litres in cows provided with sprinklers and ventilators.

Satya Pal *et al.* (1973) observed that water intake in buffaloes increased by 25% during summer (May-June) and that lactating buffaloes require 1 kg extra water for every kg milk production per 100 kg metabolic body size.

## **2.12 Body Weight Changes During Summer and Winter Seasons:**

Lactating dairy cows were exposed to  $31^{\circ}\text{C}$ , placed what remained uneaten at this temperature through a rumen fistula. The energy at higher feed level was directed toward increased body weight gain, resulting in a lower efficiency of feed utilization for milk production (Kibler *et al.*, 1966).

Morrison *et al.* (1973) reported that sprinkling of water of cattle kept under shades during summer in the Imperial Valley of California for 1 min. after every 30 min., when the temperature was above  $27^{\circ}\text{C}$  resulted in higher rate of body weight gain (2.40 lb vs. 3.06 lb) compared with cattle under shade and not sprinkled.

Karki, (1981) studied the effect of three housing systems viz. (a) calves tethered in a shed during day and in the open during night (b) tethered inside shed throughout and (c) kept loose in loose house throughout hot-humid season. They found the highest daily weight gain in group c (550g) followed by group b (455 g), respectively.

Armstrong *et al.* (1985) observed effect of fine mist injected into the air stream from fans mounted in a shade roof for cooling the cattle. Control group was placed in corral with conventional shade with 4.6 sq

meters shaded space per cow and the other group was in corral with equivalent space under shade with evaporative cooling system during May-September. Body weight changes from May-September were - 25 kg in control and + 23 kg in cooled cows.

Huang *et al.* (1986) also reported loss in body weight in ventilated and sprinkled Holstein Friesian cows as compared to control group.

Further, body weight of cows kept in spray and fans (SF) was higher than the cows cooled evaporatively (EC), suggesting that feed intake for the SF cows may have been directed more towards weight gain than toward milk production (Ryan *et al.*, 1992).

Campos *et al.* (1993) reported significantly higher average daily gain in Holstein calves housed in conventional brick building during summer than calves in open shelter.

Body weight at different ages and feed conversion efficiency had overall positive correlation with microclimate variables except THI whereas weight gain had overall negative correlation with microclimate variables except THI (Patel *et al.*, 1995).

Tripathi *et al.* (1972) found that sprinkling of water and provision of shed during summer resulted in faster body weight gain in Murrah heifers as compared to control ones. Sastry *et al.* (1981) reported sixteen percent higher weekly gains in live weight in young buffalo heifers by providing additional shelter and sprinkling of water.

To study the effect of low temperature on body weight of Holstein cows, Broucek *et al.* (1995) kept one group (trial group) in an open free-stall barn and the another (control) group in a warm enclosed tie-stall barn. Cows of control group showed higher body weight during the whole experiment compared to trial group, however the differences were not significant.

### **2.13 Economics of Modified Management:**

Thatcher *et al.* (1974) used air conditioning for promotion of heat loss which was expensive (\$50,000 initial investment for environmentally controlled structure plus \$600 to \$1000 monthly operating expenses). However, net expense diminishes if consideration is given to both lactational and reproductive improvements. They suggested that only part of the herd need to be cooled to maximize returns efficiently. Further, cooling might be considered only for those cows in heavy production that are open. In addition, less expensive cooling systems involving shades, cooled shades, evaporative cooling, sprinklers.

Martens *et al.* (1980) reported that free standing in cubicles with herringbone parlour and slatter floor was most financially worth system.

In an experiment in cows, walls were removed and replaced by steel piping, roof raised from 2.6 m apex to 3.6 m and outside paddock provided with palm thatch shelters. Sprinklers were added for cooling and a satisfactory milk yield was obtained (Ansell, 1981). For herds of 40, 80 or 120 cows, the highest income/man hours was obtained with tie-stalls, followed by deep litter housing and finally cubicle systems. Income was higher in system with controlled natural ventilation than in those with mechanical ventilation and in cubicle systems (Laursen, 1985). Loose housing was recommended for most parts of India, however slight modification of structure or use of extra fitting was often necessary to protect animals during extreme cold or hot seasons (Sastry and Georgie, 1985; Thomas and Sastry, 1991).

Huang *et al.* (1986) managed Holstein Friesian cows (8) in a cowshed without (control) or with electric fans which operated when the air temperature reached 22°C and sprinklers, which operated (7.5 litres/cow at 30-50 min. intervals) when temperature reached 27°C. Calculated daily financial gain from ventilation and sprinkling was almost 1.4 yuan/cow/d.

Similar beneficial effect was reported by Igono *et al.* (1987). An economic analysis using local prices showed a net income of 22 \$/day per 100 cows for 56 day heat period. The costs of extra feed intake, spray water and fan electricity were deducted from income. Vermeulen (1988) found that cooling by air conditioning did not compensate for the cost of equipment by improvement in milk yield and reproduction.

Aii *et al.* (1988) reported that mist and fan system was economic when daily milk yield per cow was > 0.81 kg, during hot-humid summers.

Strickland *et al.* (1989) did the economic analysis of comparison between performances of cooled and uncooled cows, loose housed in an open sided barn in a hot-humid environment indicated that sprinkler and fan cooling system offer advantage in hot-humid climate.

Abadalla and Narendran (1990) observed that to utilize wind power and wetted pads to evaporatively cool dairy cow sheds under hot and arid conditions, cellulose pad gave best cooling followed by 3 layer and 2 layer plastic fiber pad. In another report, Abadalla and Narendran (1991) found high pressure fogging system for cooling open dairy shade in hot and arid conditions as effective. Abadalla and Eknah (1991) recommended wind powered evaporative cooling system.

Lin *et al.* (1996) found that barn with fan and mister system was the best system for cooling the cows during summer as compared to use of showers because water use and run off was less in mister system.

# **CHAPTER 3**

## **MATERIALS AND METHODS**

### 3. MATERIALS AND METHODS

#### 3.1 Location of the Study:

The study was conducted at the livestock farm of National Dairy Research Institute, Karnal which is situated 250 m above mean sea level. The maximum temperature of the city goes up to 46°C during summer and in winter lowest may touch 2°C.

#### 3.2 Selection of Experimental Animals:

The study was conducted on 12 each of lactating healthy crossbred cows (Holstein X Tharparker) and Murrah buffaloes during early lactation of av. 75.50±6.11 and 78.36±8.05 days in cows and buffaloes, respectively. The particulars of the selected animals were as follows:

##### 3.2.1 Details of experimental cows:

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
<b>Hot-dry season</b>					
<b>Group-I (control)</b>					
1.	KF5761	7.9	5	130	26.0
2.	KF5931	6.3	4	156	17.5
3.	KF5979	5.1	4	068	25.0
4.	KF5169	13.4	8	109	22.0
5.	KF6183	4.1	2	034	17.5
6.	KF5881	6.8	4	027	21.5
<b>Average ± S.E.</b>		<b>7.27±1.34</b>	<b>4.50±0.81</b>	<b>87.33±21.57</b>	<b>21.58±1.47</b>
<b>Group-II (experimental)</b>					
1.	KF5788	7.6	5	119	21.5
2.	KF5818	7.4	5	165	21.0
3.	KF5955	6.1	3	085	21.0
4.	KF5889	6.8	4	092	16.5
5.	KF5598	9.1	7	033	26.0
6.	KF5569	8.6	8	026	21.5
<b>Average± S.E.</b>		<b>7.60±0.45</b>	<b>5.33±0.76</b>	<b>86.66±21.5</b>	<b>21.25±1.23</b>

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
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**Hot-humid season**

**Group-I (control)**

1.	KF5808	6.8	4	037	5.5
2.	KF5587	8.5	6	025	8.0
3.	KF6056	4.6	3	027	12.5
4.	KF6157	3.7	1	082	11.5
5.	KF6151	3.7	1	088	10.0
6.	KF6183	3.4	1	107	10.5
<b>Average±S.E.</b>		<b>5.12</b> <b>±0.84</b>	<b>2.67</b> <b>±0.84</b>	<b>61</b> <b>±14.56</b>	<b>9.67</b> <b>±1.04</b>

**Group-II (experimental)**

1.	KF6258	2.7	1	033	7.5
2.	KF6098	4.2	2	023	11.5
3.	KF6211	3.2	1	108	10.0
4.	KF5947	5.4	3	065	11.0
5.	KF6035	4.8	3	092	8.0
6.	KF6071	4.5	2	042	10.5
<b>Average±S.E.</b>		<b>4.13</b> <b>±0.41</b>	<b>2</b> <b>±0.36</b>	<b>60.05</b> <b>±13.92</b>	<b>9.75</b> <b>±0.67</b>

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
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**Winter season**

**Group-I (control)**

1.	KF5672	7.11	3	066	14.0
2.	KF5912	6.0	2	076	21.5
3.	KF5956	5.8	1	066	16.5
4.	KF6117	4.4	1	071	17.5
5.	KF6146	4.1	1	074	22.0
6.	KF6048	6.4	1	086	16.5
<b>Average±S.E</b>		<b>5.64</b> <b>±0.51</b>	<b>1.5</b> <b>±0.34</b>	<b>73.17</b> <b>±3.07</b>	<b>18.00</b> <b>±1.28</b>

**Group-II (experimental)**

1.	KF5886	6.3	1	102	13.0
2.	KF5926	5.1	2	090	19.5
3.	KF6160	3.11	1	043	18.5
4.	KF6086	4.7	1	096	14.0
5.	KF6158	3.11	1	083	19.5
6.	KF6175	3.99	1	092	22.5
<b>Average±S.E.</b>		<b>4.37</b> <b>±0.51</b>	<b>1.17</b> <b>± 0.17</b>	<b>84.33</b> <b>±8.69</b>	<b>17.83</b> <b>±1.48</b>

*[Handwritten signature]*



### 3.2.2 Details of experimental buffaloes:

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
<b>Hot-dry season</b>					
<b>Group-I (control)</b>					
1.	M4479	6.8	3	168	7.0
2.	M4872	3.8	1	046	8.5
3.	M4900	3.8	1	105	6.5
4.	M4774	4.7	1	120	7.0
5.	M4635	5.8	3	199	9.0
6.	M3942	9.8	6	137	8.0
<b>Average±S.E.</b>		<b>5.78</b> <b>±0.93</b>	<b>2.5</b> <b>±0.81</b>	<b>129.17</b> <b>±21.70</b>	<b>7.67</b> <b>±0.40</b>
<b>Group-II (experimental)</b>					
1.	M 4409	7.3	4	168	6.5
2.	M3402	15.0	8	046	8.0
3.	M397	6.3	2	101	10.0
4.	M4772	4.7	1	113	6.0
5.	M4730	4.1	2	196	6.5
6.	M4357	7.8	3	139	7.0
<b>Average±S.E.</b>		<b>7.48</b> <b>±1.60</b>	<b>3.33</b> <b>±1.02</b>	<b>127.17</b> <b>±21.70</b>	<b>7.33</b> <b>±0.60</b>

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
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**Hot-humid season**

**Group-I (control)**

1.	M4373	6.1	3	051	10.5
2.	M5103	5.6	2	059	5.5
3.	M4461	6.0	3	034	6.0
4.	M4721	3.11	2	042	8.0
5.	M4389	6.1	3	046	6.5
6.	M4633	4.11	1	083	7.5
<b>Average±S.E.</b>		<b>5.17</b> <b>±0.43</b>	<b>2.33</b> <b>±0.33</b>	<b>52.5</b> <b>±7.02</b>	<b>7.33</b> <b>±0.74</b>

**Group-II (experimental)**

1.	M4657	4.1	2	071	8.0
2.	M4874	3.1	1	035	6.5
3.	M4455	6.1	3	034	5.5
4.	M4426	6.4	3	032	8.5
5.	M4423	6.5	3	051	6.5
6.	M4695	4.7	2	043	8.0
<b>Average±S.E.</b>		<b>5.13</b> <b>±0.58</b>	<b>2.33</b> <b>±0.33</b>	<b>44.33</b> <b>±6.08</b>	<b>7.17</b> <b>±0.48</b>

Sr. no.	Animal no.	Age (years)	Parity	Stage of Lactation (days)	Milk yield(kg)
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**Winter season**

**Group-I (control)**

1.	M4231	8.2	2	088	8.0
2.	M4570	5.6	1	062	5.5
3.	M4696	2.11	1	062	10.0
4.	M4120	9.0	2	039	11.0
5.	M4730	4.5	1	041	8.0
6.	M 399	6.1	1	108	10.5
<b>Average±S.E.</b>		<b>5.92</b> <b>±1.02</b>	<b>1.33</b> <b>±0.21</b>	<b>66.67</b> <b>±11.04</b>	<b>8.83</b> <b>±0.84</b>

**Group-II (experimental)**

1.	M4417	6.9	2	072	10.5
2.	M4538	5.1	1	063	11.0
3.	M4635	5.3	1	044	11.0
4.	M4710	4.6	1	046	6.0
5.	M4773	4.2	1	048	6.0
6.	M4551	5.9	1	029	10.0
<b>Average±S.E.</b>		<b>5.33</b> <b>±0.39</b>	<b>1.17</b> <b>±0.17</b>	<b>50.33</b> <b>±6.21</b>	<b>9.08</b> <b>±0.99</b>

### **3.3 Classification of Seasons:**

The study was conducted in three seasons viz. hot-dry, hot-humid and winter. The seasons were defined based on last ten year's data of ambient temperature and relative humidity pattern as follows:

- ❖ Extreme hot-dry season (May-June) - Maximum temperature  $>36^{\circ}\text{C}$   
- Relative humidity (25-40%)
- ❖ Extreme hot-humid season (July- August) - Maximum temperature  $>33^{\circ}\text{C}$   
- Relative humidity (70-90%)
- ❖ Extreme winter season (December- January) - Minimum temperature  $<8^{\circ}\text{C}$   
- Relative humidity (35-55%)

### **3.4 Environmental Variables:**

The macroclimatic data for maximum, minimum, dry and wet bulb temperatures, relative humidity, sunshine and wind speed were obtained from the observatory unit of Central Soil Salinity Research Institute, Karnal located at about 1.0 km from the Institute. In addition, microclimatic data of the experimental sheds and of loose housing system (control) were recorded during the seasons as mentioned under section 3.3.

#### **3.4.1 Maximum and minimum temperature:**

During the experimental period of three seasons maximum and minimum temperatures were recorded using maximum and minimum thermometer for both the group of animals kept under two sets of management.

#### **3.4.2 Dry and wet bulb temperature:**

Dry bulb temperature was recorded using dry bulb thermometer and wet bulb temperature from a wet bulb thermometer, whose bulb was covered with a clean absorbent muslin wick. The wick was thoroughly wetted with clean distilled water.

### **3.4.3 Relative humidity:**

Relative humidity was calculated from dry bulb and wet bulb temperatures by using Psychrometric chart (Indian Meteorological Department).

The environmental variables were recorded twice a day i.e. at 8.00 A.M. and 2.00 P.M., as they represent approximately the epochs of maximum and minimum temperature, respectively (Ramdas, 1960). These environmental variables were recorded in the sheds of control and experimental group of animals. During winter the environmental variables were recorded both in sheds and inside the houses.

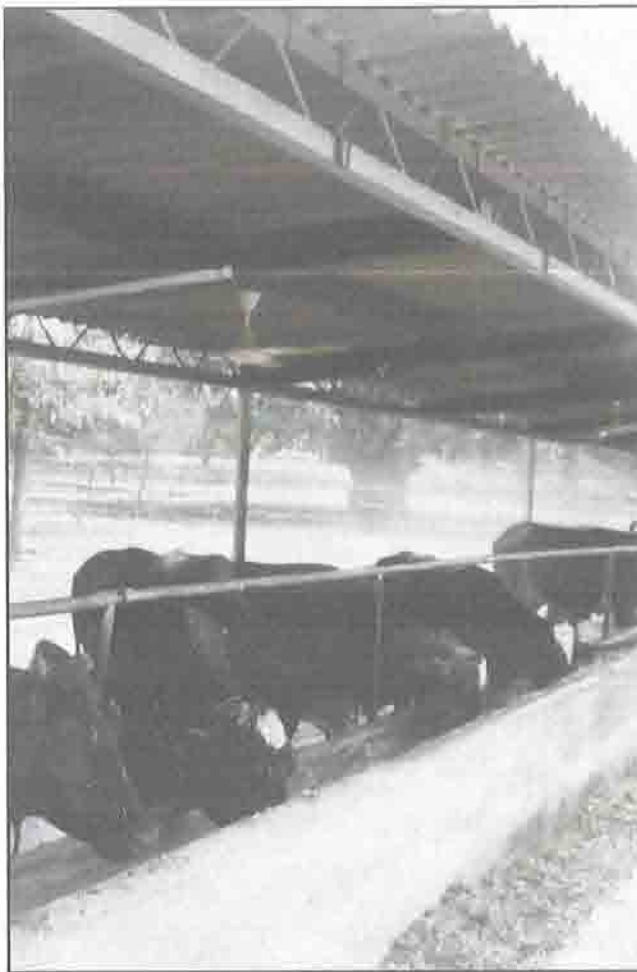
### **3.5 Management of Experimental Animals:**

#### **3.5.1 Management of cows during hot-dry and hot-humid season:**

All the cows were kept under loose housing system with brick floor and were provided *ad lib* green maize (*Zea mays*) fodder and water to drink. The concentrate mixture (72% TDN, 20% CP) was offered based on milk production (@ 1.0 kg/2.5 kg milk yield) and was offered only at the time of milking. The cows were machine milked during morning (6 A.M.), noon (12.00 A.M.) and evening (7 P.M.) and the milk yield of individual cow was recorded at each milking. The experiment was conducted for 30 days during the month of May-June (hot-dry season) and during the month of August- September (hot-humid season). During both seasons, the cows were divided in two groups of six each. Cows of Group-I were kept without mist and fan (control), while Group-II cows were provided with mist and fan from 11.00 A.M. to 4.00 P.M. daily.

#### **3.5.2 Management of cows during winter season:**

For the experiment, 12 crossbred KF cows were selected and divided in two groups of six each. Group-I was kept under loose housing system (control group) as per the management practices followed in the Institute herd. The cows of Group-II were managed separately in pucca



*Photo 1. Cows of experimental group with fan and mister system during summer*



*Photo 2. Cows of control group without fan and mister system during summer*

houses (experimental group) with cement flooring. The floor of the house was covered with paddy straw. The cows were provided *ad lib* green berseem (*Trifolium alexandrium*) fodder and water to drink in both sets of management. The concentrate was offered based on milk production @ 1.0 kg/2.5 kg milk yield during morning (6 A.M.), noon (12.00 A.M.) and evening milking (7 P.M.). The cows were machine milked and the milk yield of individual cow was recorded at each milking.

### **3.5.3 Management of buffaloes during hot-dry and hot-humid season:**

All the experimental buffaloes were kept under loose housing system and were provided *ad lib* green maize fodder and water to drink. The concentrate was offered based on milk production @ 1.5 kg/2.5 kg milk yield during morning (6 A.M.) and evening milking (7 P.M.) as per the management practices. The experiment was conducted for 30 days during the month of May-June (hot-dry season) and during the month of August- September (hot-humid season). During both the seasons, buffaloes were divided in two groups of six each. Group-I buffaloes were kept under showers (control) from 11.00 A.M. to 4.00 P.M while Group-II buffaloes were allowed to wallow in a water pond (experimental) from 11.00 A.M. to 4.00 P.M. The buffaloes were handmilked by the expert milker and the milk yield of individual buffalo was recorded at each milking.

### **3.5.4 Management of buffaloes during winter season:**

Twelve lactating Murrah buffaloes were selected and divided in two groups of six each. Group-I buffaloes were kept under loose housing system (control group) with brick floor while Group-II buffaloes were kept in pucca houses (experimental group) with concrete floor. The floor of house had a bedding of paddy straw which was changed on alternate days. The buffaloes were provided *ad lib* green berseem fodder and water to drink. The concentrate was offered based on milk production @ 1.5

kg/2.5 kg milk yield during morning (6 A.M.) and evening milking (7 P.M.). The buffaloes were handmilked by the expert milker and milk yield of individual buffalo was recorded at each milking.

### **3.6 Collection of Milk Samples:**

Milk samples were collected from cows and buffaloes of both the groups during the experiment at 3 day interval for 30 days and were defined as period 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. The aliquots of milk from A.M., noon and P.M. milking of individual cows for the day were composited in proportion to yield. The analysis of milk in pooled sample of cows was carried out. In case of buffaloes, the aliquots of milk of A.M. and P.M. milking were pooled.

### **3.7 Collection of Blood Samples:**

Jugular blood samples were collected in heparinized vacutainer tubes at 8.30 A.M. from experimental cows and buffaloes at 3 day interval as mentioned under section 3.6 for milk sampling. Blood samples were centrifuged at 3000 rpm for 30 minutes and the plasma was divided into suitable aliquots and stored in deep freezer (- 21°C) till analysis of hormones.

### **3.8 Parameters Observed:**

#### **3.8.1 Physiological responses:**

The Physiological responses viz. rectal temperature, respiration rate, heart rate and skin temperature were recorded at 8.00 A.M. in the morning of both the groups before providing mister cooling to cows of Group-II. Thereafter observations were recorded at 4.00 P.M. from control and experimental cows during hot-dry and hot-humid seasons. In buffaloes, the physiological responses as mentioned above were recorded at 8.00 A.M. in the morning before subjecting them to showers or wallowing in pond and after the treatments i.e. showers or wallowing at 4.00 P.M. during hot-dry and hot-humid seasons.



*Photo 4. Buffaloes of control group under showers during summer*



*Photo 3. Buffaloes of experimental group (wallowing) during summer*



*Photo 5. Inside view of house with bedding during winter*



*Photo 6. Buffaloes kept in houses during winter*

During winter season the physiological responses viz. rectal temperature, respiration rate, heart rate and skin temperature were recorded at same time (8.00 A.M. and 4.00 P.M.) from cows and buffaloes.

### **3.8.2 Skin temperature:**

The skin temperature of all the animals was recorded at trunk region by a non-contact temperature measurement instrument (Raytek, model Raynger ST™). The skin temperature was recorded by keeping the instrument about 6 inches away from the animal's surface directing towards the specific site where temperature was to be measured.

### **3.8.3 Milk yield:**

During the experimental period, milk yield of individual cows and buffaloes was recorded daily for the different sets of the experiments conducted during hot-dry, hot-humid and winter seasons.

### **3.8.4 Body weight:**

Body weight of control and experimental group of animals were recorded on a weigh bridge at weekly intervals during the experimental period in cows and buffaloes during hot-dry, hot-humid and winter seasons.

### **3.8.5 Feed and water intake:**

The amount of feed offered and residue left was recorded on group basis at weekly intervals during different sets of experiments conducted during hot-dry, hot-humid and winter seasons.

### **3.8.6 Economics of modified management during different seasons:**

The cost of modified management inputs like – fan, electricity and water charges for mister/showers in relation to feed intake and milk production was calculated. However, other variables like labor cost etc. remained constant.

### **3.9 Analysis of Blood Metabolites:**

In plasma samples collected during different experiments, NEFA was estimated by extraction method of Shipe *et al.* (1980) and urea by using kits. Glucose was estimated by test kits (GOD/POD method).

### **3.10 Analysis of Milk Samples:**

Milk fat was determined by Milkotester (M-III), protein by formaldehyde method (Singhal and Desraj, 1989) and lactose by picric acid method (Perry and Doon, 1950). Non esterified fatty acids (NEFA) was measured by extraction method (chloroform: heptane: methanol; 49:49:2) of Shipe *et al.* (1980) and the milk urea by dimethylaminobenzaldehyde reagent method of Bector *et al.* (1998). Total solids were estimated by the gravimetric method recommended by the Indian Standards Institution (I.S.I., 1961). Somatic cell count, epithelial cells and TLC of milk was measured microscopically by the method of Singh and Ludri (2001). 10 $\mu$ l fresh milk sample was spread over a glass slide having a marked area of 10 mm x 10 mm using a micropipette. The fine smear so prepared was dried at room temperature for 24 hours. The slide was dipped in xylene for 1 to 2 minutes to remove fat globules and dried subsequently. The slide was then stained using methylene blue dye for a period of 15 minutes and was dried at room temperature. The excess stain was removed from the smears in tap water and the slide was again dried at room temperature. Somatic cell counts were measured under microscope with a magnification of 10 x 40 in 50 fields and were multiplied by microscopic factor (1.1111) to get cells per ml of milk. The microscopic field was determined using ocular and stage micrometer.

Somatic cell counts/ml of milk = Average cell count in 50 fields x 1.1111

## **Composition and Preparation of Stain for SCC:**

### **Composition of dye:**

Methylene blue dye	- 0.6 g
Ethyl alcohol (95%)	- 54 ml
Tetrachloroethane	- 40 ml
Glacial acetic acid	- 6 ml

### **Preparation of dye:**

Ethyl alcohol (54 ml) and tetrachloroethane (40 ml) was mixed in a bottle and heated in a water bath at 60-70°C for 15 minutes. Methylene blue dye was added to the solution carefully and the solution was kept in a refrigerator at 4°C for 30 minutes and then glacial acetic acid was added. The dye solution so prepared was filtered using a filter paper with a pore size of 10 to 12 micron and was stored in a coloured bottle.

### **3.11 Radioimmunoassay of T<sub>4</sub>, T<sub>3</sub> and Insulin:**

Plasma levels of thyroxine (T<sub>4</sub>), triiodothyronine (T<sub>3</sub>) and insulin were estimated by radioimmunoassay method using RIA kits as per the standard procedure specified along with kits supplied by Board of Radiation and Isotope Technology (BRIT), Mumbai.

The intraassay and interassay coefficient of variation for T<sub>4</sub> and T<sub>3</sub> were 8.5, 9.8, 6.7 and 10.2%, respectively. For insulin, the respective values were 7.9 and 11.2%.

### **3.12 Radioimmunoassay of Cortisol:**

Plasma concentration of cortisol was estimated by magnetic immunosorbent method using kits purchased from M/s Stat Diagnostics Services, Mumbai. The procedure of the assay used is briefly described.

**Assay procedure:**

In the assay procedure, 10  $\mu$ l each of standards, control and plasma samples were pipetted in plastic RIA tubes (10 x 75 mm) and 100  $\mu$ l each of tracer (15000 cpm) and antiserum was added to all the tubes followed by vortexing of tubes for 2 min. The content of tubes were incubated at room temperature (24<sup>o</sup>C) for 2 hours and 500  $\mu$ l of magnetic immunosorbent was added after shaking until homogeneity. The tubes were vortexed, incubated for 15 min. and the bound fraction was separated by centrifugation at 3000 rpm for 15 min. The radioactivity in the pellets was counted using a gamma- counter (Model IC4702A). The interassay and intraassay coefficient of variation were 6.72 and 9.40%, respectively.

**3.13 Statistical Analysis:**

Statistical analysis of data was carried out using Least Square Analysis of Variance (ANOVA) with interactions. Mean and standard errors were calculated as per Snedecor and Cochran (1980). The correlations among the various parameters were also found out and the significant values were also tested using Duncan's Multiple Range Test.

# CHAPTER 4

## RESULTS

## 4. RESULTS

The results of various experiments conducted during hot-dry, hot-humid and winter season along with climatic data have been presented in this chapter.

### 4.1 Environmental Variables:

Amongst the environmental variables (macroclimatic) maximum and minimum temperatures,  $D_b$  and  $W_b$  temperatures, relative humidity, wind velocity and sunshine have been presented in tables 1, 2 and 3. Microclimatic variables were recorded viz. maximum and minimum temperatures,  $D_b$  and  $W_b$  temperatures and relative humidity and have been presented in tables 4, 5 and 6.

During hot-dry season (May-June) the maximum temperature reached  $>36^{\circ}\text{C}$  and relative humidity ranged between 25-40%. Maximum and minimum temperatures were higher by  $0.41^{\circ}\text{C}$  and  $0.29^{\circ}\text{C}$  in control shed (without mister) as compared to experimental shed.  $D_b$  and  $W_b$  temperatures in the morning were similar in both the sheds, however, in the afternoon,  $D_b$  temperature of control shed increased by  $1.58^{\circ}\text{C}$ . Net difference in  $W_b$  temperature in the two sheds observed was  $0.35^{\circ}\text{C}$ . Relative humidity was found to be more by 2.18% in experimental shed (Table 4).

During hot-humid season (August-September) the maximum temperature was  $>33^{\circ}\text{C}$  and relative humidity was  $>90\%$ . The overall average maximum temperature during the experimental period was  $33.65$  and  $33.39^{\circ}\text{C}$  in control and experimental shed, respectively. The minimum temperature was  $0.27^{\circ}\text{C}$  higher in control shed. In the morning  $D_b$  and  $W_b$  temperatures were similar in both the sheds, however, in the afternoon overall average  $D_b$  temperature in control and experimental sheds was  $33.5^{\circ}\text{C}$  and  $32.49^{\circ}\text{C}$ , respectively. The net difference in  $W_b$  temperature in both the sheds was lesser ( $0.33^{\circ}\text{C}$ ). Relative humidity was higher in the experimental shed than in control shed (81.1 vs. 79.7%) in

the afternoon. Further, THI was higher in control shed indicating more discomfort (Table 5).

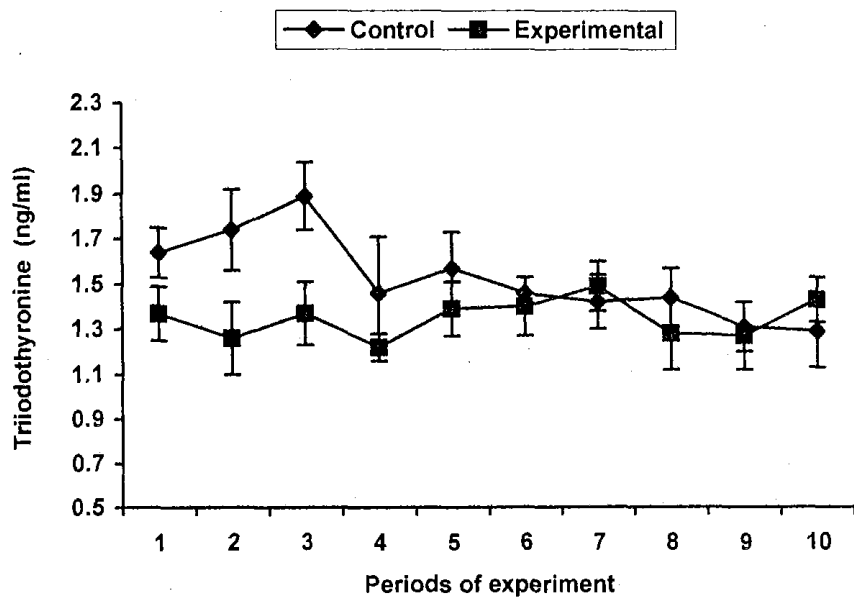
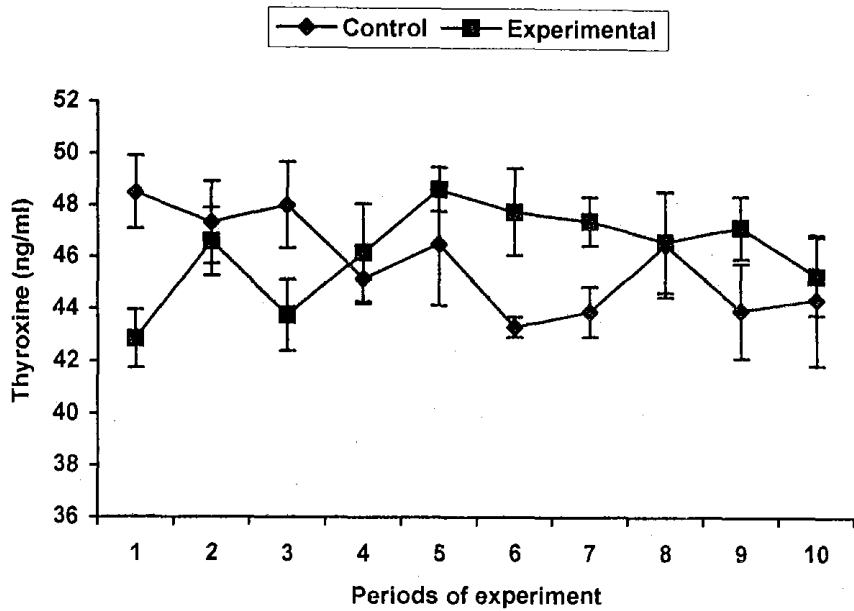
During winter (December- January) experiment was started when minimum temperature reached to  $<8^{\circ}\text{C}$  and the relative humidity ranged between 35-55%. Microclimatic data was recorded during the winter season in open shed and in houses (Table 6). Maximum temperature was  $16.17^{\circ}\text{C}$  and  $18.74^{\circ}\text{C}$  in open and in house, respectively. Mean minimum temperature was lower in open shed indicating that houses were warmer and more comfortable during winter season. The value of THI was 60.60 and 57.68 in-house and in open shed, respectively.

## **4.2 Effect of Mister System During Hot-Dry Season in**

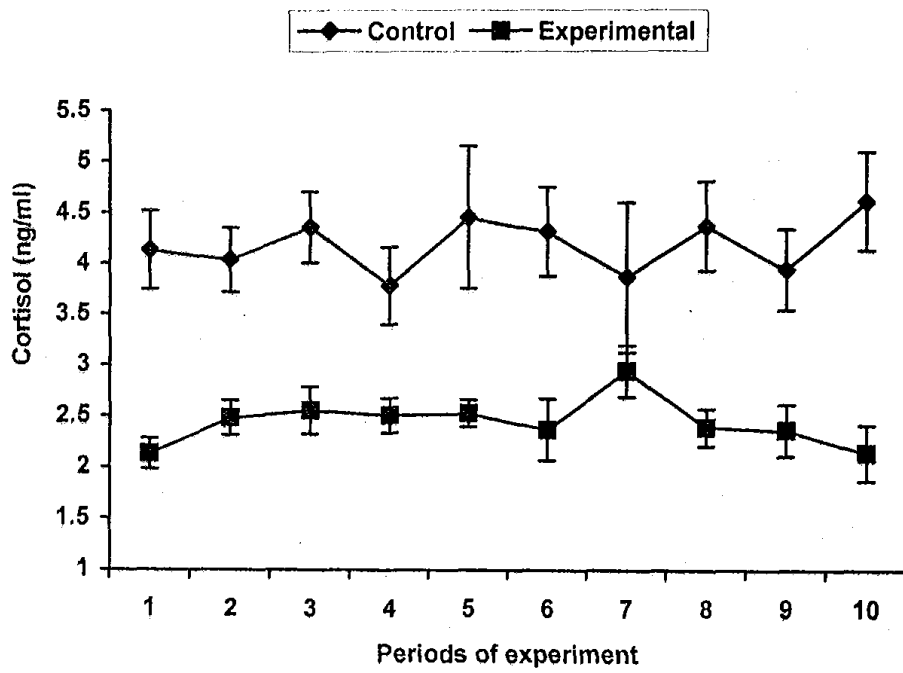
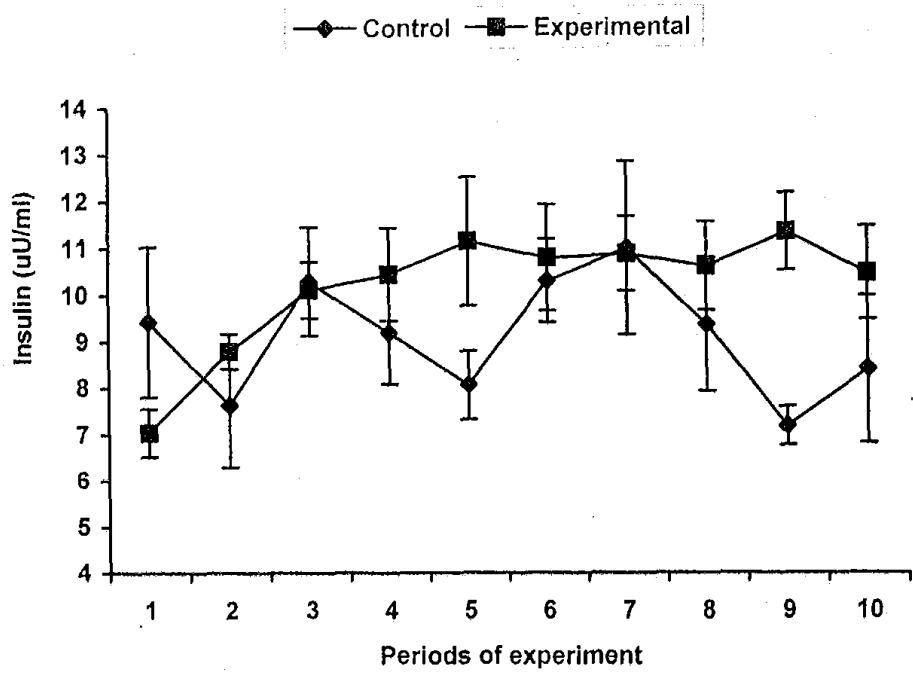
### **Cows:**

#### **4.2.1 Hormones :**

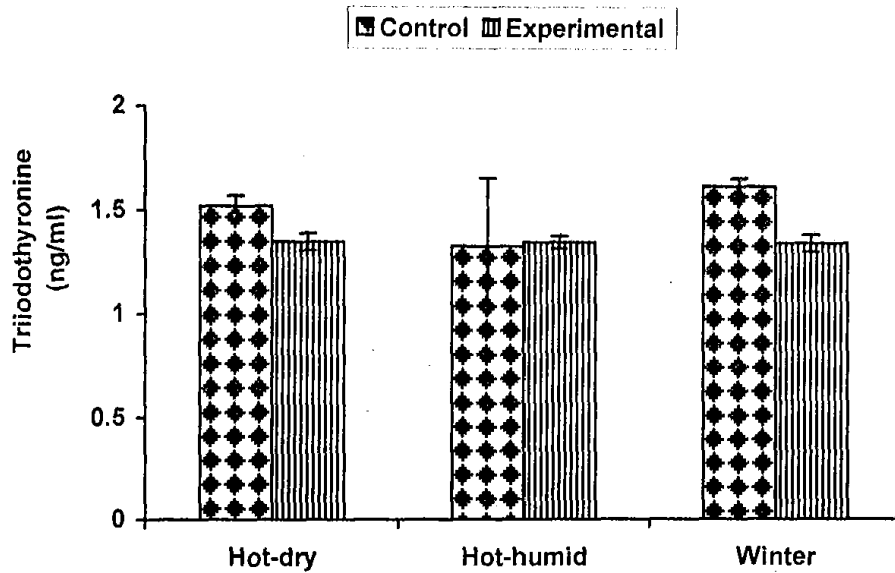
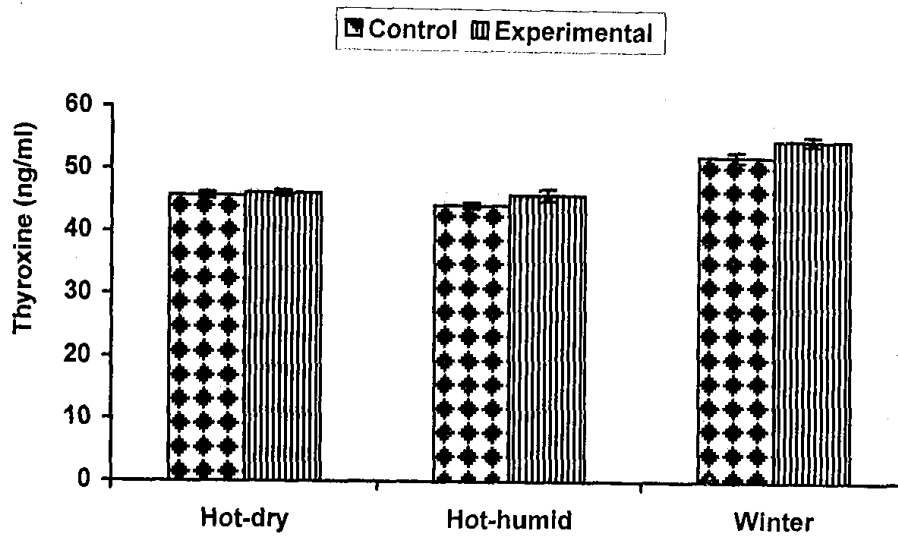
Mean plasma  $T_4$  levels were between 43.33 to 48.50 ng/ml in control group, in comparison to experimental cows (42.85 to 48.68 ng/ml; Figure 1). The overall average  $T_4$  level was  $45.79 \pm 0.54$  vs.  $46.26 \pm 0.47$  ng/ml in control and experimental group of cows, respectively (Table 7; Figure 3). The changes in  $T_4$  levels between the animals during the experiment were significant ( $P < 0.05$ ), however there was no significant difference between the two groups of cows and between different periods (Table 14). Plasma  $T_3$  and insulin levels were non-significantly different between the animals. The overall average value of  $T_3$  was significantly higher ( $1.52 \pm 0.05$  ng/ml) in control than the experimental animals ( $1.35 \pm 0.04$  ng/ml; Table 7). Analysis of variance of the data revealed significant ( $P < 0.01$ ) changes in  $T_3$  and insulin between the groups. Average insulin concentration was higher in experimental than the control group, the respective values were  $10.15 \pm 0.30$  vs.  $9.08 \pm 0.40$   $\mu\text{U/ml}$ . However, there was no significant variation in insulin and  $T_3$  concentration between animals and between periods (Table 14). Plasma cortisol concentration was significantly higher ( $P < 0.01$ ) in control



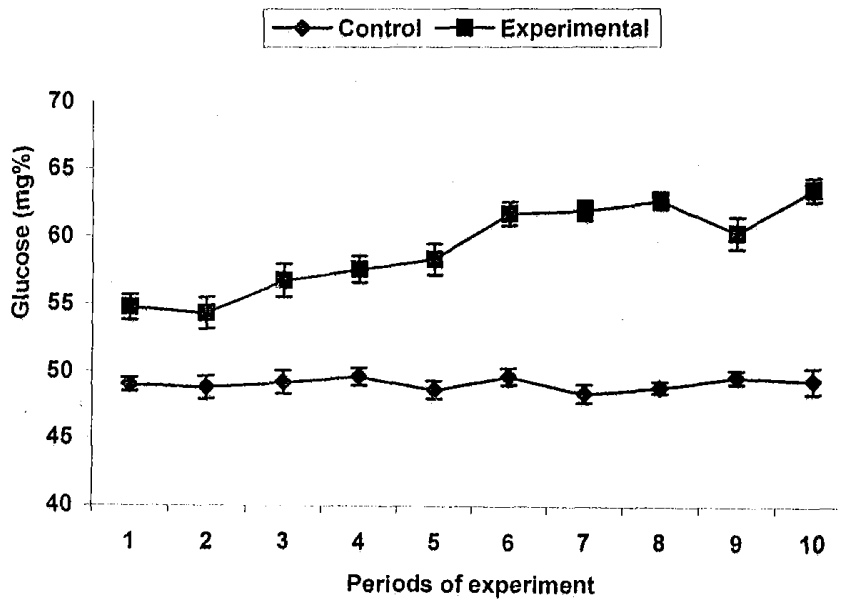
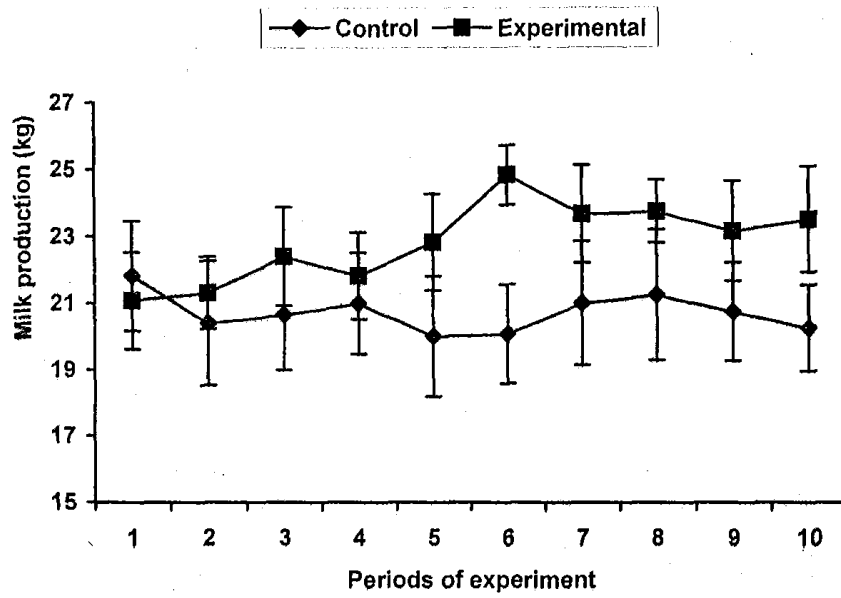
**Figure 1. Plasma thyroxine and triiodothyronine levels during hot-dry season in cows**



**Figure 2. Plasma insulin and cortisol levels during hot-dry season in cows**



**Figure 3. Overall average plasma thyroxine and triiodothyronine levels during different seasons in cows**



**Figure 4. Milk production and glucose levels during hot-dry season in cows**

in comparison to experimental cows during different periods of the study (Figure 2). The overall average value in control and experimental group was 4.17 vs. 2.38 ng/ml (Table 7). The significant changes between the groups were also reflected in significant variation ( $P < 0.01$ ) between the animals (Table 14).

#### **4.2.2 Blood metabolites:**

Plasma glucose, NEFA and urea levels varied ( $P < 0.01$ ) between two groups of cows, however, between animals variation was significant ( $P < 0.01$ ) for NEFA and urea only (Table 14). Plasma glucose increased ( $P < 0.01$ ) in experimental cows provided with mist system and varied from 54.34 to 63.37 mg%. But in control group, glucose levels varied from 48.38 to 49.65 mg%, respectively (Figure 4). Therefore, overall average plasma glucose was significantly higher ( $P < 0.01$ ) in experimental than the control (59.16 vs. 49.11 mg%). The net increase in glucose level was 20.46% in experimental cows. Plasma NEFA and urea decreased ( $P < 0.01$ ) in cows of experimental group. The decline in NEFA concentration was observed from period 2 (day 6) onwards, however in control animals, NEFA concentration increased. Plasma urea also showed similar pattern of change and was lowest by the end of experiment. The overall average values of NEFA and urea were 282.16 vs. 232.00  $\mu\text{mol/l}$  and 67.55 vs. 55.16 mg% in control and experimental groups, respectively (Table 8; Figure 5). Further, interaction of animals  $\times$  groups  $\times$  periods was significant ( $P < 0.01$ ) for plasma glucose, NEFA and urea (Table 14).

#### **4.2.3 Physiological responses:**

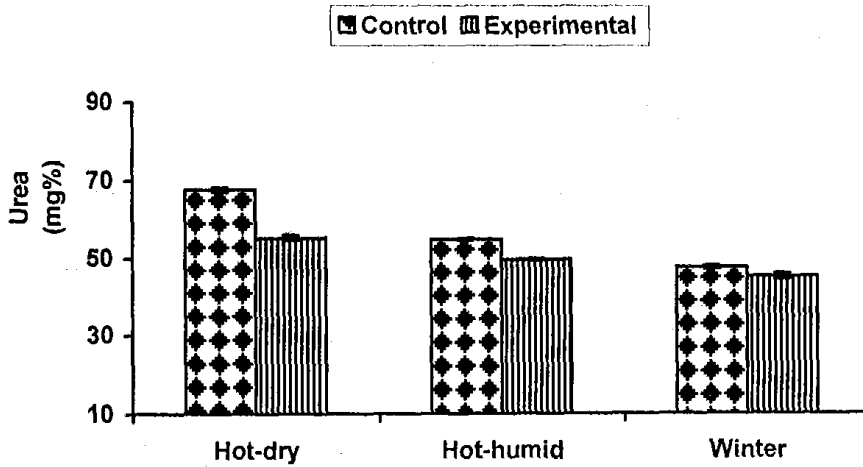
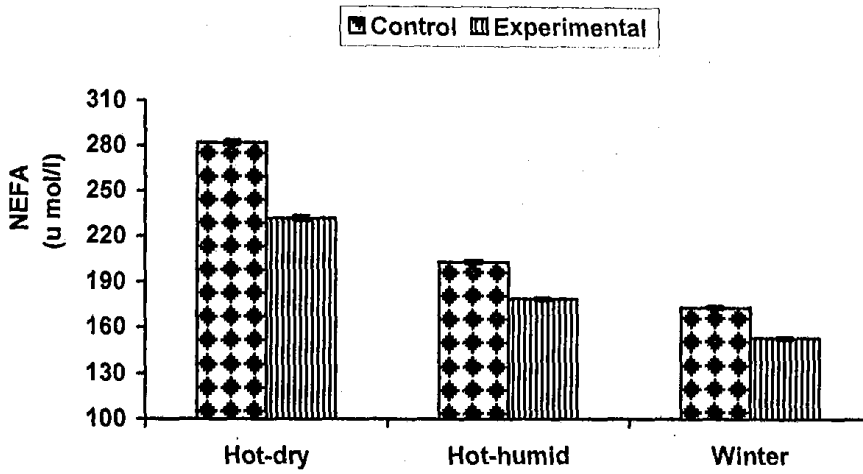
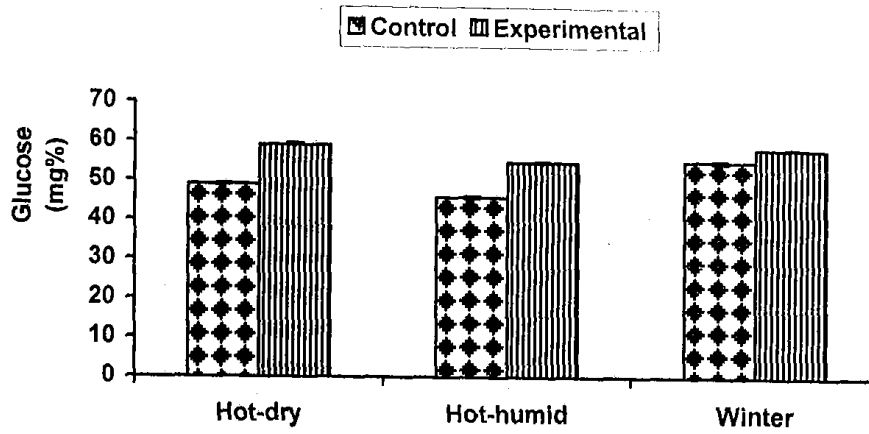
The average RT in control and experimental cows was similar ( $102.34 \pm 0.08^{\circ}\text{F}$  vs.  $102.49 \pm 0.08^{\circ}\text{F}$ ) during morning. RT significantly ( $P < 0.01$ ) increased to  $103.84 \pm 0.08^{\circ}\text{F}$  in the evening in control group, however, in experimental cows RT was maintained ( $102.22 \pm 0.04^{\circ}\text{F}$ ; Table 9 & 10). RT varied ( $P < 0.05$ ) between the cows and during different periods ( $P < 0.01$ ) of the experiment in the morning but varied significantly

( $P < 0.01$ ) between the two groups and periods in the evening (Table 16). Average RR during morning was similar in control and experimental cows ( $32.70 \pm 0.40$  and  $32.06 \pm 0.38$  breaths/min) which further increased to  $45.43 \pm 0.40$  breaths/min. in the evening in control group, however, in experimental group the RR increased to  $40.20 \pm 0.36$  breaths/min. in the evening (Figure 6 & 7). RR varied ( $P < 0.01$ ) during different periods of study in the morning and between the two groups ( $P < 0.01$ ) and between periods in evening, but changes in RR between animals during morning and evening was not significant. An increase in HR from morning values of  $62.70 \pm 0.41$ /min to  $76.43 \pm 0.44$ /min. in evening was observed in control group, however in experimental group HR increased from  $62.40 \pm 0.39$ /min to  $71.76 \pm 0.47$ /min. in the evening (Table 16). The pooled analysis of morning and evening of physiological responses (RT, RR and HR) and ST revealed significant ( $P < 0.01$ ) variation between groups (Table 15). The variations in morning and evening ST of cows was significant ( $P < 0.05$ ) during the different periods of study. Further, ST varied ( $P < 0.01$ ) between two groups of cows and between animals during evening only. ST was  $36.68$  to  $38.20$  and  $39.13$  and  $41.03^\circ\text{C}$  in morning and evening in control group in comparison to  $37.06$  to  $38.85$  and  $36.46$  to  $37.63^\circ\text{C}$  in experimental group (Table 9 & 10). The overall average morning ST was similar in both the groups but in evening ST was more in control than in experimental cows ( $39.87$  vs.  $37.13^\circ\text{C}$ ).

#### **4.2.4 Body weight changes, feed and water intake:**

The body weight of cows was significantly different in both the groups due to the initial differences in the body weight (Table 38 b).

Feed intake during 1<sup>st</sup> week was similar in both the groups, however, it increased during 2<sup>nd</sup> and 3<sup>rd</sup> week of the study in experimental group of cows. In control group, feed intake declined during 2<sup>nd</sup> and 3<sup>rd</sup> week. The overall average feed intake was  $36.76 \pm 1.18$  kg and  $41.59 \pm 0.83$  kg and was significantly ( $P < 0.01$ ) different (Table 38b).



**Figure 5. Overall average plasma glucose, NEFA and urea levels during different seasons in cows**

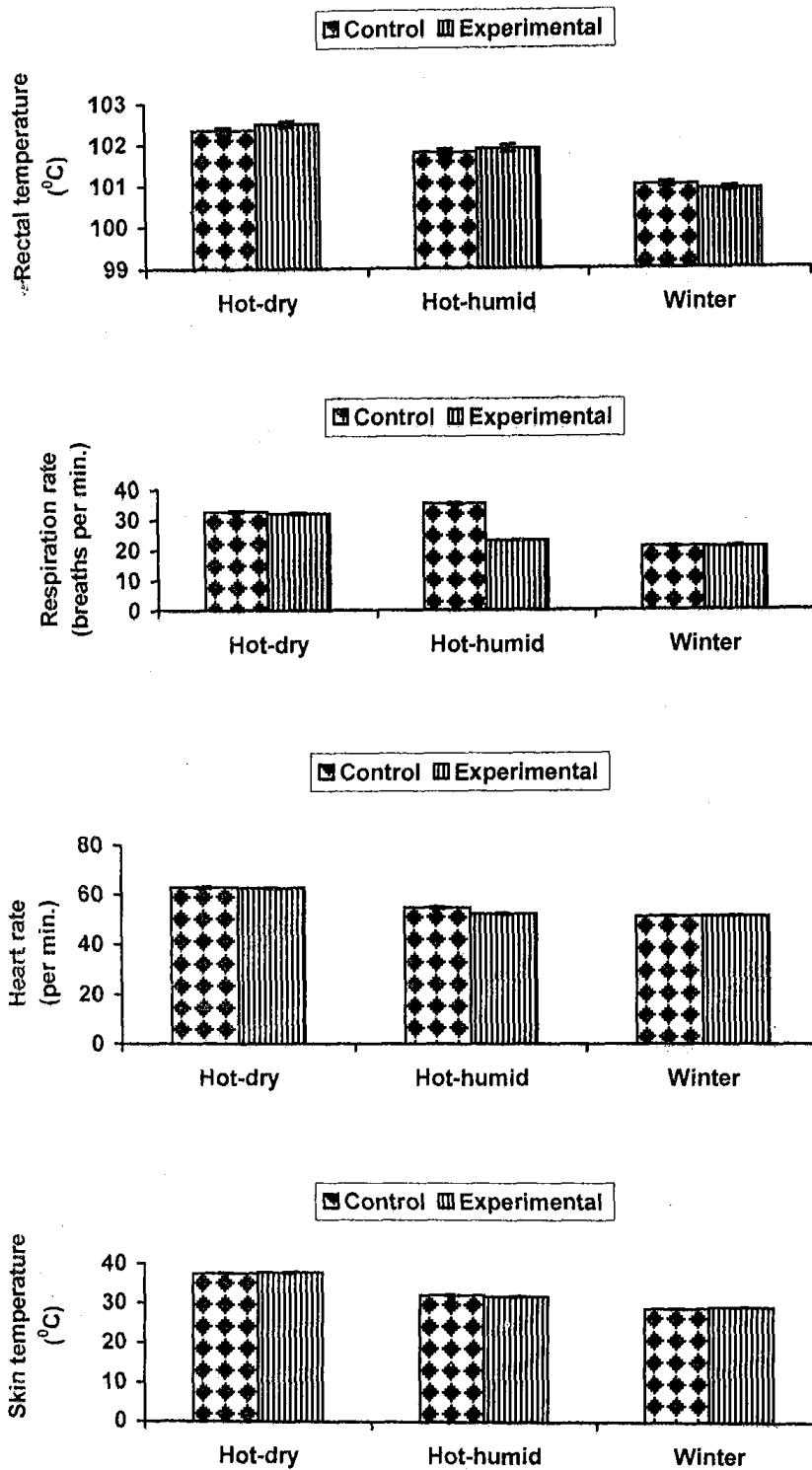
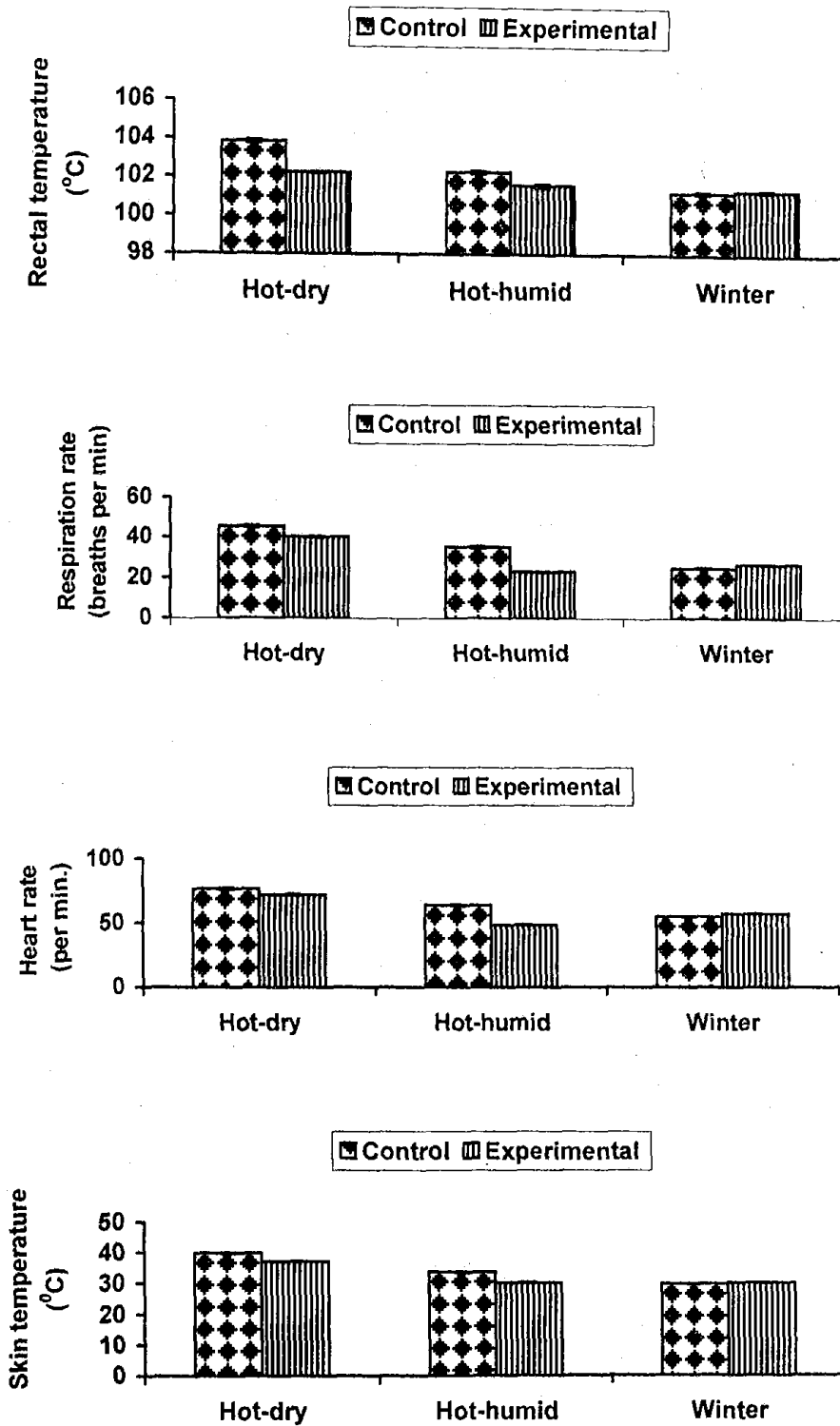
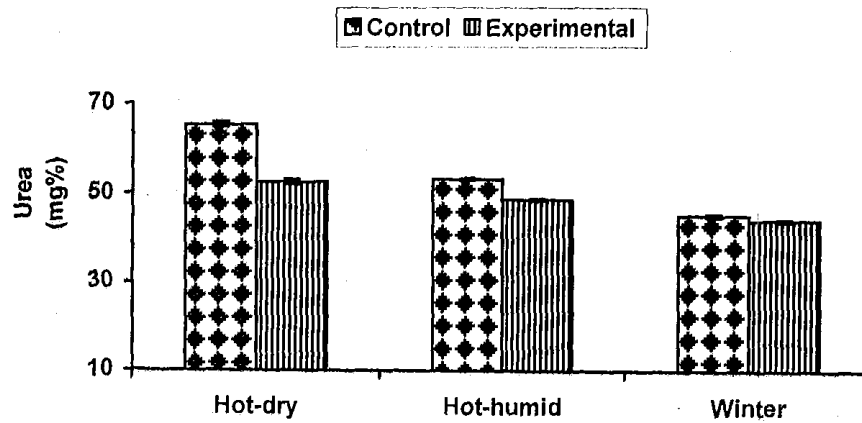
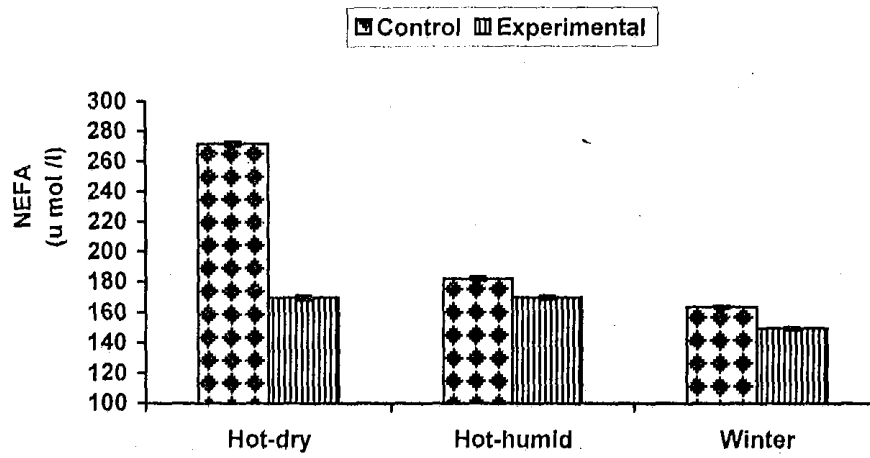
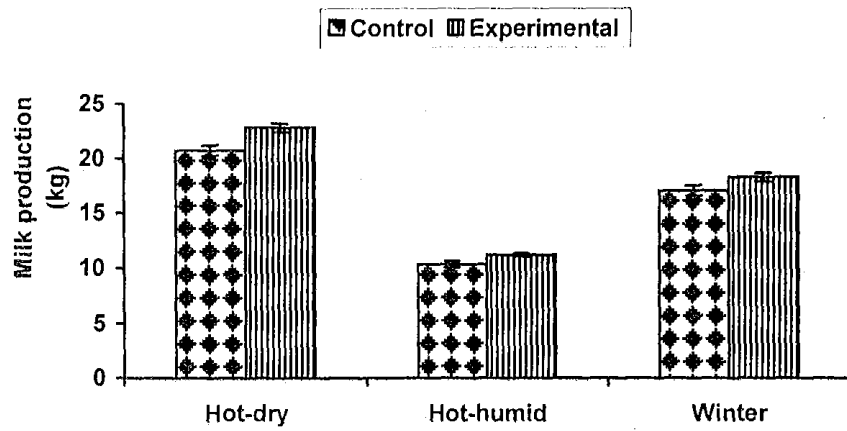


Figure 6. Overall average morning rectal temperature, respiration rate, heart rate and skin temperature during different seasons in cows



**Figure 7. Overall average evening rectal temperature, respiration rate, heart rate and skin temperature during different seasons in cows**



**Figure 8. Overall average milk production, milk NEFA and urea levels during different seasons in cows**

Water intake of cows was similar in both the groups and the intake was decreased during 2<sup>nd</sup> week of study in comparison to control in which water intake was non-significantly higher. The overall average water intake was 58.20 vs. 56.50 litres in control and experimental groups (Table 38b).

#### **4.2.5 Milk production:**

The average values of milk production on day 1 of experiment were  $21.83 \pm 1.65$  and  $21.08 \pm 1.45$  kg/day in control and experimental cows (Table 11). The milk production increased in experimental cows with mist and fan from period 2 of the study but in control, milk production was maintained (Figure 4). Overall average milk production in cows of both the groups was 20.72 vs. 22.84 kg/day, respectively (Figure 8). The changes in milk production between groups and between animals were significant ( $P < 0.01$ ). During the study, experimental cows produced on an average 2.12 kg/day/animal more milk in comparison to control cows. The changes in the milk production between periods and the interaction of animals x groups x periods were non-significant (Table 17).

#### **4.2.6 Milk composition:**

The milk composition data indicated significant ( $P < 0.01$ ) changes in values of fat, protein, lactose, total solids and milk urea between two groups of cows, however, between animals variation was significant ( $P < 0.05$ ) for protein only (Table 17). Because of non-significant changes in milk composition between animals, the milk compositional changes and their interactions were non-significantly different during different days of the study. Total solids, fat, protein and lactose increased ( $P < 0.01$ ) in experimental cows provided with mist and fan. The overall values of fat, protein and lactose were more in experimental than the control cows, the respective values were 4.20 and 3.62, 4.63 and 4.08 and 5.21 and 4.94% (Figure 9 & 10). Fat content of milk was similar in control group during the study but in experimental cows, fat increased from 4<sup>th</sup> period

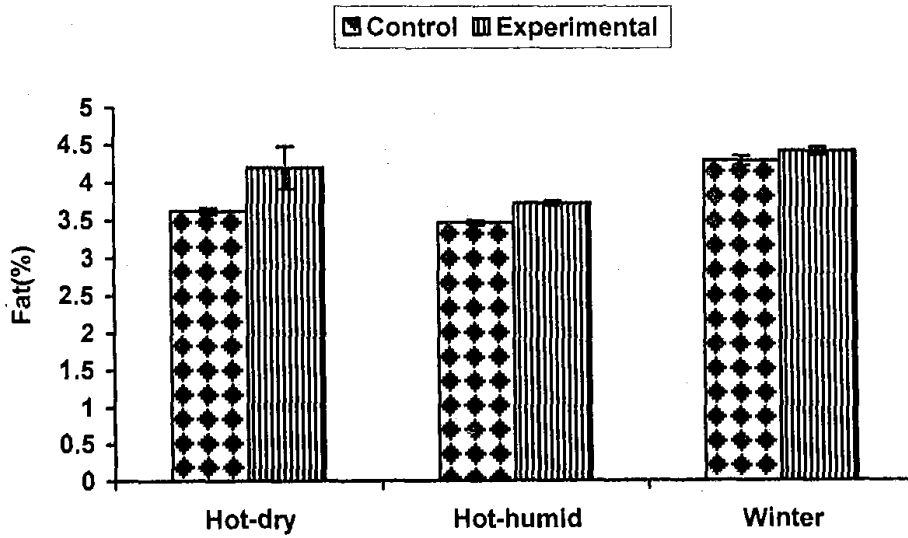
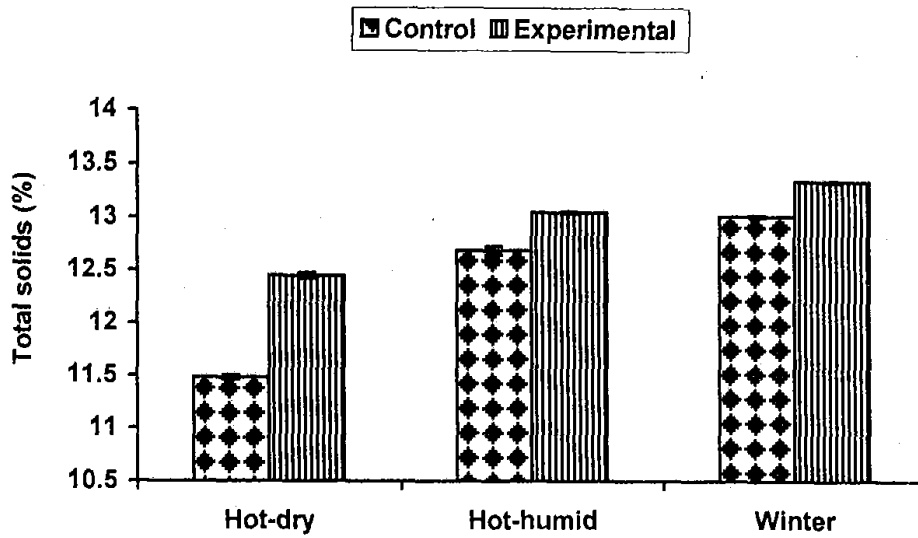
onwards (Table 12). Such effect of mist cooling was evident on protein and lactose of milk from 2<sup>nd</sup> and 6<sup>th</sup> period of experiment. The net increases in fat, protein and lactose were 16.02, 13.48 and 5.46%, respectively. These increases were due to more milk production in experimental cows (by 10.23%). Milk NEFA concentrations significantly ( $P<0.01$ ) decreased in experimental group and overall average NEFA in control and experimental cows was 271.58 vs. 169.58 $\mu$  mol/ l (Table 11). The higher levels of milk NEFA concentration in control cows was observed during the study period. But in cows provided with mist, NEFA decreased gradually and was lowest at the end of the experiment. The overall average values of urea significantly declined ( $P<0.01$ ) in experimental cows than the control, the respective values were 52.55 vs. 65.28 mg% (Table 11; Figure 8).

#### **4.2.7 Somatic cell count (SCC):**

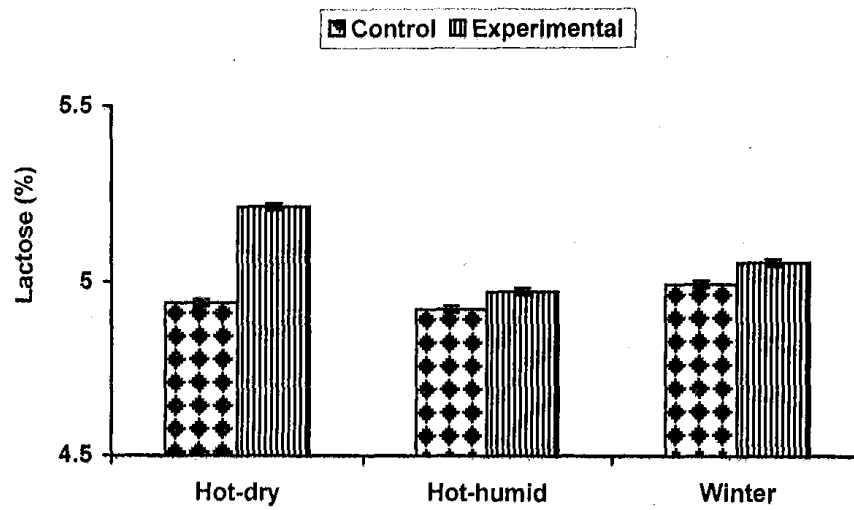
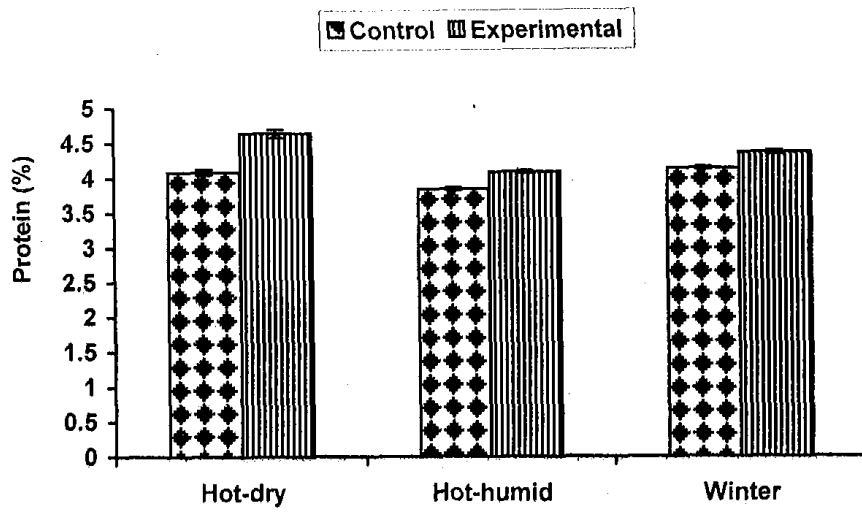
Average SCC were 1.55 vs. 1.52  $\times 10^5$  cells/ml of milk in control and experimental group of cows (Figure 11). In experimental cows, SCC was highest during period 1 (1.61 $\pm$ 0.93  $\times 10^5$  cells/ml) and thereafter declined (Table 13). But in control group SCC was maintained. Epithelial cells of milk were 56.12% in control and 57.89% in experimental group of cows. There was no significant difference in epithelial cells between the two groups, between animals and between periods. The overall average values of TLC were 43.22 and 41.44% in control and experimental group of cows. TLC percent varied ( $P<0.05$ ) between animals and decreased significantly ( $P<0.01$ ) in experimental group of cows (Table 17).

#### **4.2.8 Correlation coefficients:**

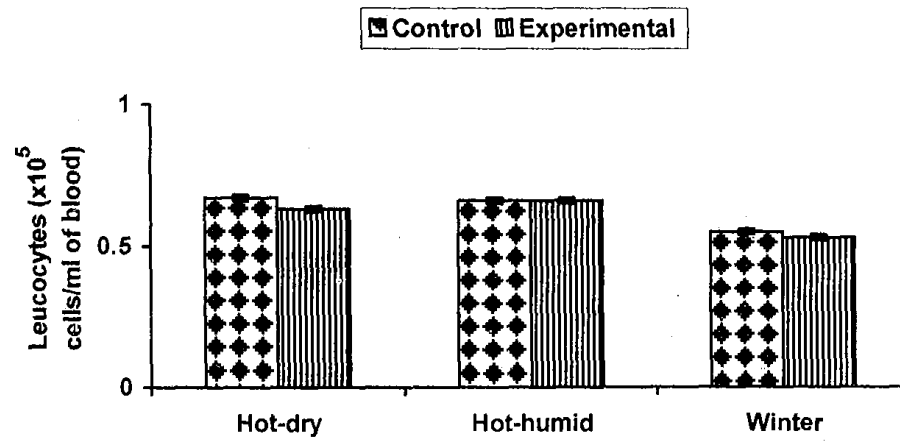
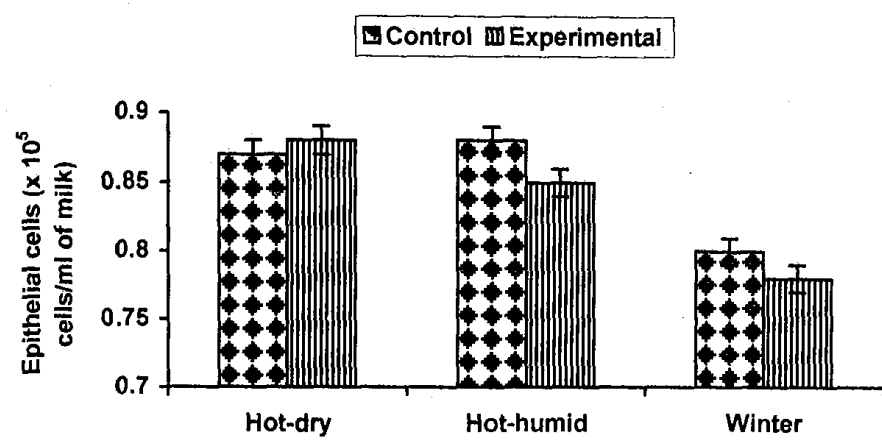
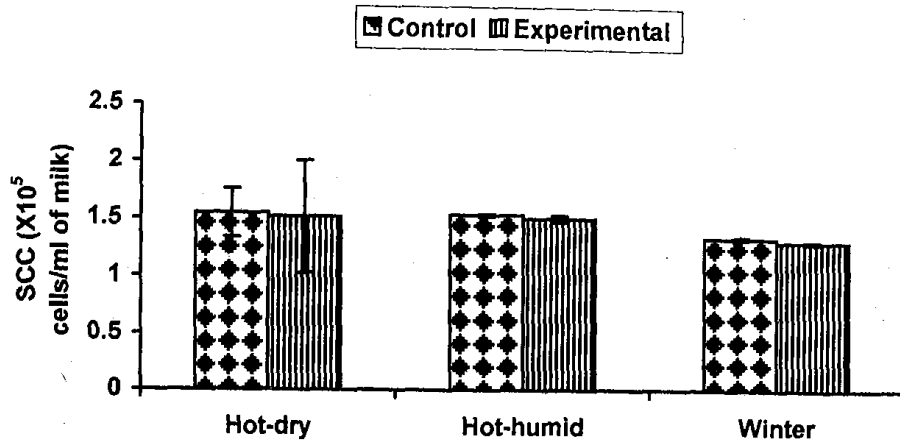
Correlation coefficient analysis indicated that in control group, skin temperature was negatively correlated with milk production ( $P<0.01$ ) and positively to RT, RR and HR ( $P<0.01$ ). Milk production was negatively correlated to RT ( $P<0.01$ ), RR and HR ( $P<0.05$ ; Table 19). RT was positively correlated to RR and HR ( $P<0.01$ ). In experimental cows,



**Figure 9. Overall average total solids and fat content of milk during different seasons in cows**



**Figure 10. Overall average protein and lactose content of milk during different seasons in cows.**



**Figure 11. Overall average somatic cell count (SCC), epithelial cells and TLC during different seasons in cows**

similar sets of correlations were found. Milk production was negatively correlated to ST ( $P<0.01$ ) and positively to RR ( $P<0.05$ ) and HR ( $P<0.01$ ). RR was positively correlated to HR ( $P<0.01$ ) and negatively ( $P<0.05$ ) with ST (Table 19). When the data of control and experimental cows was pooled the correlation analysis (Table 18) indicated positive association of RT with RR and HR ( $P<0.01$ ) and also with ST ( $P<0.01$ ). RR was also found to be positively correlated to HR and ST ( $P<0.01$ ). Milk production was negatively correlated with RT ( $P<0.01$ ) and ST ( $P<0.01$ ; Table 18).

During hot-dry season maximum temperature was not correlated to any of the parameters studied (Table 20). Plasma concentration of  $T_4$  was positively correlated to lactose ( $P<0.05$ ) and the level of  $T_3$  was positively correlated to cortisol ( $P<0.01$ ) and negatively with plasma glucose and TS ( $P<0.05$ ) of milk. Insulin concentration was negatively correlated with plasma NEFA ( $P<0.05$ ), plasma urea ( $P<0.01$ ), TLC ( $P<0.05$ ), milk NEFA ( $P<0.05$ ) and milk urea ( $P<0.01$ ). Plasma cortisol concentration had negative correlation with plasma glucose ( $P<0.01$ ), TS ( $P<0.01$ ), fat, protein and lactose ( $P<0.01$ ) and a positive correlation with plasma and milk NEFA and urea ( $P<0.01$ ) was found. Milk production was positively correlated to lactose and TS ( $P<0.01$ ) and had a negative correlation with milk NEFA and urea ( $P<0.01$ ). SCC of milk was positively correlated with epithelial cells and TLC ( $P<0.01$ ). TLC of milk was positively related to milk NEFA and milk urea ( $P<0.01$ ) and negatively with fat ( $P<0.05$ ) and lactose ( $P<0.01$ ). Milk fat content was correlated positively to protein and lactose content of milk ( $P<0.01$ ) and negatively with milk NEFA and urea ( $P<0.01$ ). Plasma glucose was negatively correlated to plasma NEFA, urea, TLC, milk NEFA and milk urea ( $P<0.01$ ) and positively with milk production, TS, fat, protein and lactose ( $P<0.01$ ).

### **4.3 Effect of Mister System During Hot-Humid Season in Cows:**

#### **4.3.1 Hormones:**

The overall average values of T<sub>4</sub> varied non-significantly between control and experimental group, the respective values were 44.49 vs. 46.21 ng/ml (Table 21; Figure 3). Further, there was no significant variation in T<sub>4</sub> levels between different cows and during different periods of study. Plasma T<sub>4</sub> varied from 41.70 to 46.66 ng/ml in control and from 42.88 to 49.88 ng/ml in experimental animals (Figure 12). Similarly, plasma T<sub>3</sub> concentration non-significantly varied between two groups of the animals during different periods of the study. Further, T<sub>3</sub> levels were non-significantly different between animals. Overall insulin concentration in control and experimental animals was 6.00 vs. 8.86 μU/ml (Table 21; Figure 13). The concentration of insulin in control and experimental group ranged from 4.84 to 7.05 and from 7.96 to 9.66 μU/ml (Figure 14) and was significantly (P<0.01) different between groups, however, between periods and between animals the effect was non-significant (Table 28). Circulatory levels of cortisol decreased (P<0.01) due to mist and fan cooling in experimental cows (2.83±0.04 ng/ml) in comparison to control (4.35±0.08 ng/ml). The decreased level of cortisol during the different periods of the experiment was maintained in experimental cows (Table 21).

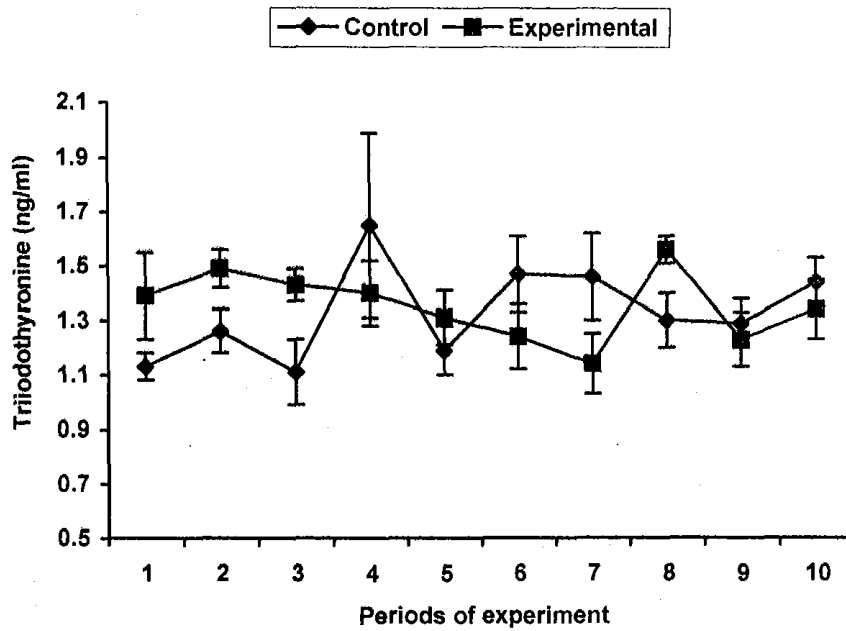
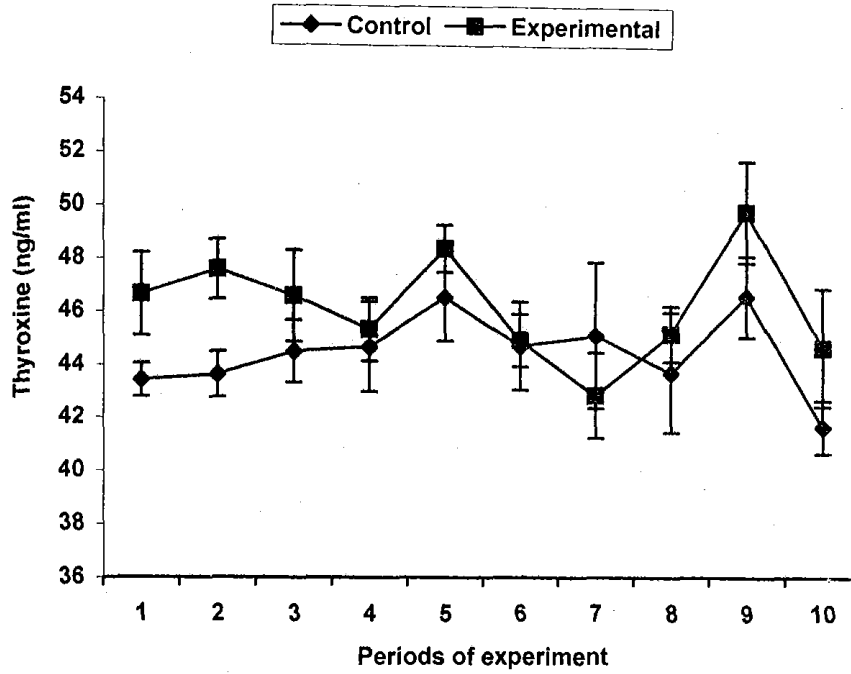
#### **4.3.2 Blood metabolites:**

Plasma glucose levels were non-significantly different between the cows but increased (P<0.01) in experimental (cooled) cows in comparison to control ones (Table 28). The overall average glucose was 46.03±0.26 and 54.93±0.33 mg% in control and experimental animals (Table 22). The effect of mister cooling on glucose level was evident from period 1 of experiment which increased significantly (P<0.05) from period 5 onwards (Figure 15). Glucose concentration was significantly (P<0.01) higher

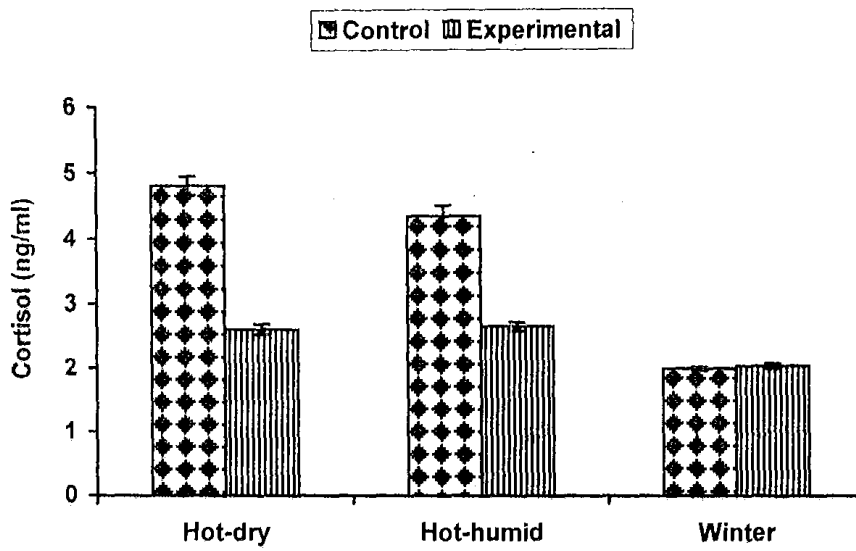
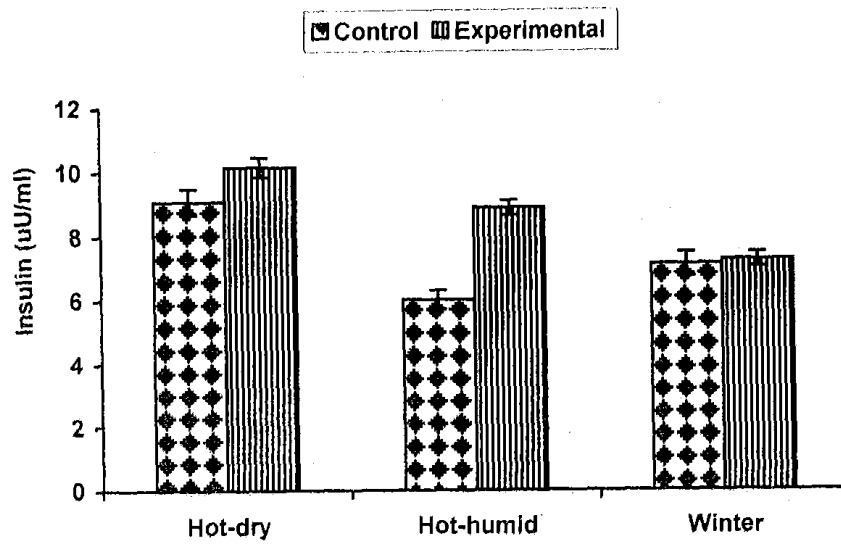
(52.39 to 57.79 mg%) in experimental animals in comparison to control (44.56 to 47.37 mg%). Glucose concentration was non-significantly different between the animals. Plasma NEFA was  $203.16 \pm 1.12$  and  $178.66 \pm 1.20$   $\mu$  mol/l in control and experimental cows, respectively (Table 22). NEFA levels decreased significantly ( $P < 0.01$ ) in experimental group and were from 169.16 to 189.16  $\mu$ mol/l in comparison to control (195.00 to 210.00  $\mu$ mol/l; Table 22). The changes in NEFA between periods ( $P < 0.01$ ) and the interaction of animals x groups x periods were significant ( $P < 0.05$ ). A similar trend of decline in plasma urea concentration was found in experimental cows. The overall average values significantly ( $P < 0.01$ ) decreased in experimental cows, the respective values were 54.83 vs. 49.52 mg%, respectively (Tables 22 & 28; Figure 5). Plasma urea concentration in control increased during the periods of experiment, however, in experimental level decreased gradually and was lowest in period 10 of study.

#### **4.3.3 Physiological responses:**

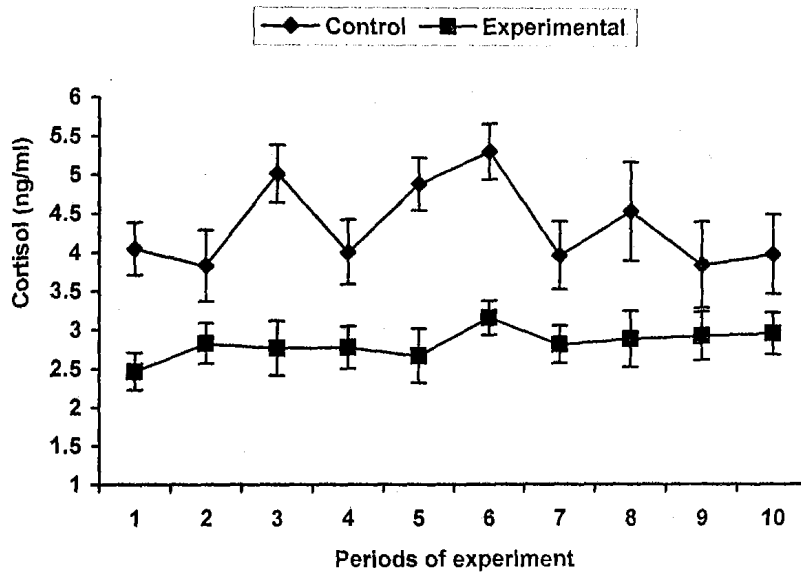
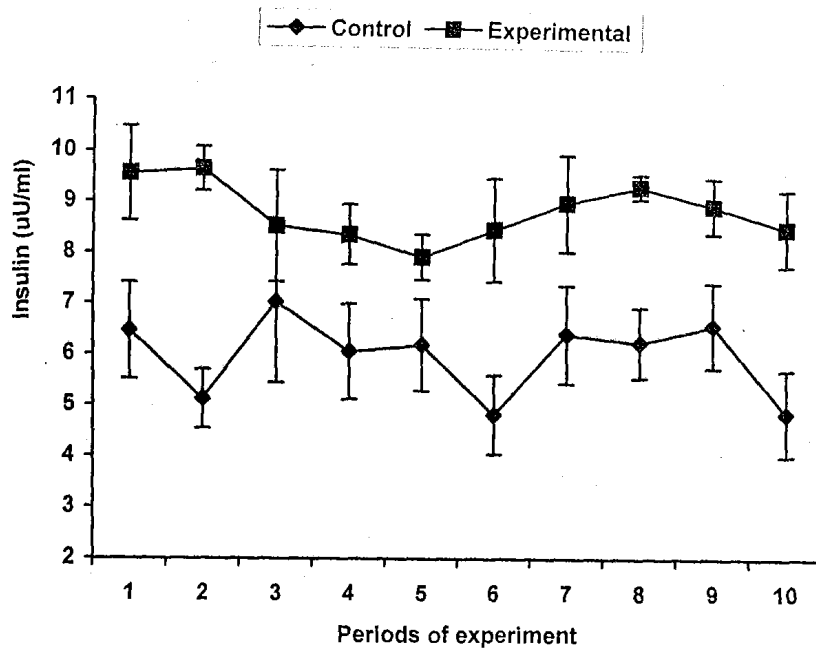
Average RT in control and experimental cows was similar ( $101.79 \pm 0.08^{\circ}\text{F}$  vs.  $101.87 \pm 0.09^{\circ}\text{F}$ ) during morning (8.00 A.M.) and increased to  $102.27 \pm 0.07^{\circ}\text{F}$  in the evening (4.00 P.M.) in control group of cows, however, in experimental cows a decrease in RT ( $P < 0.01$ ) was observed from  $101.87 \pm 0.09$  to  $101.59 \pm 0.10^{\circ}\text{F}$ . RT varied ( $P < 0.01$ ) between the cows and during periods of the experiment in the morning (Table 30). A significant ( $P < 0.05$ ) variation in RT was observed between animals, between the groups and during periods ( $P < 0.01$ ), Further, group x periods interaction was significant ( $P < 0.05$ ) in the evening. The average RR during morning was more in control ( $27.10 \pm 0.28$  /min) in comparison to experimental cows ( $25.49 \pm 0.29$ /min.) which further increased to  $35.26 \pm 0.39$ /min. in the evening, however, in experimental group the RR decreased to  $23.10 \pm 0.21$ /min. in the evening (Tables 23 & 24). RR in both the groups of cows was maximum during period 4 and 5, the



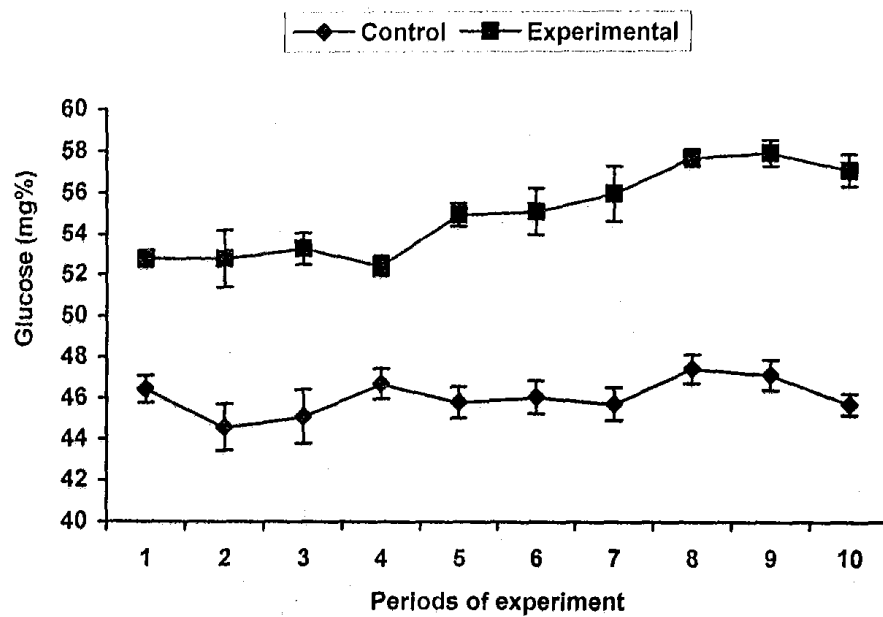
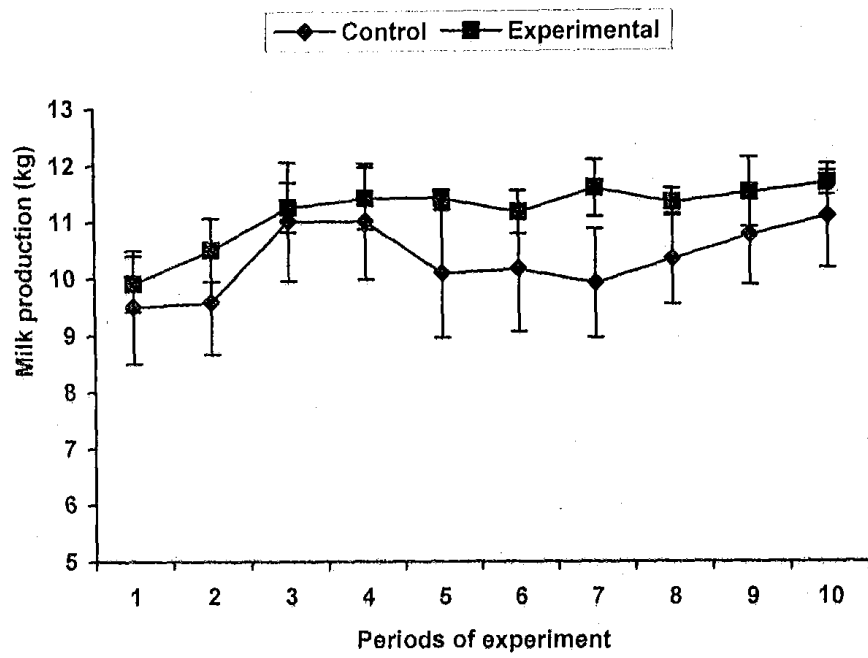
**Figure 12. Plasma thyroxine and triiodothyronine levels during hot-humid season in cows**



**Figure 13. Overall average plasma insulin and cortisol levels during different seasons in cows**



**Figure 14. Plasma insulin and cortisol levels during hot-humid season in cows**



**Figure 15. Milk production and glucose levels during hot-humid season in cows**

respective values in morning were 30.00 and 29.66 vs. 28.00 and 27.66 in control and experimental animals (Figure 6 & 7). RR varied during different periods of the study and between the two groups ( $P < 0.01$ ) during morning and evening, but RR changes between animals was not significant. An increase in HR from  $54.53 \pm 0.44$ /min to  $63.66 \pm 0.58$ /min. was observed in control group, however in experimental group HR decreased from  $52.00 \pm 0.39$ /min to  $48.55 \pm 0.30$ /min (Tables 23 & 24). HR during morning was non-significant between animals, however, during evening the changes were significant ( $P < 0.05$ ). Further, variation in HR was significant between groups ( $P < 0.01$ ) during different periods of the study ( $P < 0.01$ ) and the interaction of periods x groups was also significant ( $P < 0.01$ ). Average HR of animals in the evening remained low (47.00 to 51.00 /min.) in comparison to control (56.66 to 68.33/min.) during different periods of the study. The average morning ST in control group cows remained fluctuated between 29.75 and 33.66°C and was significantly ( $P < 0.01$ ) higher during morning in comparison to experimental group (29.66 to 33.41°C). The pooled analysis of morning and evening physiological responses (RT, RR and HR) and ST revealed significant ( $P < 0.01$ ) variation between groups (Table 29). The changes in ST during evening were consistent during different periods of the study, however, in control it fluctuated. ST varied significantly ( $P < 0.01$ ) between groups, between periods ( $P < 0.01$ ) and the interaction of periods x groups was significant ( $P < 0.01$ ), however, between animal variation of ST was non-significant (Table 30).

#### **4.3.4 Body weight changes, feed and water intake:**

During hot-humid season the body weight of the cows non-significantly varied with overall average body weight of  $361.79 \pm 13.86$  vs.  $357.99 \pm 21.61$  kg in control and experimental cows (Table 38b).

Feed intake during 1<sup>st</sup> week of experiment was similar in both the groups. In experimental cows feed intake increased during 2<sup>nd</sup> week

onwards but in control feed intake was decreased. Feed intake of experimental group was higher ( $P<0.01$ ) than control (Table 38b).

The water intake during hot-humid season did not exhibit a set pattern of change in control group, however, in experimental group water intake was high during 1<sup>st</sup> week and declined thereafter. The water intake was significantly higher in control than the experimental group ( $57.13\pm 0.77$  vs.  $52.47\pm 1.23$  litres; Table 38b).

#### **4.3.5 Milk production :**

The average milk production of cows in experimental group increased gradually during the experimental period but such pattern was not found in control group (Figure 15). The milk production of control group rather fluctuated during different periods of the experiment. Due to the mister and fan effect the milk production of experimental cows increased with average percent of 2.27 to 16.85% (Figure 16). Milk production varied between the groups ( $P<0.01$ ) and the overall average milk production was more in experimental cows ( $11.17\pm 0.14$  kg/d) in comparison to control ( $10.34\pm 0.29$  kg/d). The variation in milk production between the animals ( $P<0.01$ ) was significant (Table 31).

#### **4.3.6 Milk composition:**

Total solids of milk varied between the groups ( $P<0.01$ ) and was higher in experimental ( $13.06\pm 0.01\%$ ) than control ( $12.70\pm 0.04\%$ ; Figure 9). Average total solids of milk were higher throughout the study in experimental cows and varied ( $P<0.05$ ) between animals, however, there was no significant difference in total solids of milk between periods of the study (Table 31).

Average value of fat in control group of animals was less ( $3.31\pm 0.11\%$ ) in comparison to experimental cows ( $3.54\pm 0.08\%$ ) in period 1 of the experiment (Table 26). There was an increasing trend in fat content of milk in both the groups. However, fat was non-significantly different between animals. Further, variation in fat was significant

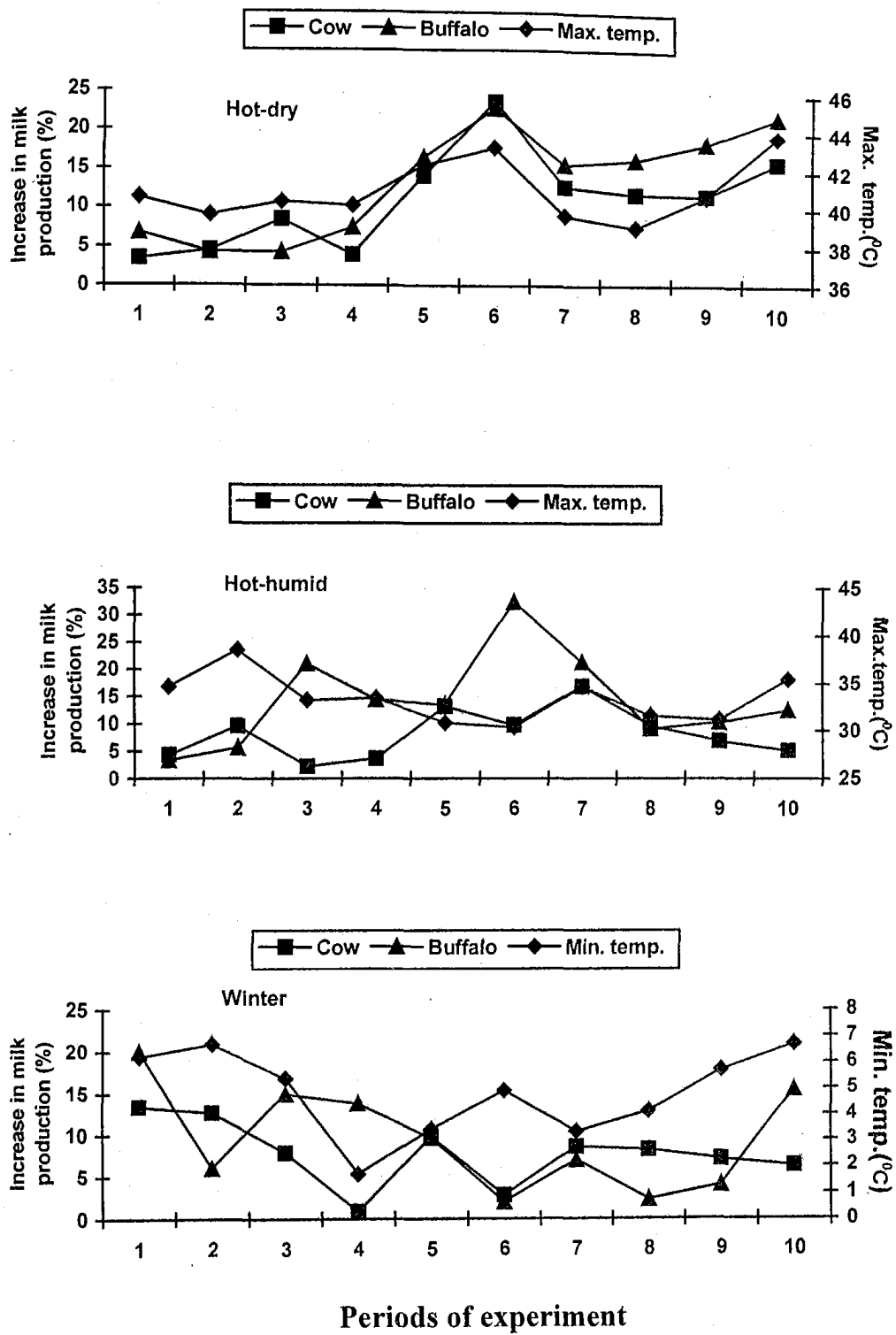


Figure 16. Percent increase in milk production in experimental group of animals

( $P < 0.01$ ) between groups and between the periods ( $P < 0.01$ ). Overall average value of fat was high in experimental in comparison to control cows (3.73 vs. 3.47%; Table 26).

Average protein content of milk during period 1 of experiment was similar in control and experimental cows. Thereafter, average protein values increased gradually in both the groups. The overall average protein was significantly higher ( $P < 0.01$ ) in experimental than control cows (4.07 vs. 3.83%; Table 26) and increase in protein % in experimental cows was 6.26%. Protein content of milk also varied ( $P < 0.01$ ) between animals and during different periods ( $P < 0.01$ ) of the study (Table 31).

Lactose content of milk varied between the groups ( $P < 0.01$ ) and the overall average values were higher in experimental animals ( $4.97 \pm 0.01\%$ ) in comparison to control ( $4.92 \pm 0.01\%$ ; Table 26; Figure 10). Further, variation in lactose content of milk between the cows during different periods of the study was non-significantly different.

The concentration of NEFA in milk was higher ( $P < 0.01$ ) in control than the experimental cows. The NEFA concentration was 6.67% more in control group in comparison to experimental cows (Figure 8). However, between animals and between periods variations were non-significant. Average NEFA varied between 176.66 to 188.33 and 162.50 to 180.00  $\mu\text{mol/l}$  in control and experimental cows during different periods of the study (Table 25).

Milk urea concentration varied ( $P < 0.01$ ) between the two groups of animals (Table 31) and the average value was more in control ( $53.14 \pm 0.25 \text{ mg}\%$ ) than the experimental group ( $48.50 \pm 0.24 \text{ mg}\%$ ). The variability in urea concentration between animals and between periods was non-significantly different. Average value of milk urea was higher from period 1 till the end of experiment in control group and was from 51.47 to 54.39  $\text{mg}\%$  (Table 25).

#### **4.3.7 Somatic cell count (SCC):**

SCC of milk was significantly different ( $P < 0.01$ ) between groups and between periods ( $P < 0.01$ ). However, SCC variation between animals was non-significant (Table 31). SCC was similar during period 1 of the study in both the group of cows and increased gradually during different periods of the study in control group. Average SCC was maximum ( $1.62 \pm 0.02 \times 10^5$  cells/ml of milk) during period 10 of the study in control cows, however, in experimental cows average SCC declined by period 4 of the study and thereafter fluctuated (Table 27). The overall average SCC was more in control than the experimental cows, the respective values being 1.54 vs.  $1.51 \times 10^5$  cells/ml of milk (Figure 11). On an average, SCC was higher by 1.94% in milk of control cows. The average epithelial cells were more in control than in experimental cows during period 1 of the study and thereafter fluctuated between 0.81 to  $1.00 \times 10^5$  cells/ml of milk. The experimental cows, which were provided with mist and fan, did not indicate a set pattern of decline in epithelial cell secretion in milk. However, average values during different periods of the study were less. Epithelial cell percent was 57.14 and 56.29 in control and experimental groups respectively (Table 27). The epithelial cells varied between the groups ( $P < 0.01$ ) and between periods ( $P < 0.01$ ). Further, variability in epithelial cells between the animals was also significant ( $P < 0.01$ ; Table 31). Average TLC content of milk was similar in control and experimental group. However, TLC was significantly ( $P < 0.01$ ) different between periods of the study (Table 31). There was no effect of mister cooling and fan on TLC of milk of both the groups of animals. Further, between animal variation of milk TLC was non-significant. On percent basis TLC was 42.85 vs. 43.70% in control and experimental groups, respectively. On an average, the ratio of epithelial cell to TLC was 57:43 and 56:44 in control and experimental groups respectively.

#### 4.3.8 Correlation coefficients:

In control group of animals, ST was positively correlated with maximum temperature ( $P<0.01$ ), and negatively with RT ( $P<0.01$ ). HR had a significant positive correlation with RT ( $P<0.05$ ) and RR ( $P<0.01$ ). RR was positively correlated to RT ( $P<0.01$ ; Table 33).

In experimental group, ST was positively correlated with RT ( $P<0.01$ ), RR ( $P<0.01$ ) and HR ( $P<0.01$ ). The other correlations were found to be similar as for control.

When the data of control and experimental group was pooled, it was found that ST was positively correlated with maximum temperature ( $P<0.01$ ), RR ( $P<0.01$ ) and HR ( $P<0.01$ ). Further, RT was positively correlated ( $P<0.01$ ) to HR. HR had a positive correlation with RR ( $P<0.01$ ; Table 32).

During hot-humid, positive correlation of cortisol with plasma NEFA and urea ( $P<0.01$ ) and negative correlation with plasma glucose, TS, fat and protein ( $P<0.01$ ) was found. Further, cortisol was negatively correlated to milk production ( $P<0.05$ ). Plasma  $T_4$  was negatively correlated to SCC and epithelial cells ( $P<0.05$ ). Insulin concentration had negative correlation with cortisol, plasma NEFA, urea, milk NEFA and urea ( $P<0.01$ ) and was positively correlated to glucose, TS, fat ( $P<0.01$ ) and protein, lactose and milk production ( $P<0.05$ ). Further, a negative correlation of cortisol with plasma glucose, milk production, TS, fat and protein ( $P<0.01$ ) and negatively associated with milk and plasma NEFA and urea ( $P<0.01$ ). Milk production during hot-humid was positively associated with TS, fat, protein and lactose ( $P<0.01$ ). SCC of milk was found to be positively correlated with epithelial cells and TLC ( $P<0.01$ ) of milk. Epithelial cells were negatively correlated with TLC ( $P<0.01$ ). Fat content of milk was positively correlated with protein ( $P<0.01$ ) and negatively with milk urea ( $P<0.01$ ). Milk protein was positively correlated

to lactose ( $P<0.05$ ) and negatively with milk NEFA and urea ( $P<0.01$ ; Table 34).

#### **4.4 Effect of Housing and Straw Bedding During Winter Season in Cows:**

##### **4.4.1 Hormones:**

Average value of  $T_4$  was higher in experimental cows ( $55.42\pm 0.77$  ng/ml) and varied significantly ( $P<0.01$ ) between the animals and between periods ( $P<0.01$ ) of study (Table 35 & 42). The significant variation between the groups ( $P<0.01$ ) in  $T_4$  concentration was due to higher basal concentration of  $T_4$  during different periods of study. Plasma  $T_4$  increased from 50.70 to 58.20 ng/ml during period 1 to 10 of study in control cows. However, in experimental cows  $T_4$  concentration increased without any distinct pattern of change in relation to periods of study (Figure 17). Plasma  $T_3$  was low in experimental group ( $1.35\pm 0.04$  ng/ml) in comparison to control ( $1.62\pm 0.04$  ng/ml) and was significantly different ( $P<0.01$ ; Table 35 & 42). However, between periods and between animals variation in  $T_3$  concentration was non-significant. The effect of mist and fan cooling in experimental cows decreased plasma  $T_3$  from period 1 of the study, however, plasma  $T_3$  fluctuated during different periods of the study. In control group of cows, average basal plasma  $T_3$  concentration increased during different periods of the study. Plasma insulin non-significantly varied between the animals and between periods. There was no effect of in-house keeping of cows on plasma insulin concentration (Figure 18). The overall average value of insulin in control and experimental group was  $7.09\pm 0.38$  vs.  $7.23\pm 0.23$   $\mu$ U/ml (Table 35). Plasma cortisol concentration significantly increased ( $P<0.01$ ) in cows kept inside the houses in comparison to control (Table 42). The respective values were 2.25 vs. 2.09 ng/ml (Table 35). Further, cortisol concentration varied between animals ( $P<0.05$ ).

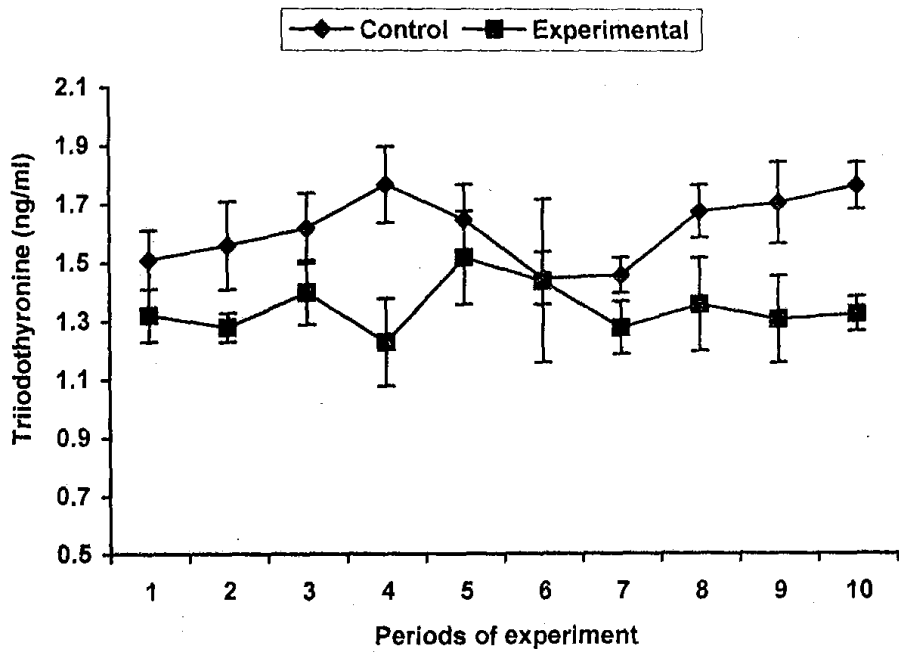
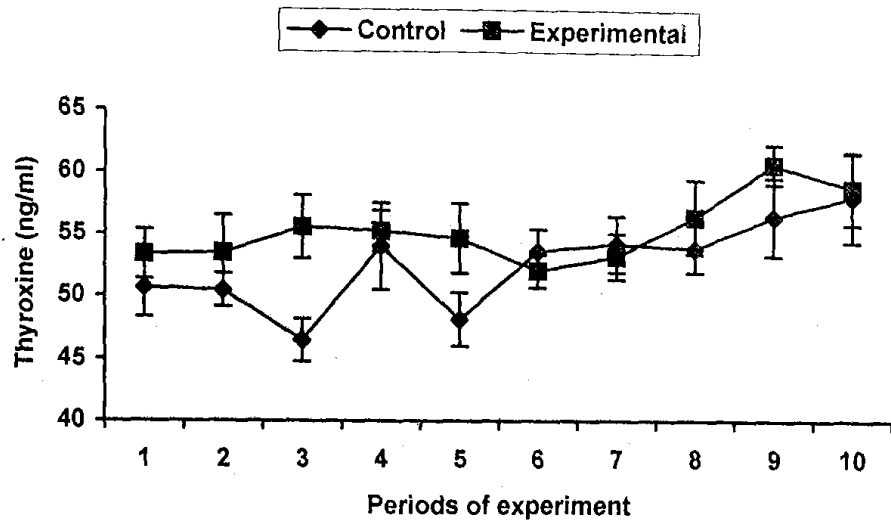
Plasma concentration of T<sub>4</sub> and cortisol was similar during hot-dry and hot-humid in control and experimental groups, however, T<sub>4</sub> was higher during winter in both the groups (Figure 3 & 13). Further, cortisol concentration during winter was higher in comparison to control group. Basal values of plasma T<sub>3</sub> concentration increased during hot-humid season and during winter season in control group of cows (Figure 3). Further, insulin concentration was high during hot-dry season and declined during hot-humid and winter seasons in control group of animals indicating thereby that season influenced plasma concentration of hormones (Figure 13).

#### **4.4.2 Blood metabolites:**

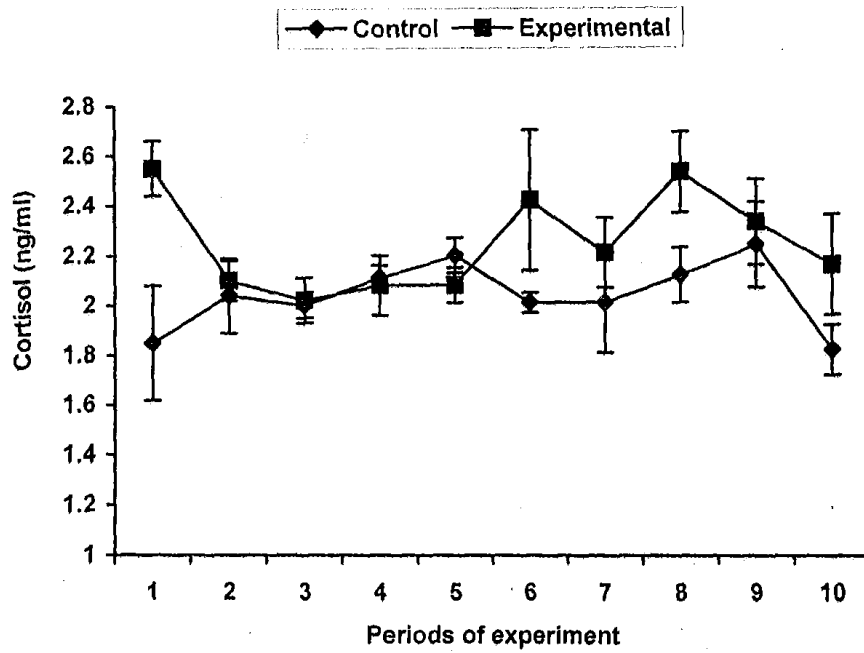
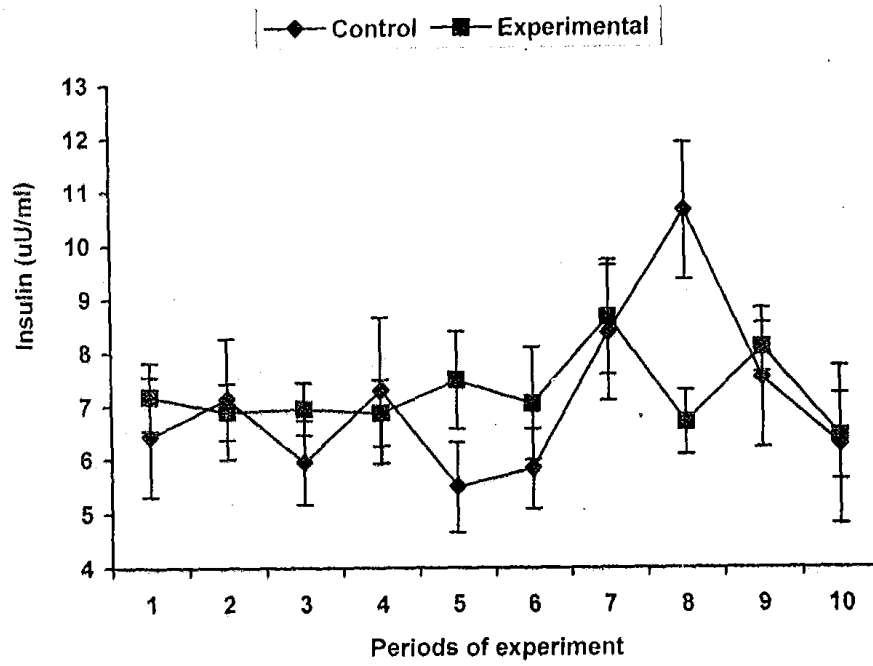
Average plasma glucose was low ( $52.98 \pm 0.57$  mg%) during period 1 in control group of cows and increased to maximum values of 58.38 mg% during 7<sup>th</sup> period of the study (Table 36). However, in experimental cows glucose remained higher during different periods of the study and was significantly different between the groups ( $P < 0.01$ ). Glucose levels non-significantly varied between animals but the changes were significant ( $P < 0.05$ ) during different periods of the study (Table 42). Further, interaction of group x period x animal was also significant ( $P < 0.01$ ).

Plasma NEFA concentration was higher in control and was significantly different ( $P < 0.01$ ) between the groups (Table 42). Further, NEFA levels varied non-significantly between the animals and between periods. In-house keeping of experimental animals resulted in decreased NEFA concentration during different periods of the study. However, in control, NEFA levels were almost maintained. On percent basis average NEFA concentration was higher by 11.68% in control group of animals.

Average plasma urea was high in control ( $47.48 \pm 0.40$  mg%) in comparison to experimental group ( $45.14 \pm 0.75$  mg%) and was significantly different ( $P < 0.01$ ; Table 36 & 42). Urea levels also varied significantly ( $P < 0.01$ ) between periods but animal to animal variation was



**Figure 17. Plasma thyroxine and triiodothyronine levels during winter season in cows**



**Figure 18. Plasma insulin and cortisol levels during winter season in cows**

non-significant. Average plasma urea concentration fluctuated between 43.75 to 49.27 mg% and 37.31 to 47.78 mg% in control and experimental groups during different periods of the study (Table 36) and was higher by 4.92% in control animals.

Basal values of glucose in control group of cows were higher in hot-dry and winter season and low during hot-humid season (Figure 5). Plasma NEFA concentration was higher and urea concentration was lower ( $P < 0.01$ ) in buffaloes than the cows (Table 91).

#### **4.4.3 Physiological responses:**

During morning average RT non-significantly varied in control and experimental cows, the respective values were 100.98 vs. 100.87<sup>o</sup>F (Table 37). However, between animal variations in RT was significant ( $P < 0.05$ ). Average RT during evening varied between animals ( $P < 0.05$ ) and was non-significant between the groups and between periods. When compared with morning temperature the average RT increased from 100.98 to 101.26<sup>o</sup>F in control and from 100.87 to 101.36<sup>o</sup>F in experimental cows in the evening (Table 38a; Figure 6 & 7). When the data was pooled for morning and evening the RT varied significantly between groups and between animals ( $P < 0.01$ ). However, between periods variation in RT was non-significant (Table 43).

RR changes between the animals, between groups and between periods were non-significant during morning. The average RR was similar in control and experimental cows (21.10 vs. 21.02 breaths/min.) and varied significantly ( $P < 0.01$ ) between 2 groups in evening. The values of RR was higher in experimental in comparison to control group (26.80 vs. 25.03 breaths/min.). Further, there was no significant variation in RR between the periods and between animals. Average RR varied in the evening from 24.00 to 26.33 and 26.00 to 28.00 breaths/min. in control and experimental animals during different periods of the study (Table 38a). The pooled analysis of morning and evening RR also revealed

significant variation in RR between the groups ( $P<0.05$ ) but between animals and periods variation in RR was non-significant (Table 43).

Average values of HR were non-significant between the groups during morning, however, it varied significantly between periods ( $P<0.01$ ). During evening, HR was more (57.36/min.) in comparison to control (55.13/min.) and significantly ( $P<0.01$ ) varied between the periods and groups (Table 44). However, between animal variation was not different. Average HR during evening was higher ( $P<0.01$ ) during all the periods of the study in experimental group in comparison to control. The pooled analysis of morning and evening data also indicated significant variation in HR between groups and between periods ( $P<0.01$ ).

Average value of ST was significantly ( $P<0.01$ ) higher in the evening ( $29.72\pm 0.09^{\circ}\text{C}$ ) in the control group in comparison to morning ST ( $28.75\pm 0.17^{\circ}\text{C}$ ). ST in both the groups varied between periods ( $P<0.01$ ) during morning but was not different between animals. Average basal morning ST was 27.83 to 30.00 and 27.16 to 29.91 $^{\circ}\text{C}$  in experimental and control groups, respectively (Table 37). During evening ST in both control and experimental group was higher ( $P<0.01$ ). ST increased from overall average value of 28.75 to 29.72 in control group and 29.09 to 30.06 $^{\circ}\text{C}$  in experimental group (Table 38a; Figure 6 & 7). The pooled data analysis of morning and evening of 2 groups of animals indicated significant variation in ST between groups and between periods ( $P<0.01$ ), however, between animal variation was non-significant (Table 43).

#### **4.4.4 Body weight changes, feed and water intake:**

During winter season, the body weight of the control and experimental group was  $371.32\pm 24.97$  kg and  $378.12\pm 8.13$  kg and was non-significantly different (Table 38 b).

Feed intake of both the groups was similar during 1<sup>st</sup> week of the study and was high ( $P<0.01$ ) in experimental group than the control (42.16 vs. 38.07 kg; Table 38b).

The overall average of water intake was  $35.66 \pm 0.63$  and  $38.65 \pm 0.46$  L in control and experimental cows (Table 38b).

#### **4.4.5 Milk production :**

Average values of milk production increased during period 1 in experimental cows kept inside the houses. However, in the remaining periods of the study the milk production was maintained but in control group milk production increased gradually upto 4<sup>th</sup> period and thereafter fluctuated (Figure 19). The overall average value of milk production in control and experimental group was significantly ( $P < 0.01$ ) different ( $18.26$  vs.  $17.00$  kg/day; Tables 39 & 45). Milk production on percent basis increased by 7.41 percent in experimental cows. The milk production changes between animals also varied significantly ( $P < 0.01$ ). The percent increase in milk production in experimental group varied from 0.88 to 13.42% during different periods of the study (Figure 16). But the effect was more pronounced during period 1 of experiment.

#### **4.4.6 Milk composition:**

Average values of total solids in both the groups of animals fluctuated and overall average total solids was lower in control than experimental group ( $13.03$  vs.  $13.36\%$ ). Total solids varied between periods ( $P < 0.01$ ) of the study but there was no significant difference in total solids of milk between the animals (Table 45).

Average fat content of milk varied significantly ( $P < 0.05$ ) between groups and between animals ( $P < 0.01$ ) however, between period variation was non-significant. Average fat in control group fluctuated between 4.10 to 4.35 during different periods of study and was low in comparison to experimental group (4.14 to 4.56%; Table 40). A gradual increase in fat content of milk was found during different periods of the study in experimental cows. Average protein varied significantly between groups and periods ( $P < 0.01$ ) and between animals also ( $P < 0.05$ ). Further, the animal x group x period interaction was also significant ( $P < 0.01$ ). Average

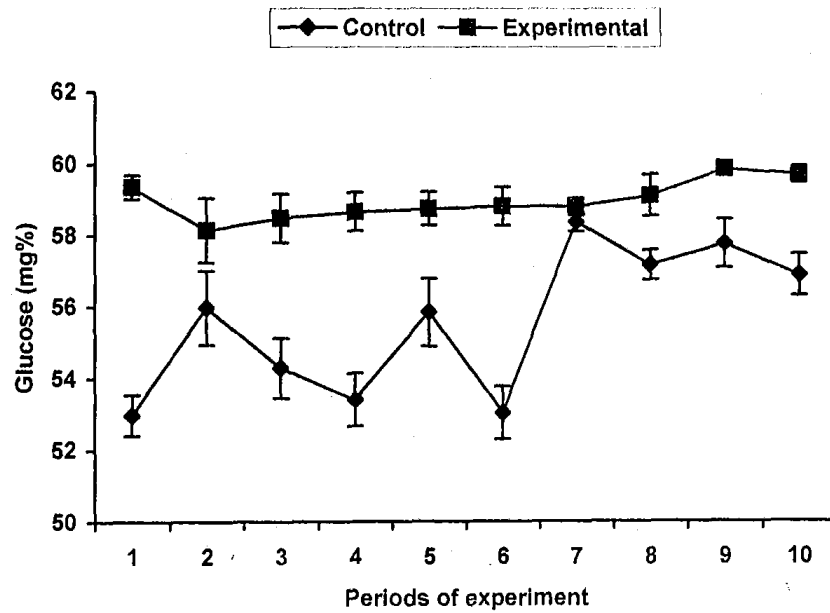
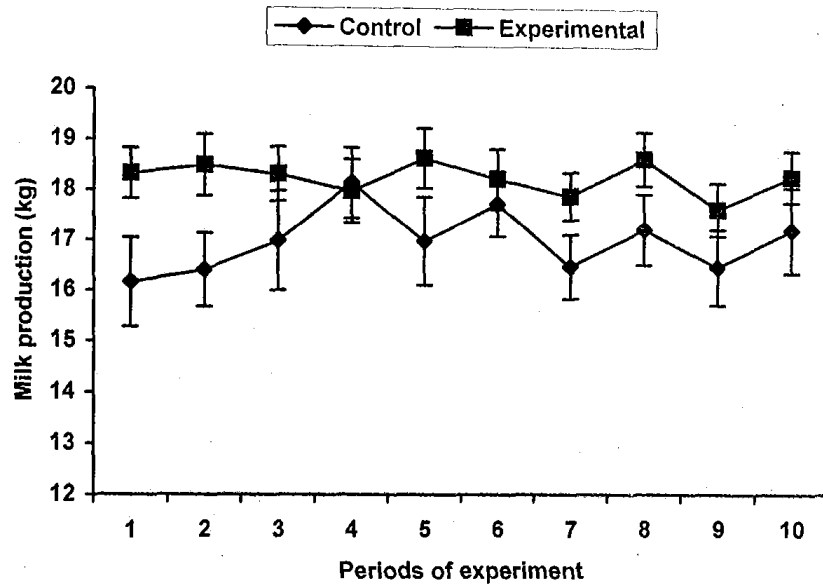
protein content of milk increased gradually during the different periods of study in experimental group but such pattern of change was not found in control group. Average protein content was low in experimental group during period 1, however, an increase in protein occurred from period 3 onwards in experimental cows.

Lactose content of milk was high (5.05%) in experimental than the control group (4.99%) and was significantly different between groups and between animals ( $P < 0.01$ ) but the variation during different periods of the study was not significant (Table 45). Like fat and protein, the average values of lactose also exhibited an increasing trend from period 1 onwards, however, in control it fluctuated.

Average NEFA concentration was higher in control than the experimental group, the respective values were 163.25 vs. 149.25  $\mu\text{mol/l}$ . NEFA level was significantly different between the groups and between animals ( $P < 0.01$ ) but non-significant between periods (Table 45). Average values of milk urea decreased ( $P < 0.05$ ) in experimental group during different periods of study and the overall average milk urea was less ( $43.75 \pm 0.34 \text{ mg\%}$ ) in experimental than control group ( $44.91 \pm 0.36 \text{ mg\%}$ ). Urea levels non-significantly varied between animals and between periods of the study. Average urea levels were 43.03 to 44.61 and 44.02 to 46.26  $\text{mg\%}$  in experimental and control groups during different periods of the study (Table 39; Figure 8).

#### **4.4.7 Somatic cell count (SCC):**

SCC of milk fluctuated in control and experimental group during different periods of the study and was  $1.35 \pm 0.01 \times 10^5$  cells/ml and  $1.32 \pm 0.01 \times 10^5$  cells/ml in control and experimental groups, respectively (Table 41; Figure 11). SCC varied between two groups ( $P < 0.05$ ) of cows, however, between animal and between periods variation was non-significant. Epithelial cells of milk varied between animals ( $P < 0.01$ ), however, there was no significant difference in milk epithelial cells



**Figure 19. Milk production and glucose levels during winter season in cows**

between the groups and between periods. The overall average epithelial cells were  $0.80 \pm 0.01 \times 10^5$  cells/ml and  $0.78 \pm 0.01 \times 10^5$  cells/ml in control and experimental group. Average TLC was low in experimental ( $0.53 \pm 0.01 \times 10^5$  cells/ml) than the control group ( $0.55 \pm 0.01 \times 10^5$  cells/ml) and non-significantly varied. TLC varied between animals ( $P < 0.01$ ) but between period variation was non-significant (Table 45).

The comparative pattern of change in SCC of milk in cows indicated that basal value of SCC in control group of cows was higher ( $P < 0.01$ ) and similar during hot-dry and hot-humid season in comparison to winter season (Table 93; Figure 11). Further, epithelial cells and TLC also varied ( $P < 0.01$ ) during different seasons and the basal values of TLC was low in winter and high in hot-dry and hot-humid season.

#### **4.4.8 Correlation coefficients:**

In control group, the correlation of physiological responses and milk production indicated a negative correlation of milk production with RT ( $P < 0.01$ ). RT was positively correlated with RR ( $P < 0.05$ ) and ST ( $P < 0.01$ ). RR was positively correlated with HR and ST ( $P < 0.01$ ). ST was positively correlated with HR ( $P < 0.01$ ). Minimum temperature was found to be positively associated with RR ( $P < 0.01$ ; Table 47).

In experimental group, minimum temperature correlation with RR was not found. Milk production was positively correlated to RR and HR ( $P < 0.01$ ). RT was positively correlated with RR, HR and ST ( $P < 0.01$ ). Further, RR was positively correlated with ST. HR was also positively ( $P < 0.01$ ) correlated with ST (Table 46). The pooled analysis of data of control and experimental group indicated a positive correlation of RT with HR and ST ( $P < 0.01$ ). Further, ST was positively correlated to HR ( $P < 0.01$ ; Table 46).

During winter season, the correlation of cow's data (Table 48) revealed that minimum temperature was negatively correlated with

plasma levels of T<sub>4</sub> and lactose (P<0.05) and with insulin, glucose and protein (P<0.01). Plasma T<sub>4</sub> was correlated to glucose (P<0.01), protein and milk NEFA (P<0.05). Plasma T<sub>3</sub> level had negative correlation with cortisol, glucose (P<0.01) and was positively correlated with plasma NEFA, epithelial cells and milk urea (P<0.05). Plasma insulin was not correlated to any of the parameters studied. However, cortisol level was positively correlated with glucose and protein (P<0.01) and negatively to milk NEFA (P<0.01). Plasma glucose was positively correlated with TS (P<0.05), protein (P<0.01) and negatively to milk and plasma NEFA (P<0.01), SCC and epithelial cells (P<0.05). Milk production of cows was positively associated with SCC (P<0.05) and TLC (P<0.01) and negatively with fat content (P<0.01). TS was positively correlated (P<0.01) with protein and lactose content of milk. SCC was positively correlated with epithelial cells and TLC (P<0.01). TLC of milk was negatively correlated (P<0.05) with fat. Protein was positively correlated with lactose (P<0.01) and negatively with milk NEFA (P<0.01).

#### **4.5 Effect of Showers and Wallowing During Hot-Dry Season in Buffaloes:**

##### **4.5.1 Hormones:**

Average plasma T<sub>4</sub> was high (52.27±0.67 ng/ml) in wallowing buffaloes (experimental) than control (50.65±0.50 ng/ml) and was significantly different (P<0.01; Table 49 & 56). Further, T<sub>4</sub> concentration varied (P<0.01) between periods of the study and was non-significantly different between animals. Average plasma T<sub>4</sub> was high from period 1 onwards till period 10 of the study but in control group it fluctuated (Figure 20). The average levels of T<sub>4</sub> was less in experimental than the control group during period 1 of the study, the respective values were 47.36 vs. 50.03 ng/ml.

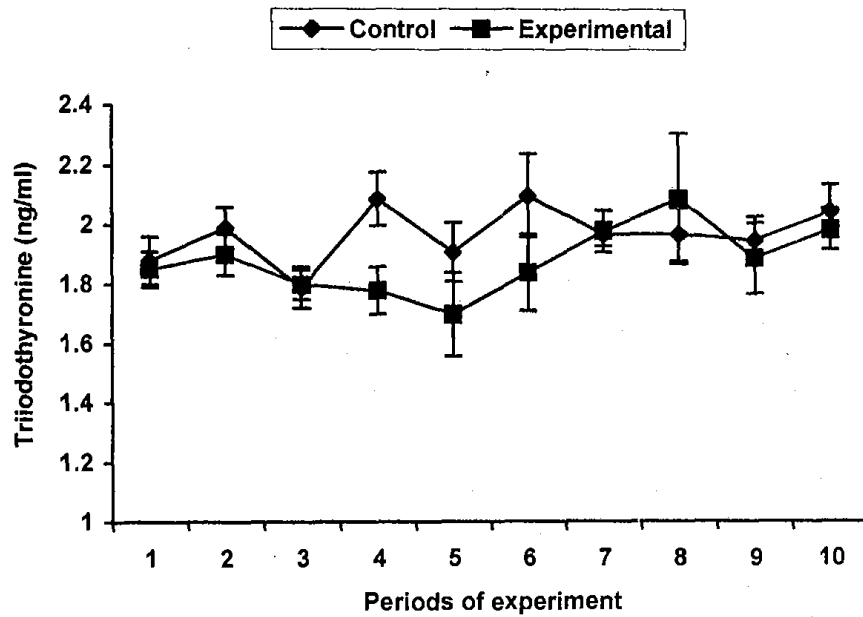
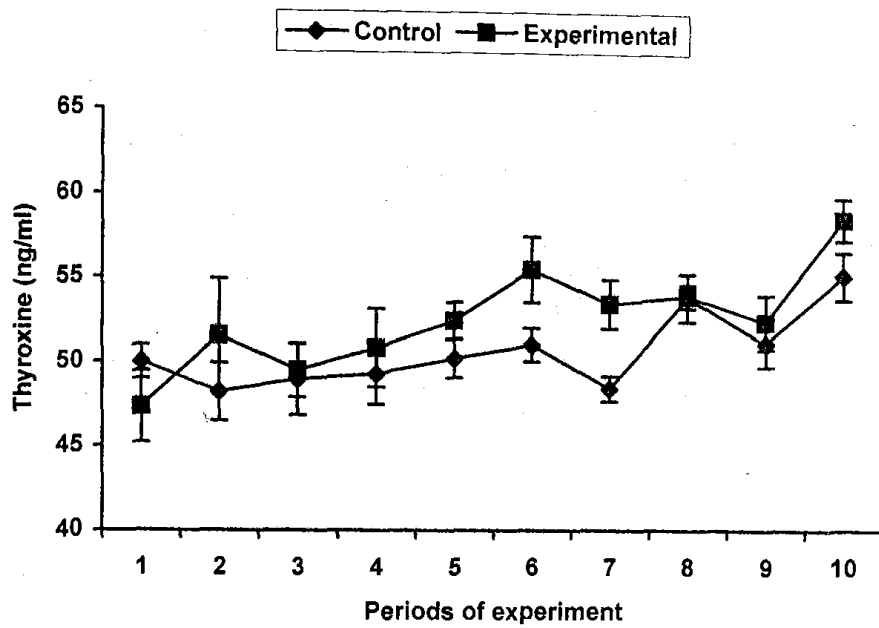
Plasma T<sub>3</sub> did not vary between groups and between periods of the study. Further, there was no significant variation in T<sub>3</sub> concentration

between animals. During the experiment, T<sub>3</sub> concentration fluctuated in both groups and average T<sub>3</sub> concentration was 1.97±0.03 and 1.88±0.03 ng/ml in control and experimental groups, respectively (Table 49; Figure 22). Plasma insulin increased from period 3 to period 10 in experimental group, however, in control buffaloes kept under showers, insulin concentration decreased and was lowest (6.96±0.40 ng/ml) during period 6 in comparison to experimental (11.85±0.85 ng/ml; Figure 21). The overall average plasma insulin was significantly different between the groups (P<0.01) and was 10.86 vs. 8.30 μU/ml in experimental and control groups, respectively (Table 49; Figure 23). Plasma insulin did not vary between periods and between animals, however, the interaction of animal x group x period was significant (P<0.01; Table 56). Plasma cortisol concentration in control group was higher (P<0.01) in comparison to experimental (4.80 vs. 2.60 ng/ml; Table 49). The animal to animal variability in cortisol concentration was not significant (Table 56).

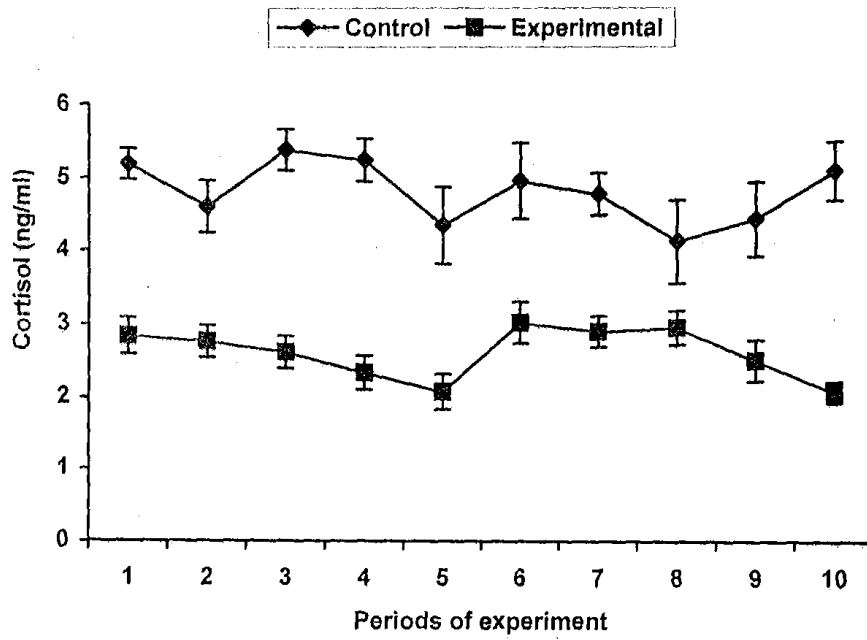
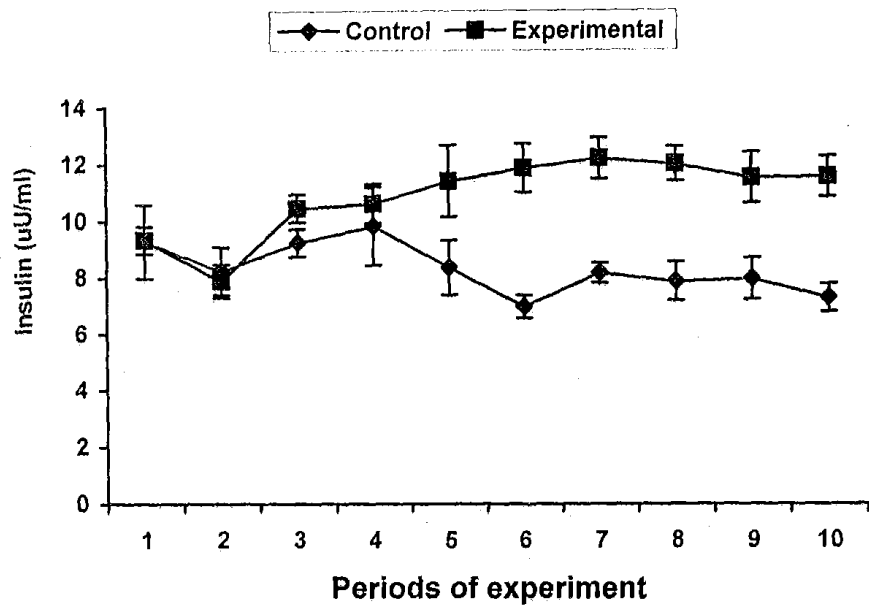
#### **4.5.2 Blood metabolites:**

In experimental buffaloes, wallowing increased glucose levels during period 1 and a steady increase was found till period 10 of study. But in control group, glucose varied during different periods (Figure 25). The overall average glucose was 63.95 vs. 53.19 mg% in experimental and control group (Table 50; Figure 24) and was significantly different (P<0.01). Further, glucose concentration varied between animals (P<0.01) but variation in glucose during different periods of the study was non-significant (Table 56).

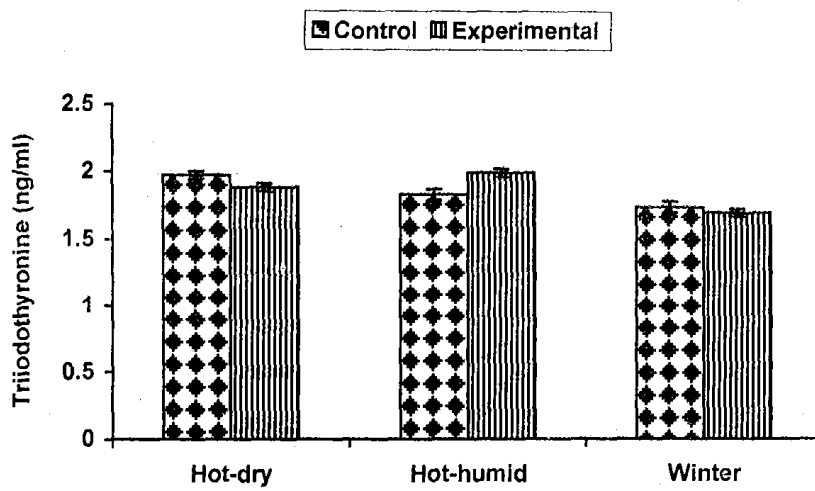
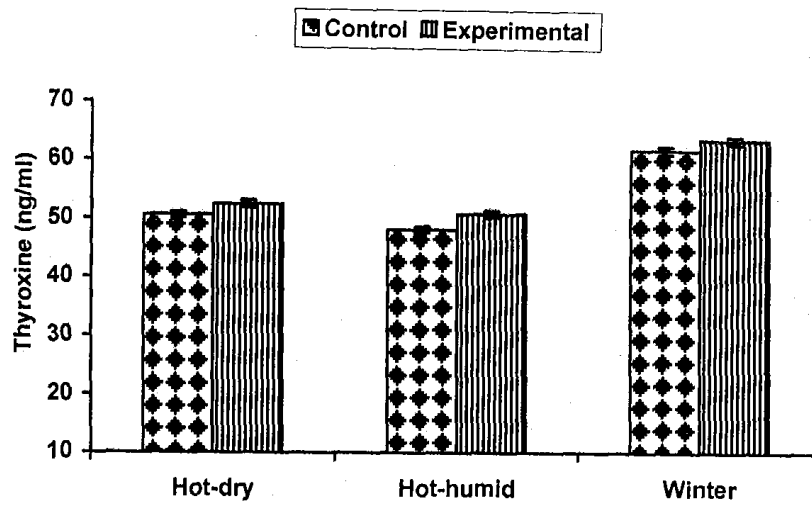
Average plasma NEFA concentration was lower 253.50 μmol/l in experimental than control group 292.50 μmol/l and was significantly different (P<0.05). Further, between animal variation was also significant (P<0.01; Table 56). Wallowing did not influence the glucose levels with a



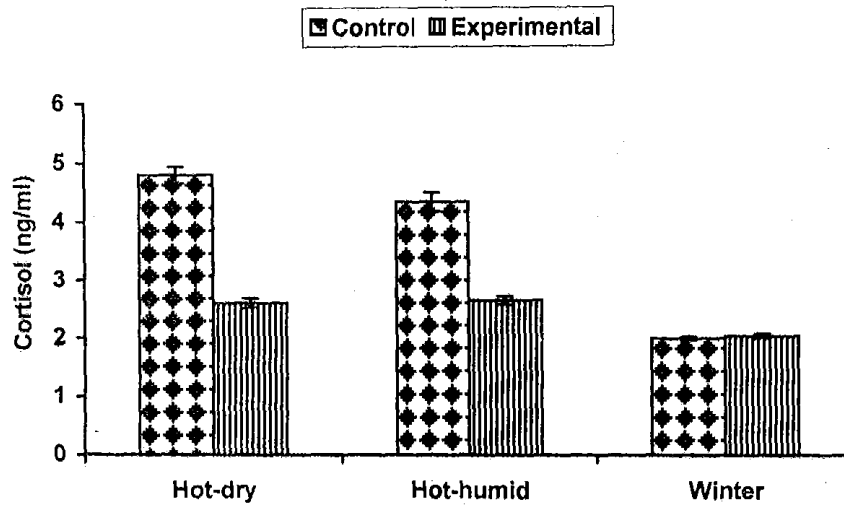
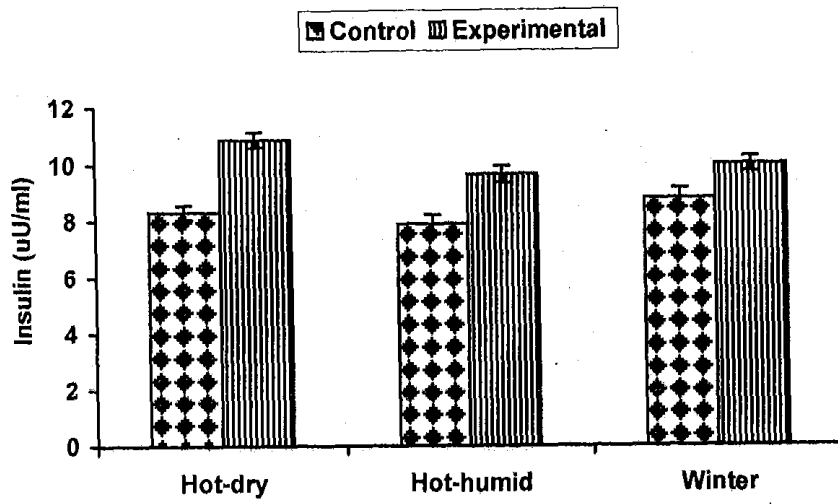
**Figure 20. Plasma thyroxine and triiodothyronine levels during hot-dry season in buffaloes**



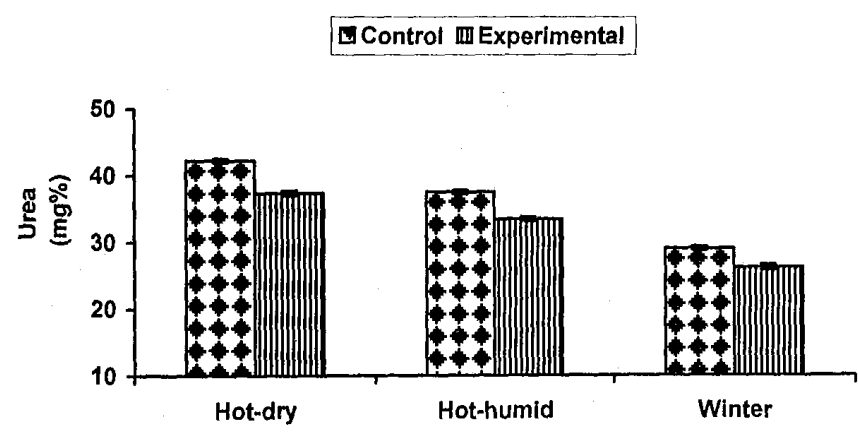
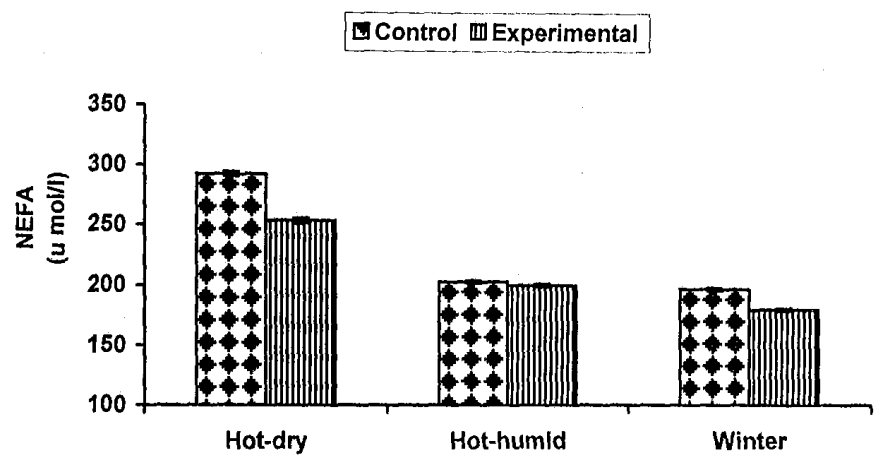
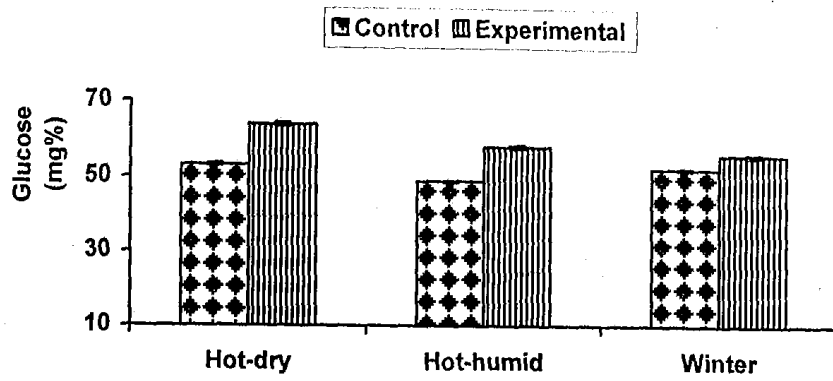
**Figure 21. Plasma insulin and cortisol levels during hot-dry season in buffaloes**



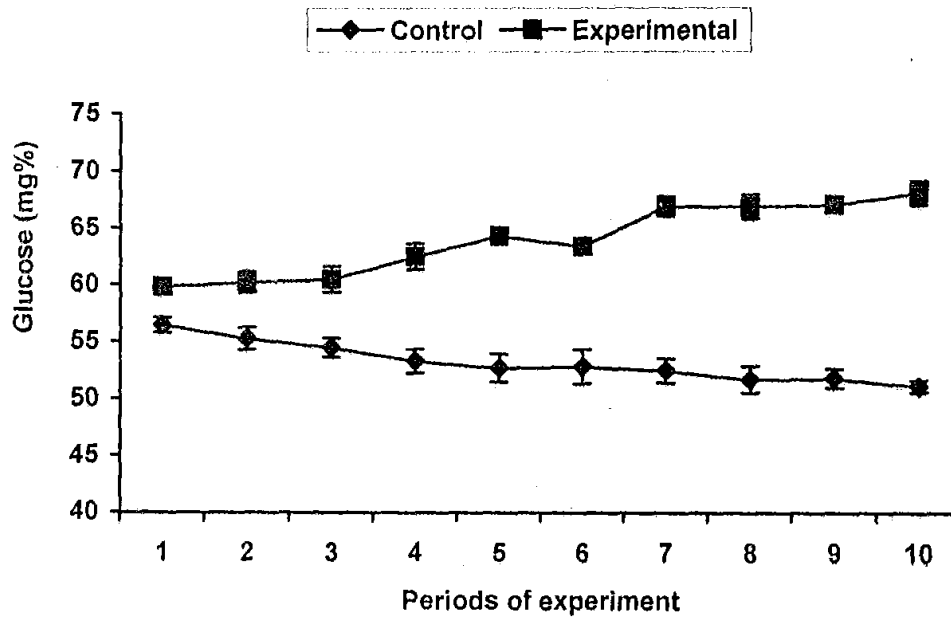
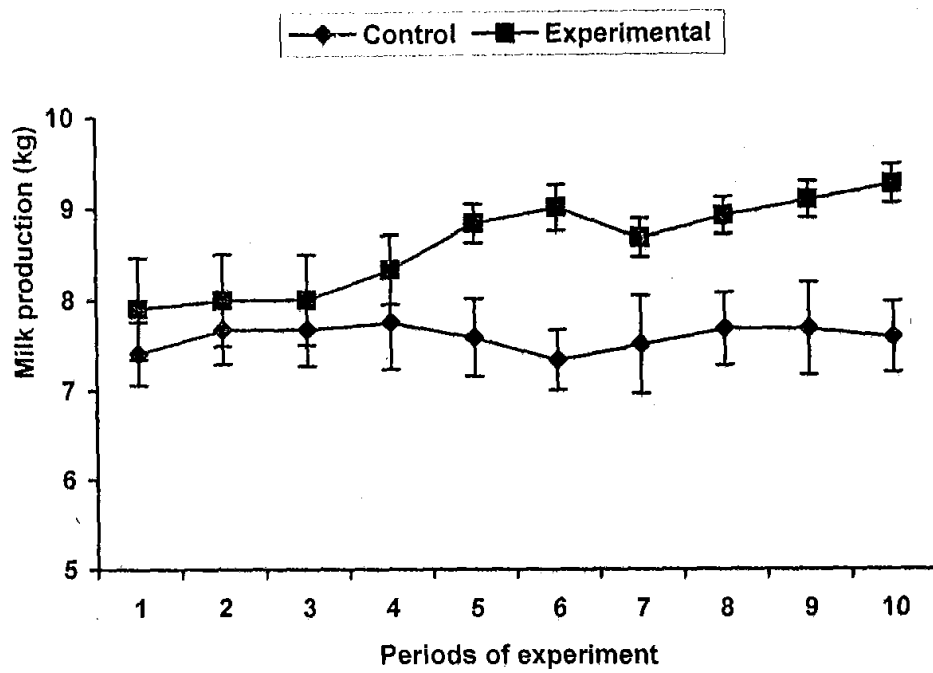
**Figure 22. Overall average plasma thyroxine and triiodothyronine levels during different seasons in buffaloes**



**Figure 23. Overall average plasma insulin and cortisol levels during different seasons in buffaloes**



**Figure 24. Overall average plasma glucose, NEFA and urea levels during different seasons in buffaloes**



**Figure 25. Milk production and glucose levels during hot-dry season in buffaloes**

distinct pattern of change, however, in control group NEFA increased continuously.

Average plasma urea was similar in control and experimental group during period 1 (40.01 vs. 40.32 mg%; Table 50). Thereafter, plasma urea decreased in experimental group of animals in comparison to control in which an increase in plasma urea occurred. Overall average plasma urea was less in experimental in comparison to control (37.33 vs. 42.19 mg%; Figure 24) and was significantly different ( $P < 0.01$ ). Further, plasma urea non-significantly varied between periods of the study but changes were significant between animals ( $P < 0.01$ ) and interaction of animal x group x period was significant ( $P < 0.01$ ; Table 56).

#### **4.5.3 Physiological responses:**

Average RT was similar in control and experimental group (101.40 vs. 101.34<sup>0</sup>F) during morning. However, changes in RT of two groups of animals were significant ( $P < 0.01$ ) during evening. During morning RT non-significantly varied between animals, however, between periods changes were significant ( $P < 0.01$ ; Table 58). Average RT decreased from 101.40 to 101.24<sup>0</sup>F during evening in control group but in experimental group it decreased from 101.34 to 100.47<sup>0</sup>F (Table 51 & 52). The variation in RT between periods ( $P < 0.05$ ) and between periods x groups was significant ( $P < 0.01$ ) but between animals variation in RT was non-significant. The average RT during evening was more ( $P < 0.01$ ) in control in comparison to experimental group. The pooled data of morning and evening of control and experimental group indicated significant variation in RT of two groups of animals ( $P < 0.01$ ) but between animals and between periods effect was non-significant (Table 57).

Average RR in control and experimental was 24.13 vs. 23.33 breaths/min. and was significantly different ( $P < 0.01$ ) during morning (Table 51 & 58). Further, variation in RR between the animals and between periods was not significant. During evening RR decreased from

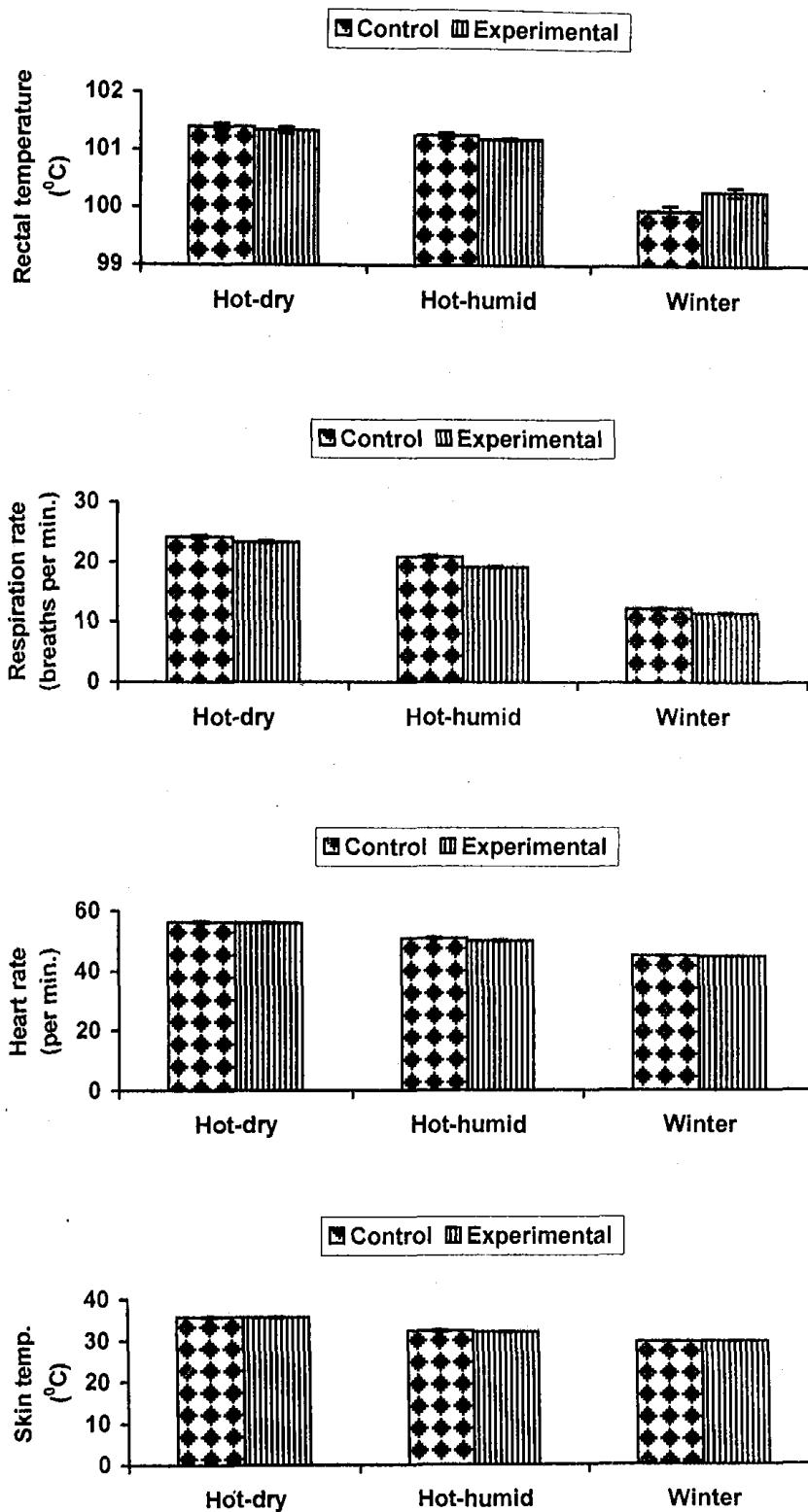
24.13 to 21.20 breaths/min. in control group in comparison to 23.33 to 16.33 breaths/min. in experimental group (Figure 26 & 27). Further, between periods and interaction of periods x groups was significant ( $P < 0.05$ ). RR varied non-significantly between animals. Average HR was higher in control ( $53.83 \pm 0.29$  /min.) in comparison to experimental ( $46.00 \pm 0.34$  /min.) and was significantly different ( $P < 0.01$ ) during evening (Table 52 & 58). However, during morning the changes in HR between the animals, between groups and during different periods of study were non-significant. The overall morning average HR was 56.30 vs. 56.00/min. in control and experimental groups, respectively. The average HR decreased due to wallowing during different periods of the study in comparison to control. The average HR varied from 52.00 to 56.00 and 43.00 to 49.00/min. in control and experimental groups during evening (Table 52).

ST non-significantly varied between groups during morning but the changes in ST was significant ( $P < 0.01$ ) during evening (Table 58). Average ST in control and experimental group was similar during morning ( $35.70$  vs.  $35.68^{\circ}\text{C}$ ) and decreased to  $33.89^{\circ}\text{C}$  in control group kept under showers, however, in experimental average ST decreased to  $31.42^{\circ}\text{C}$  (Table 52; Figure 26 & 27). During evening ST varied non-significantly between animals and between periods of the study.

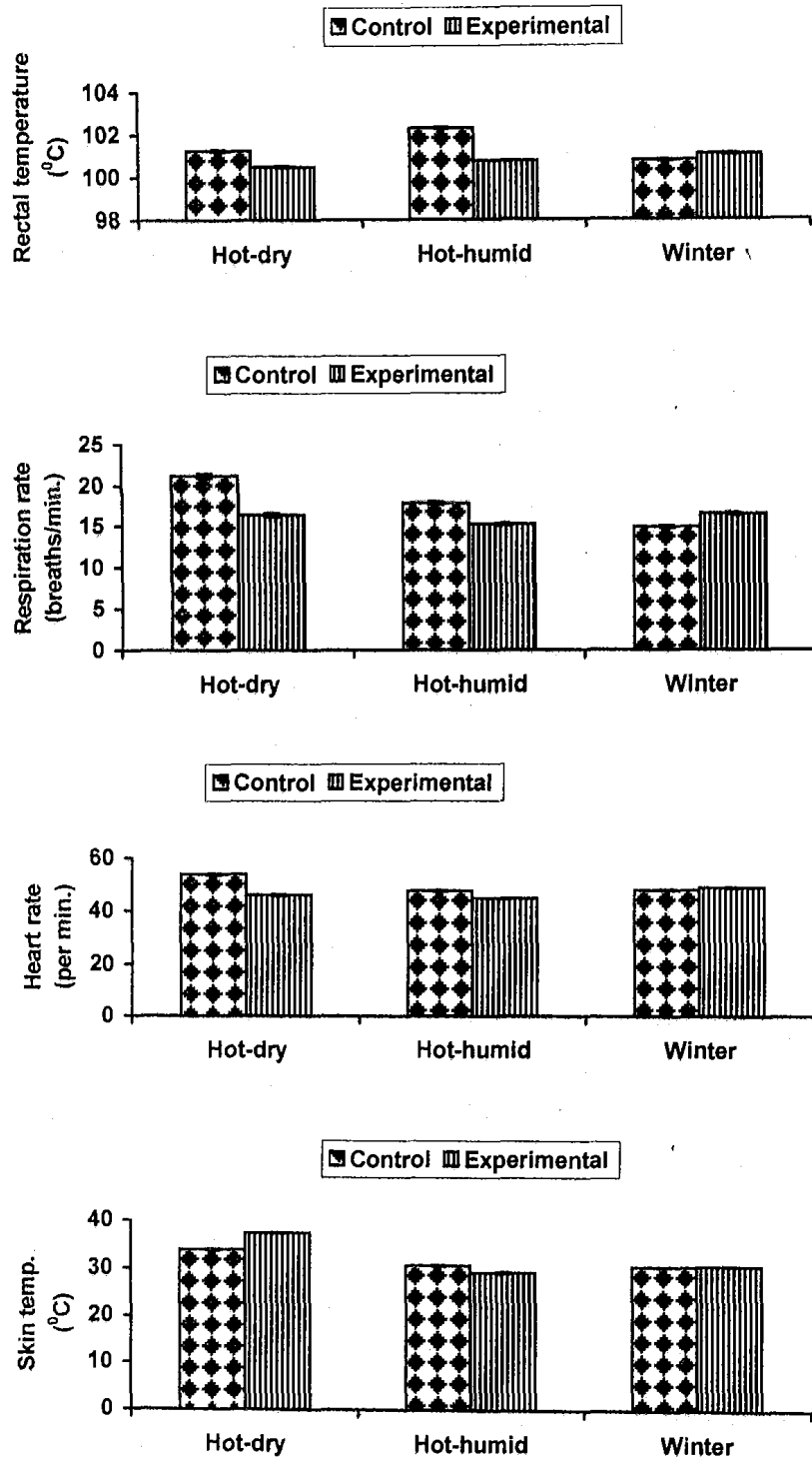
#### **4.5.4 Body weight changes, feed and water intake:**

During hot-dry season, body weight of buffaloes was significantly different due to the initial differences in the body weight of both the groups. The body weight averaged 504.13 vs. 523.14 kg in control and experimental buffaloes (Table 80b).

Feed intake increased virtually during the different weeks of the study in experimental buffaloes, however, in control, it declined during 2<sup>nd</sup> week and was maintained thereafter. Overall feed intake was higher



**Figure 26. Overall average morning rectal temperature, respiration rate, heart rate and skin temperature during different seasons in c buffaloes**



**Figure 27. Overall average evening rectal temperature, respiration rate, heart rate and skin temperature during different seasons in buffaloes**

( $P < 0.05$ ) in experimental buffaloes ( $43.91 \pm 0.87$  kg) than the control group ( $39.34 \pm 0.54$  kg; Table 80b).

Water intake was initially similar in both the groups. However, water intake decreased ( $P < 0.05$ ) in experimental buffaloes in comparison to control. The overall water intake was  $64.56 \pm 1.05$  litres against  $59.26 \pm 1.93$  litres in experimental buffaloes (Table 80b).

#### **4.5.5 Milk production :**

Milk production of the experimental group of animals was high ( $8.60 \pm 0.12$  kg) in comparison to control ( $7.58 \pm 0.12$  kg) and was significant ( $P < 0.01$ ). Milk production also varied significantly between animals ( $P < 0.01$ ) however, between periods there was no significant change in milk production (Table 59). The milk production was 13.45% higher (4.30 to 22.78%) during different periods in experimental group (Figure 16). The effect of wallowing was clearly seen on milk production of experimental buffaloes from period 1 of the study, however, in control, milk production was maintained (Table 53; Figure 25).

#### **4.5.6 Milk composition:**

The average values of total solids significantly varied between the animals and between groups ( $P < 0.01$ ) and was high in experimental ( $17.73 \pm 0.04\%$ ) in comparison to control ( $17.41 \pm 0.03\%$ ; Table 54 & 59). However, between period variation was non-significant. Average fat content of milk increased in experimental buffaloes from period 1 of the study and was significantly different between the groups ( $P < 0.01$ ). The overall average fat was  $7.42 \pm 0.04\%$  in wallowing buffaloes in comparison to  $6.92 \pm 0.05\%$  in control (Figure 33). Fat percent also varied ( $P < 0.01$ ) between the animals but between period variation was non-significant (Table 59). In control group, the average fat remained similar during different periods of the study.

Milk protein varied significantly between groups ( $P < 0.01$ ) and was high ( $5.27 \pm 0.05\%$ ) in wallowing buffaloes than control kept under

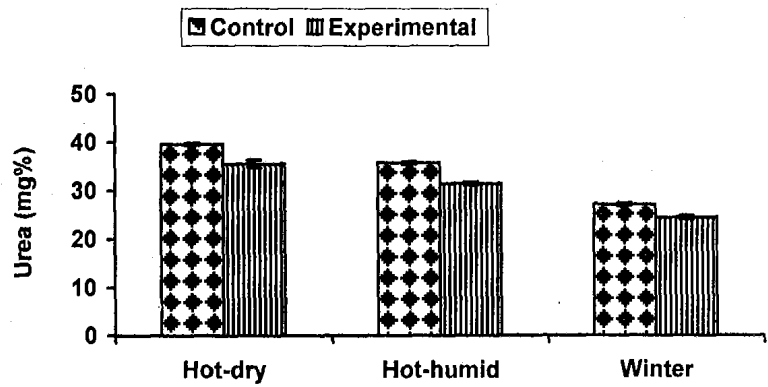
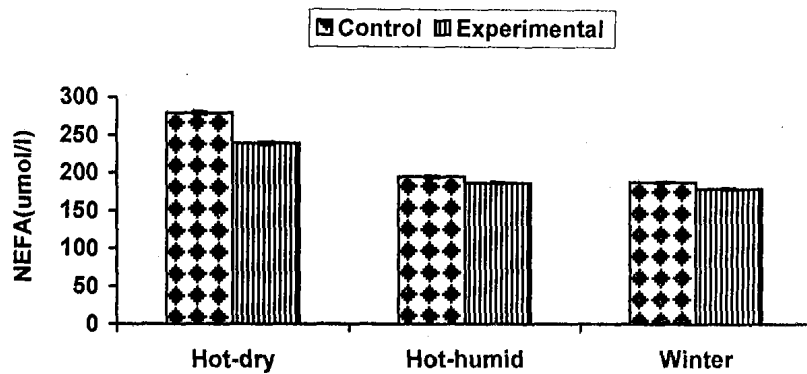
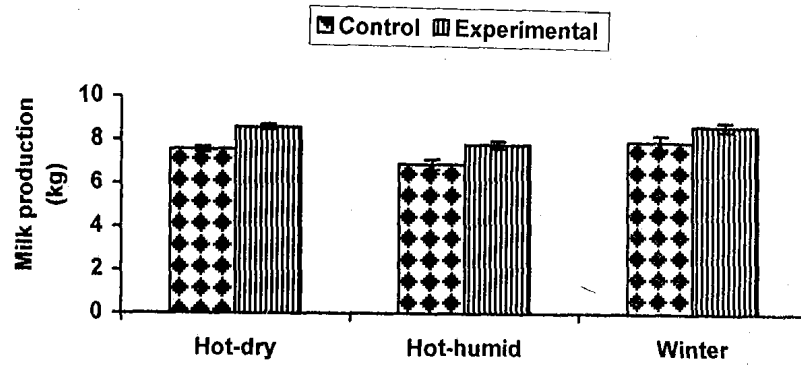
showers ( $4.38 \pm 0.04\%$ ; Table 54 & 59). Average protein increased from  $4.73 \pm 0.11$  to  $5.66 \pm 0.10\%$  from period 1 to 10 of the study. However, in control it was maintained. The variation in protein between animals and between periods was non-significant.

Like protein, lactose content of milk also varied between the groups ( $P < 0.01$ ) but between animal and between periods variation in lactose was non-significant. Average lactose also increased due to wallowing from period 1 of the study in experimental group but in control average lactose declined.

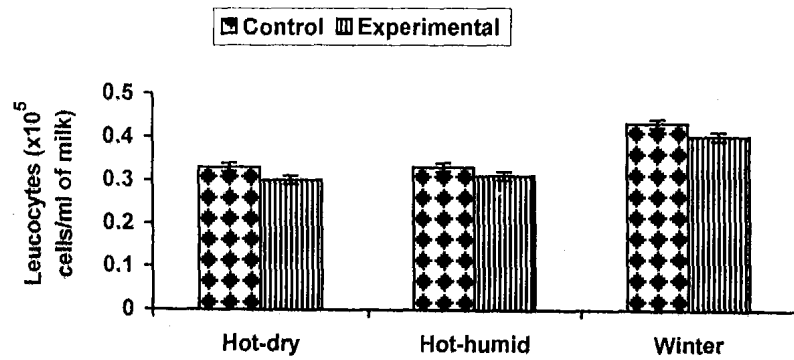
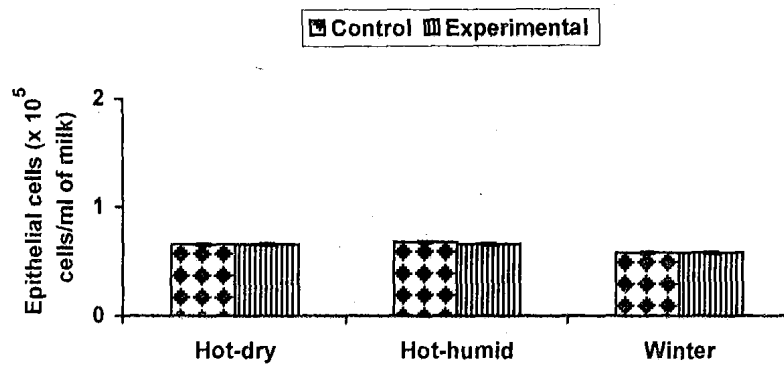
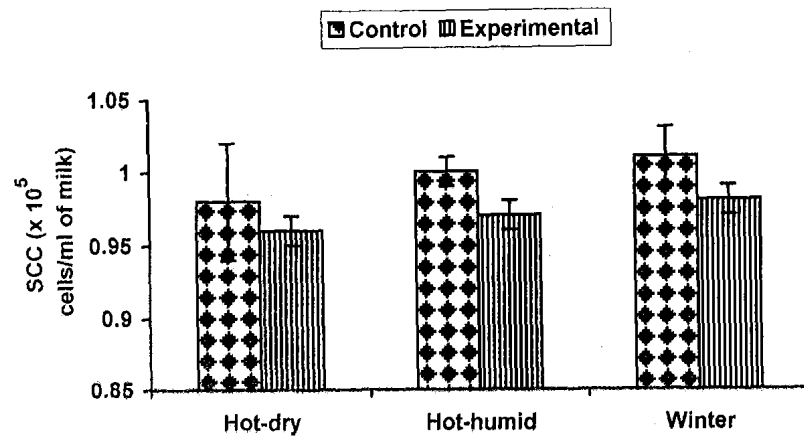
Milk NEFA varied significantly between the groups ( $P < 0.01$ ) due to less NEFA concentration in experimental group ( $239.41 \pm 2.03 \mu\text{mol/l}$ ) in comparison to control ( $279.50 \pm 1.91 \mu\text{mol/l}$ ; Table 53; Figure 28). Average NEFA concentration was high during period 1 in control and experimental group but declined to low value of  $231.67 \pm 7.14 \mu\text{mol/l}$  in experimental group during period 10 of the study, however, in control, average NEFA levels increased. A similar pattern of change was found in milk urea concentration in experimental group. The average milk urea in experimental group decreased from period 1 of the study to low levels of  $33.84 \text{ mg}\%$  by period 10, but in control group milk urea levels increased gradually from period 1 to 10 of the study. Urea varied significantly ( $P < 0.01$ ) between control and experimental group but between animal and between periods, NEFA concentration was non-significant (Table 59).

#### **4.5.7 Somatic cell count:**

SCC of milk non-significantly varied between the groups and between animals and the overall average was  $0.98 \pm 0.04$  and  $0.96 \pm 0.01 \times 10^5$  cells/ml of milk in control and experimental groups, respectively (Table 55; Figure 29). In both the groups of buffaloes SCC decreased, however, there was no distinct pattern of change. Average epithelial cells of milk were similar in control and experimental buffaloes and non-



**Figure 28. Overall average milk production, milk NEFA and urea levels during different seasons in buffaloes**



**Figure 29. Overall average somatic cell count (SCC), epithelial cells and TLC during different seasons in buffaloes**

significantly varied between groups and between animals but between periods variation was significant ( $P < 0.05$ ; Table 59).

Average TLC varied significantly between groups and between periods ( $P < 0.01$ ) and was higher in control ( $0.33 \pm 0.01 \times 10^5$  cells/ml of milk) in comparison to experimental buffaloes ( $0.30 \pm 0.01 \times 10^5$  cells/ml of milk). Further, interaction of animal  $\times$  group  $\times$  periods was also significant. On percent basis the ratio of epithelial cells to TLC was 66:34 and 67:33 in control and experimental buffaloes (Table 55).

#### **4.5.8 Correlation coefficients:**

The correlation data indicated a positive correlation of maximum temperature with RT ( $P < 0.05$ ) in control group but in experimental the correlation was non-significant. Milk production was negatively correlated to RR ( $P < 0.05$ ) in control group, however, in experimental group, milk production was negatively correlated with RT, RR, HR and ST ( $P < 0.01$ ). RT was positively correlated with ST in control ( $P < 0.05$ ) and experimental group ( $P < 0.01$ ), but in experimental group RT was positively correlated with RR and HR ( $P < 0.01$ ). RR was positively correlated with ST in both control and experimental group, however, in experimental group RR was also positively correlated to HR ( $P < 0.01$ ; Table 61). Further, HR was positively correlated with ST ( $P < 0.01$ ) in both the groups of animals. The pooled data analysis of control and experimental group (Table 60) also indicated a positive correlation of maximum temperature with RT ( $P < 0.05$ ). The ST was negatively correlated ( $P < 0.01$ ) with milk production and positively to RT, HR, RR ( $P < 0.01$ ). HR was negatively correlated with milk production ( $P < 0.01$ ) and positively with RT and RR ( $P < 0.01$ ). RR was found to be positively correlated with RT ( $P < 0.01$ ). RT was negatively correlated to milk production ( $P < 0.01$ ; Table 60).

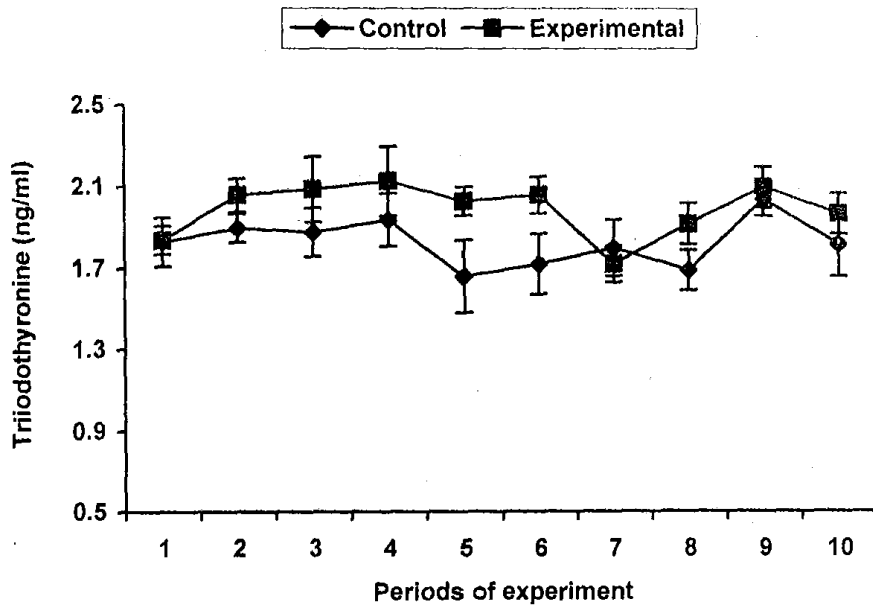
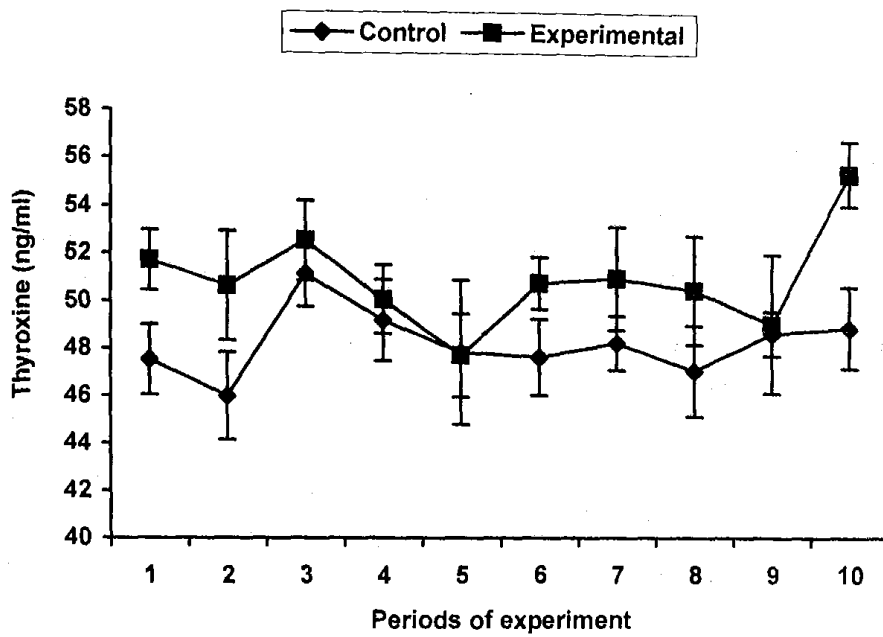
Experiments during hot-dry season in buffaloes indicated a positive correlation of maximum temperature with plasma  $T_4$  ( $P < 0.01$ ). Plasma  $T_4$  concentration was negatively correlated to cortisol, SCC,

plasma NEFA ( $P<0.05$ ) and plasma urea ( $P<0.01$ ).  $T_3$  concentration was neither correlated to milk production, metabolites nor with milk composition. Insulin level was negatively correlated to cortisol, plasma glucose, NEFA and urea ( $P<0.01$ ). During hot-dry season cortisol was positively correlated to glucose, plasma NEFA and urea ( $P<0.01$ ). Milk production was positively correlated to TS, fat, protein, lactose and milk NEFA and urea ( $P<0.01$ ). SCC of milk was positively correlated ( $P<0.01$ ) to epithelial cells and TLC of milk while epithelial cells was negatively correlated to TLC ( $P<0.01$ ). Fat content of milk was positively correlated to protein, lactose, milk NEFA and urea ( $P<0.01$ ) while protein was positively correlated to milk NEFA and urea (Table 62).

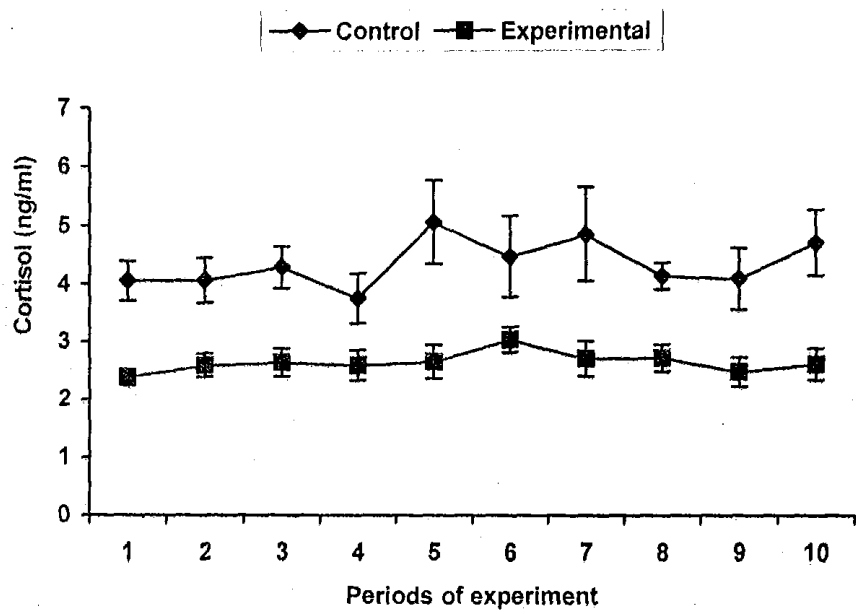
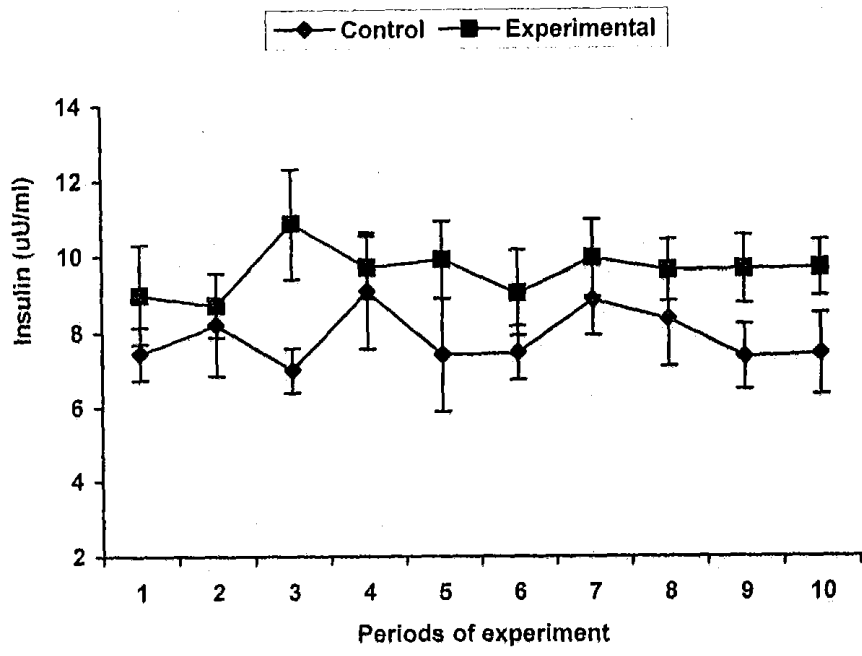
#### **4.6 Effect of Showers and Wallowing During Hot-Humid Season in Buffaloes:**

##### **4.6.1 Hormones:**

Average  $T_4$  concentration increased ( $P<0.01$ ) due to wallowing in experimental buffaloes and average  $T_4$  concentration was higher ( $P<0.01$ ) in experimental buffaloes than the control (50.97 vs. 48.25 ng/ml; Table 63).  $T_4$  concentration did not vary between periods and between animals. Plasma  $T_3$  varied significantly between groups ( $P<0.01$ ) and was high ( $1.99\pm 0.03$  ng/ml) in experimental in comparison to control group ( $1.83\pm 0.04$  ng/ml; Figure 21). Average plasma  $T_3$  increased during period 2 to 6 in experimental group, however, in control a decline was observed during the period (Figure 30). The variation in  $T_3$  between animals and between periods was non-significant (Table 70). Plasma insulin varied significantly between groups ( $P<0.01$ ) and was higher in experimental than the control group (9.62 vs. 7.86  $\mu$ U/ml) but there was no significant variation in insulin concentration between animals and between periods (Table 63 & 70). Cortisol concentration declined ( $P<0.01$ ) in wallowing buffaloes in comparison to control (Table 70; Figure 31). The overall



**Figure 30. Plasma thyroxine and triiodothyronine levels during hot-humid season in buffaloes**



**Figure 31. Plasma insulin and cortisol levels during hot-humid season in buffaloes**

average value of cortisol was  $4.33 \pm 0.16$  and  $2.64 \pm 0.32$  ng/ml (Table 63). Between animal variation for cortisol was not significant.

#### **4.6.2 Blood metabolites:**

Average plasma glucose concentration increased gradually from period 1 in experimental group but in control it fluctuated (Figure 32). The overall average glucose level was high ( $P < 0.01$ ) in experimental group ( $58.11 \pm 0.45$  mg%) than the control ( $49.08 \pm 0.28$  mg%; Table 64) and varied between periods ( $P < 0.01$ ) however, between animal variation was non-significant.

Average NEFA levels did not exhibit a distinct pattern of change in relation to wallowing in experimental group and a similar pattern was also found in control. However, average NEFA was non-significantly less ( $200.25 \pm 1.35$   $\mu\text{mol/l}$ ) in experimental buffaloes than the control ( $203.25 \pm 1.15$   $\mu\text{mol/l}$ ; Table 64 & 70). NEFA changes between animals and between periods were non-significant. Average plasma urea concentration was high in control group and significantly varied ( $P < 0.01$ ) between the groups. The overall average urea was  $37.53 \pm 0.28$  mg% in control in comparison to  $33.46 \pm 0.30$  mg% in experimental buffaloes (Figure 24). Further, urea levels varied between the periods ( $P < 0.01$ ) and between animals ( $P < 0.01$ ; Table 70).

#### **4.6.3 Physiological responses:**

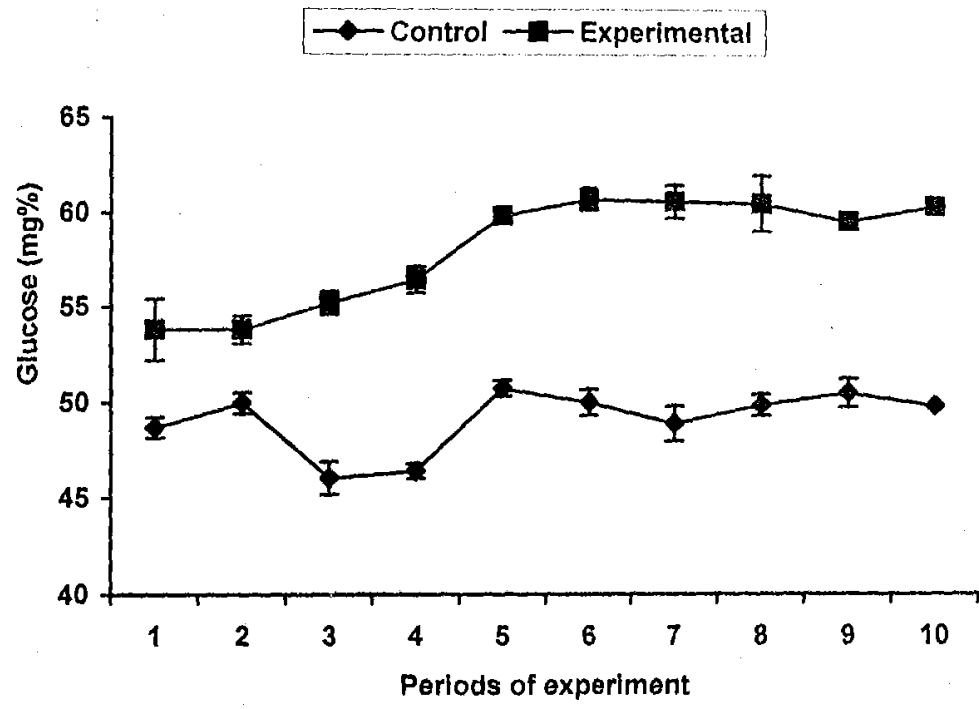
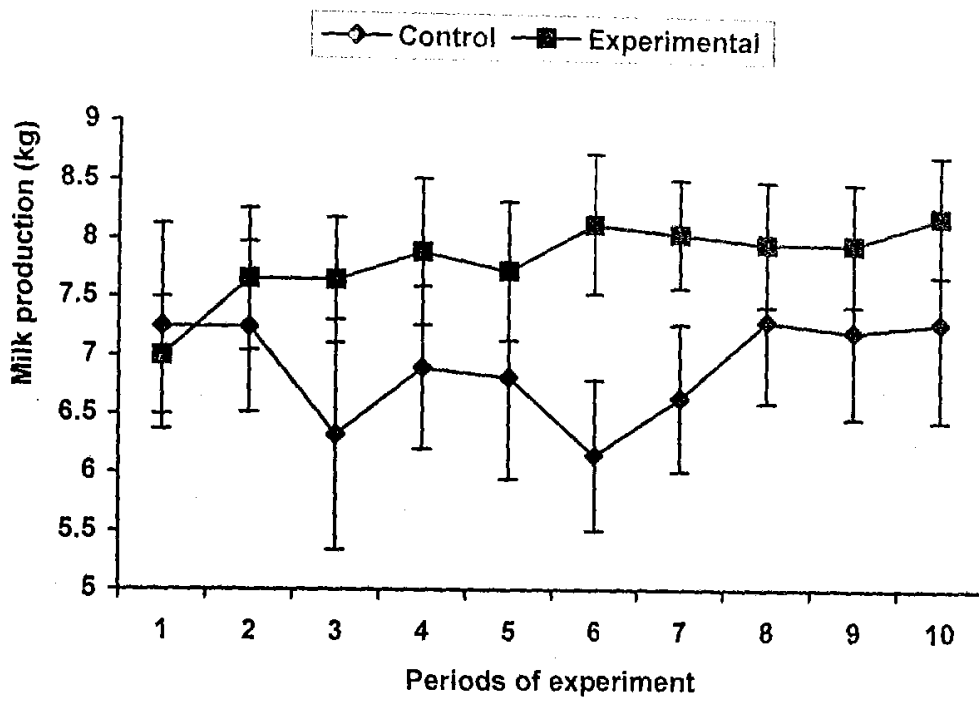
Average morning RT non-significantly varied between the groups, but changes in evening RT was significant ( $P < 0.01$ ). The average RT was  $101.27^{\circ}\text{F}$  during morning and increased to  $102.27^{\circ}\text{F}$  during evening in control group, however, in experimental group the overall average RT decreased from morning RT value of  $101.20$  to  $100.73^{\circ}\text{F}$  (Table 65 & 66). RT was high in control than the experimental buffaloes during evening and was significantly different ( $P < 0.01$ ) but between animals and between periods variation was non-significant. However, during morning

between period and interaction of period x group was significant ( $P<0.01$ ) which was not found for RT during evening (Table 72).

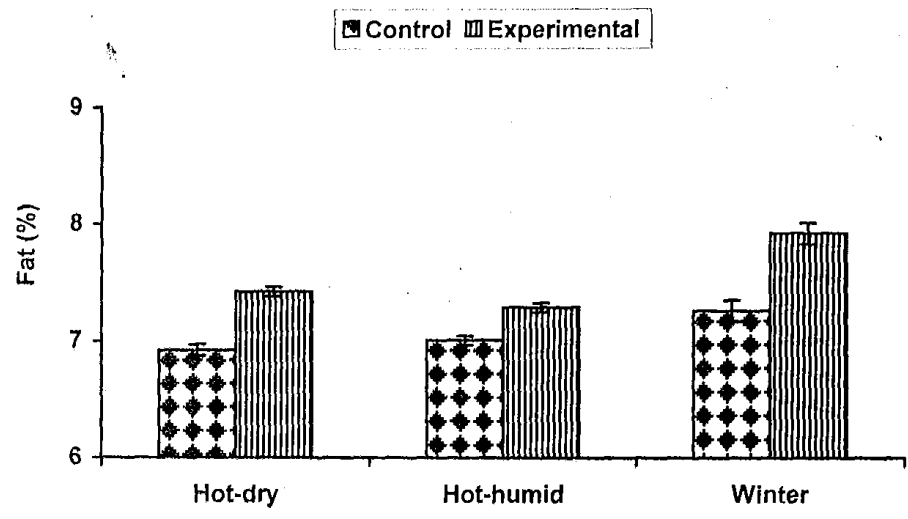
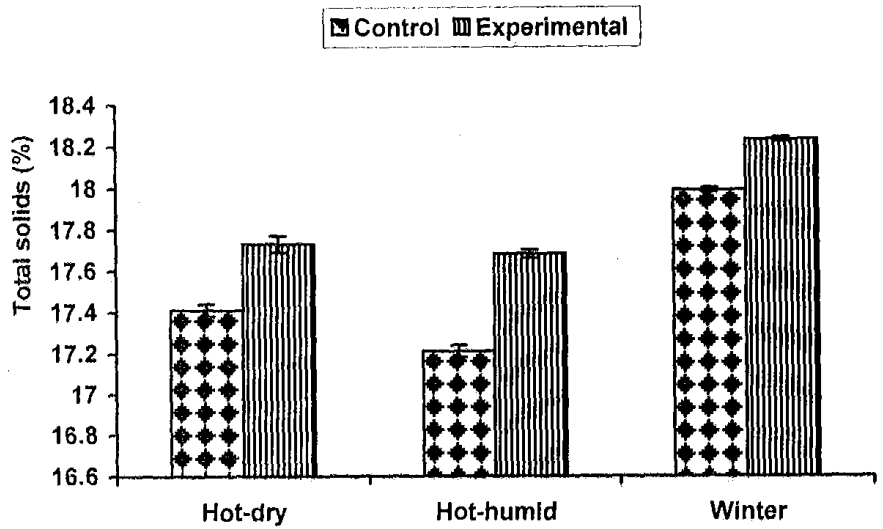
Average RR was significantly different between the groups ( $P<0.01$ ) during morning and evening hours of recording, however, between animal variation was non-significant. Further, RR also varied between periods ( $P<0.05$ ) during morning and evening. Average RR was high during morning in control ( $20.93\pm 0.22$  breaths/min.) in comparison to experimental ( $19.20\pm 0.15$  breaths/min.). The similar pattern was found during evening where RR was high in control than the experimental buffaloes (17.80 vs. 15.20 breaths/min.). RR declined from morning values of 20.93 to 17.80 and 19.20 to 15.20 breaths/min. in control and experimental buffaloes respectively (Table 65 & 66).

Average HR was significantly different ( $P<0.05$ ) between the groups during morning and the respective values were 51.03 vs. 50.16 /min. in control and experimental group. HR varied significantly between periods ( $P<0.01$ ) but there was no significant variation between animals (Table 72). Average HR in the evening also varied significantly ( $P<0.01$ ) between groups and was significantly less in experimental than the control (44.70 vs. 47.60 /min.). Average HR declined during evening in both the groups, however, decline was more in experimental (from 50.16 to 44.70 /min.) in comparison to control group (51.03 to 47.60 /min.; Figure 26 & 27). The changes in HR between the animals and between periods were non-significant.

Average ST did not vary between the groups and was  $32.37\pm 0.22$  and  $32.10\pm 0.13^{\circ}\text{C}$  in control and experimental group respectively (Table 65). There was no significant variation in ST between animals but between period variation was significant ( $P<0.01$ ). During evening ST significantly varied between groups and between periods ( $P<0.01$ ), however, between animal variation was non-significant. The average ST in experimental group during evening remained low in comparison to control during different periods of the study with overall average values



**Figure 32. Milk production and glucose levels during hot-humid season in buffaloes**



**Figure 33. Overall average total solids and fat content of milk during different seasons in buffaloes**

being less ( $29.21 \pm 0.15^{\circ}\text{C}$ ) in comparison to control ( $30.64 \pm 0.14^{\circ}\text{C}$ ; Table 66).

#### **4.6.4 Body weight changes, feed and water intake:**

During hot-humid season, the body weights of control and experimental buffaloes non-significantly varied and the respective values were  $522.91 \pm 24.62$  and  $519.60 \pm 32.68$  kg (Table 80b).

The feed intake of buffaloes was significantly higher ( $P < 0.05$ ) in experimental buffaloes ( $45.02 \pm 10.54$  kg) in comparison to control ( $40.87 \pm 0.83$  kg; Table 80b).

The water intake of experimental buffaloes was similar to control during the 1<sup>st</sup> week of the study (55.84 vs. 54.78 litres), however, water intake declined in experimental buffaloes (Table 80b).

#### **4.6.5 Milk production:**

Average milk production was less during period 1 in experimental ( $7.00 \pm 0.50$  kg) in comparison to control ( $7.25 \pm 0.88$  kg) but increased gradually in wallowing buffaloes (Figure 32). In control group of buffaloes provided with showers, the milk production varied and was significantly different ( $P < 0.01$ ) in comparison to experimental group. The overall average value of milk production was 7.85 vs. 6.93 kg in experimental and control groups, respectively (Table 67). The percent increase in milk production during different periods of study in wallowing buffaloes in comparison to control ranged from 3.44 to 32.46% with an overall value of 13.27% (Figure 16). Milk production changes between animals were significantly different ( $P < 0.01$ ), however, between periods milk production non-significantly varied (Table 73).

#### **4.6.6 Milk composition:**

Average total solids of milk was significantly different between groups ( $P < 0.01$ ) and between periods ( $P < 0.05$ ), however, between animal variation was non-significant. Average total solids was high in experimental group in comparison to control, the respective values being

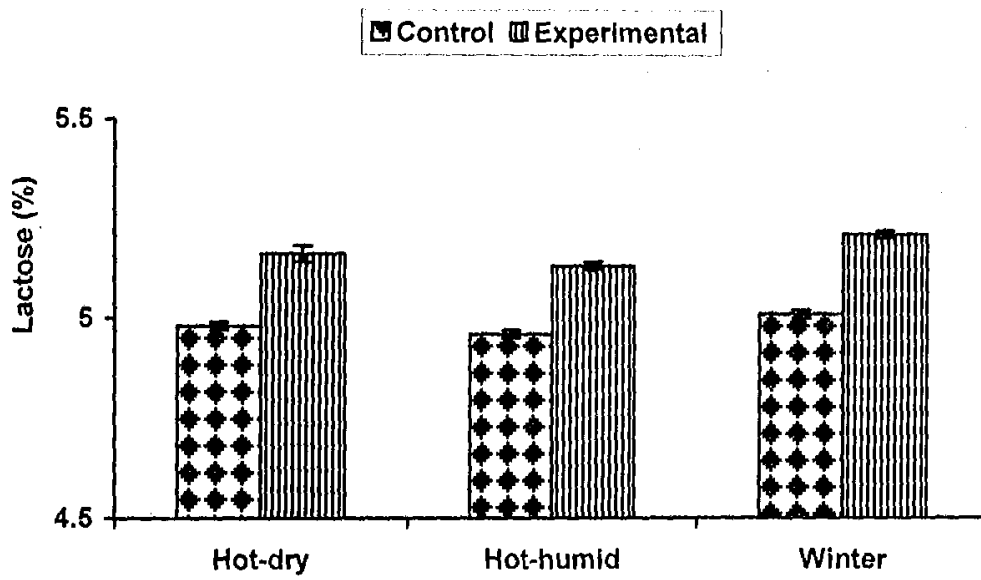
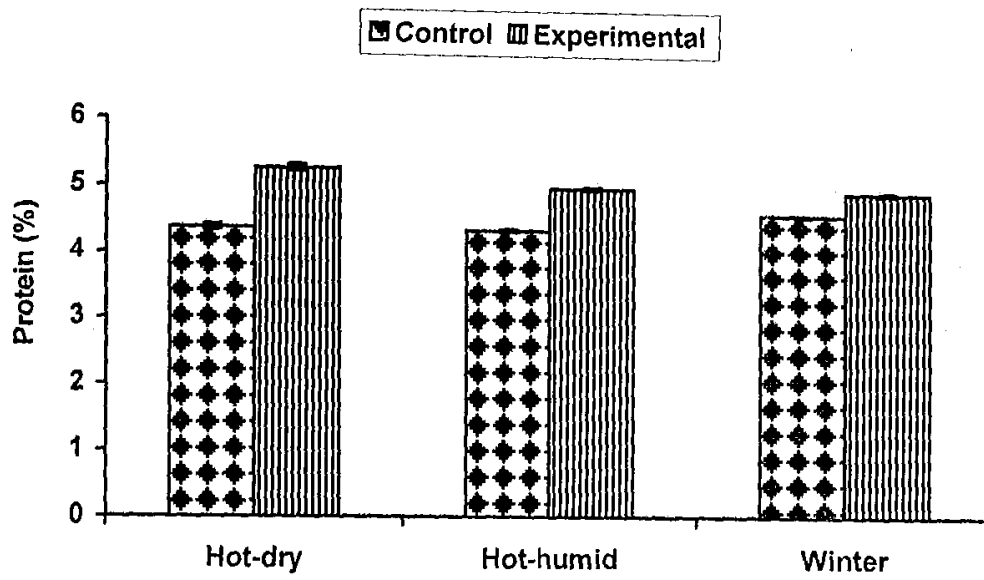
17.68 vs. 17.21% (Figure 33). Average fat was more (7.28%) in experimental than the control (7.00%) and was significantly different ( $P < 0.01$ ; Table 68 & 73). Fat content of milk also varied between animals ( $P < 0.01$ ) but between periods variation was non-significant. The effect of wallowing on fat content of milk was found during period 1 of the study.

Average protein content of milk varied significantly between groups ( $P < 0.01$ ) and between animals and between periods ( $P < 0.05$ ). Further, interaction of animal  $\times$  group  $\times$  period was also significant ( $P < 0.01$ ). Protein content of milk in experimental buffaloes increased during period 1 and such increases were maintained during different periods of the study. The overall average value of protein was high in experimental than the control group (4.99 vs. 4.35%; Table 68; Figure 34).

Lactose content of milk varied significantly ( $P < 0.01$ ) between the groups and was high in experimental ( $5.13 \pm 0.01\%$ ) than the control ( $4.96 \pm 0.01\%$ ; Table 68 & 73). Further, lactose varied between periods ( $P < 0.01$ ) and between animals ( $P < 0.05$ ). The experimental group of buffaloes had more lactose (4.97 to 5.17%) during different periods of the study in comparison to control (4.90 to 5.00%).

Average NEFA concentration in experimental buffaloes decreased during period 1 due to wallowing and during period 1 to period 5 and thereafter maintained. In control group, milk NEFA concentration was high during different periods of the study and was significantly different ( $P < 0.01$ ) than the experimental. The variability in NEFA concentration was non-significant between the animals and between periods. The overall average value of NEFA was  $195.83 \pm 1.30 \mu\text{mol/l}$  and  $187.66 \pm 1.01 \mu\text{mol/l}$  in control and experimental group, respectively (Table 67; Figure 28).

Average milk urea varied significantly between the groups ( $P < 0.01$ ) and was high in control ( $35.83 \pm 0.30 \text{ mg}\%$ ) in comparison to experimental buffaloes ( $31.50 \pm 0.32 \text{ mg}\%$ ; Table 67). Milk urea varied significantly between periods ( $P < 0.01$ ) of the study but variation in NEFA



**Figure 34. Overall average protein and lactose content of milk during different seasons in buffaloes**

concentration between animals was non-significant. There was no distinct pattern of change in milk urea in relation to wallowing in experimental buffaloes and showers in control group.

#### **4.6.7 Somatic cell count (SCC):**

Average SCC of milk non-significantly varied between animals and between periods. Further, there was no significant variation in SCC of milk between the groups. The overall average SCC was  $1.00 \pm 0.01 \times 10^5$  cells/ml of milk and  $0.97 \pm 0.01 \times 10^5$  cells/ml in control and experimental buffaloes respectively (Table 69; Figure 29). The average epithelial cells were less in experimental buffaloes in comparison to control ( $0.65$  vs.  $0.67 \times 10^5$  cells/ml) and was non-significantly different. Basal epithelial cells level was significantly lower in experimental buffaloes in comparison to control. Average TLC varied between periods of the study but there was no significant difference in TLC between the groups and between animals (Table 73). In wallowing buffaloes, average TLC was high during period 1 ( $0.39 \pm 0.03 \times 10^5$  cells/ml) and declined to  $0.29 \pm 0.02 \times 10^5$  cells/ml during period 3 and thereafter varied. The overall average TLC was high in control ( $0.33 \pm 0.01 \times 10^5$  cells/ml) than the experimental buffaloes ( $0.31 \pm 0.01 \times 10^5$  cells/ml; Table 69).

#### **4.6.8 Correlation coefficients:**

Correlation coefficient of physiological responses indicated a positive association of maximum temperature ( $P < 0.01$ ) with ST in both control and experimental buffaloes. Milk production was found to be positively correlated with RR ( $P < 0.05$ ) in control, however, milk production was negatively correlated with RT, RR ( $P < 0.05$ ) and HR ( $P < 0.01$ ). RT was positively associated to HR and ST and RR ( $P < 0.01$ ) in experimental group but in control RT alone was positively correlated to RR ( $P < 0.05$ ). RR was positively correlated to HR and ST ( $P < 0.01$ ) in experimental group but in control group RR was positively correlated

with HR ( $P < 0.05$ ) only. HR was positively correlated to ST ( $P < 0.01$ ) in experimental group only (Table 75).

The pooled analysis of morning and evening data of physiological responses indicated a positive association of maximum temperature with ST ( $P < 0.01$ ) and the milk production was negatively correlated to RT, RR, ST ( $P < 0.05$ ) and HR ( $P < 0.01$ ). RT was positively correlated to RR, HR and ST ( $P < 0.01$ ). Further, RR was positively correlated to HR and ST ( $P < 0.01$ ). ST was found to be positively correlated to HR ( $P < 0.01$ ; Table 74).

During hot-humid season, maximum temperature was negatively correlated to plasma glucose ( $P < 0.05$ ) in lactating buffaloes. Plasma concentration of  $T_4$ ,  $T_3$  and insulin was positively correlated to milk production ( $P < 0.01$ ), however, cortisol was negatively correlated with milk production ( $P < 0.01$ ). TS of milk was positively correlated with fat, protein and lactose ( $P < 0.01$ ) and negatively to milk NEFA, urea ( $P < 0.01$ ) and TLC ( $P < 0.05$ ). SCC was positively correlated to epithelial cells and TLC ( $P < 0.01$ ) and negatively with lactose ( $P < 0.01$ ). However, TLC was positively correlated to milk NEFA concentration ( $P < 0.01$ ). Fat content of milk was positively correlated with protein and lactose ( $P < 0.01$ ) and negatively with milk urea ( $P < 0.01$ ). Protein content of milk was correlated positively with lactose ( $P < 0.01$ ) and negatively with milk NEFA and urea ( $P < 0.01$ ). Lactose content of milk was found to be correlated negatively with milk NEFA and urea ( $P < 0.01$ ; Table 76).

## **4.7 Effect of Housing and Straw Bedding During Winter Season in Buffaloes:**

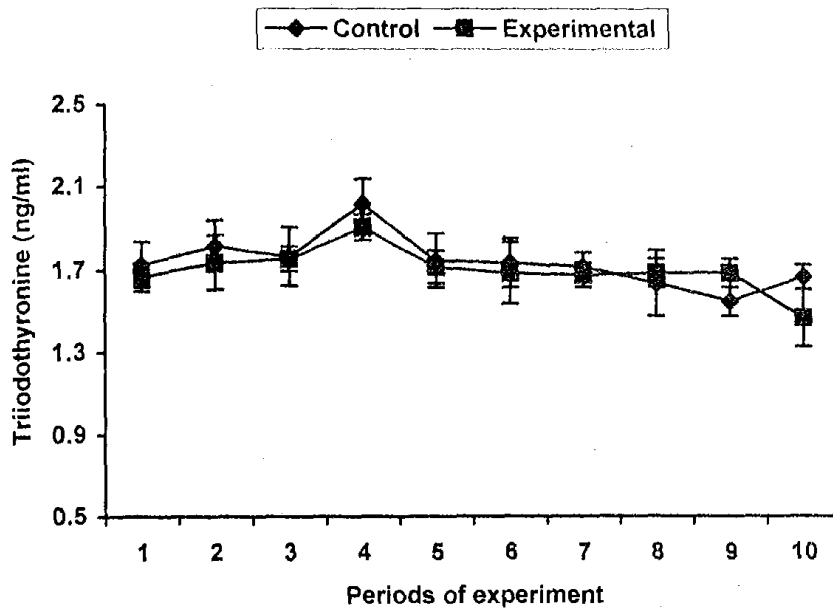
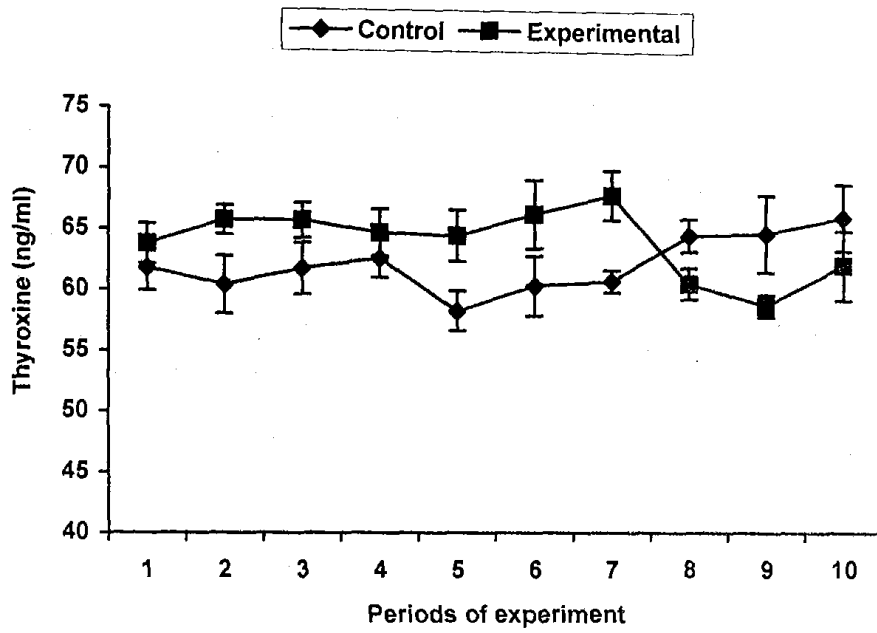
### **4.7.1 Hormones:**

Average circulatory level of  $T_4$  was significantly different ( $P < 0.01$ ) between two groups of buffaloes, however,  $T_3$  concentration non-significantly varied between the groups. Average  $T_4$  concentration was high during different periods of the study in buffaloes kept inside the houses with floor bedding and ranged from 58.86 to 67.90 ng/ml in

comparison to 58.33 to 66.21 ng/ml in control (Table 77; Figure 35). The overall average T<sub>4</sub> level was more (64.05±0.65 ng/ml) in comparison to control (62.19±0.68 ng/ml; Figure 22). There was no difference in T<sub>4</sub> concentration between periods, however, between animals concentration varied (P<0.01). Circulatory levels of T<sub>3</sub> non-significantly varied between groups with overall average levels of 1.74±0.04 ng/ml and 1.70±0.03 ng/ml in control and experimental groups, respectively (Table 77 & 84). Further, between animal and between period change was non-significant.

Insulin concentration increased in experimental buffaloes from period one of the study, however, in control insulin was maintained throughout the study (Figure 36). Average plasma insulin was low in experimental group than the control (7.79 vs. 8.48 µU/ml) during period 1 but in experimental insulin increased till period 10 of the study (Table 77). The overall average insulin was significantly higher (P<0.01) in experimental than the control groups, the respective values were 9.95±0.27 µU/ml and 8.75±0.35 µU/ml. Plasma cortisol concentration non-significantly varied between animals and between groups. However, between periods variability in cortisol was significant (P<0.05; Table 84). In both the groups cortisol concentration varied from 1.84 to 2.35 ng/ml during different periods of the study (Table 77).

Plasma T<sub>4</sub> concentration was higher during hot-dry season and declined during hot-humid season in control group of buffaloes. Further, T<sub>4</sub> concentration increased significantly during winter (Figure 22). T<sub>3</sub> concentration was high during hot-dry season and decline during hot-humid and winter season. Plasma insulin concentration in control group was also high during hot-dry and winter season and declined during hot-humid season. Cortisol concentration was also higher during hot-dry and hot-humid and was significantly low in winter in control group indicating thereby the effect of season on these hormones (Figure 23).



**Figure 35. Plasma thyroxine and triiodothyronine levels during winter season in buffaloes**

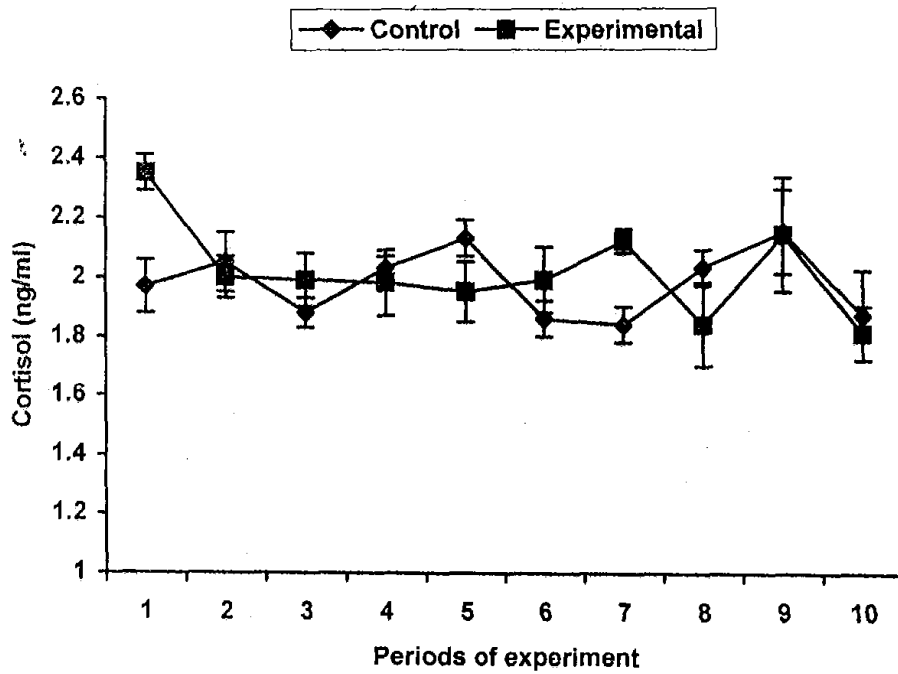
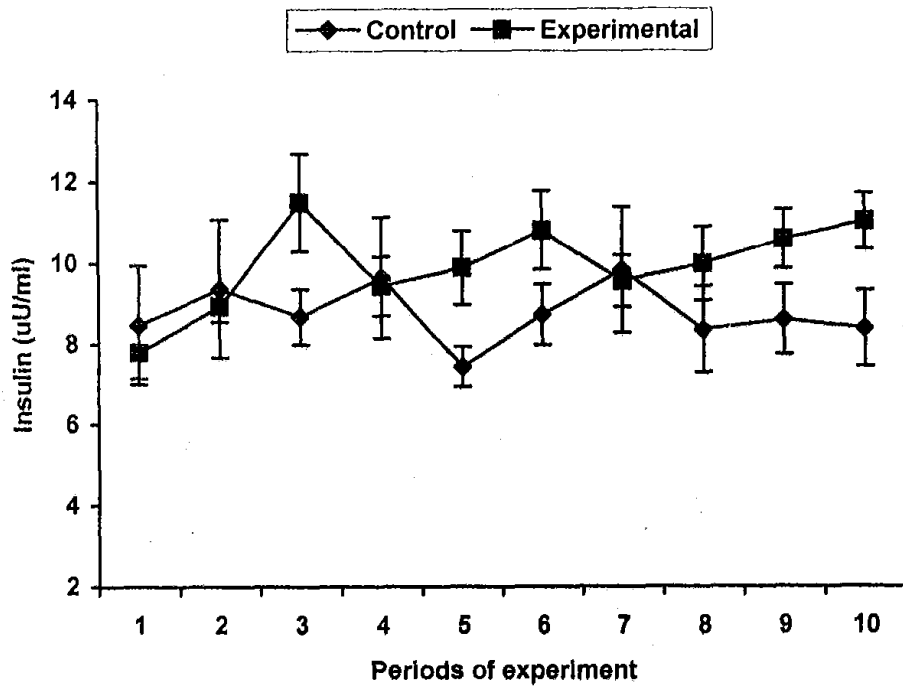


Figure 36. Plasma insulin and cortisol levels during winter season in buffaloes

The comparative account of hormonal profile (Table 91) indicated that basal concentration of T<sub>4</sub>, T<sub>3</sub> and insulin was significantly higher (P<0.01) in buffaloes than the cows.

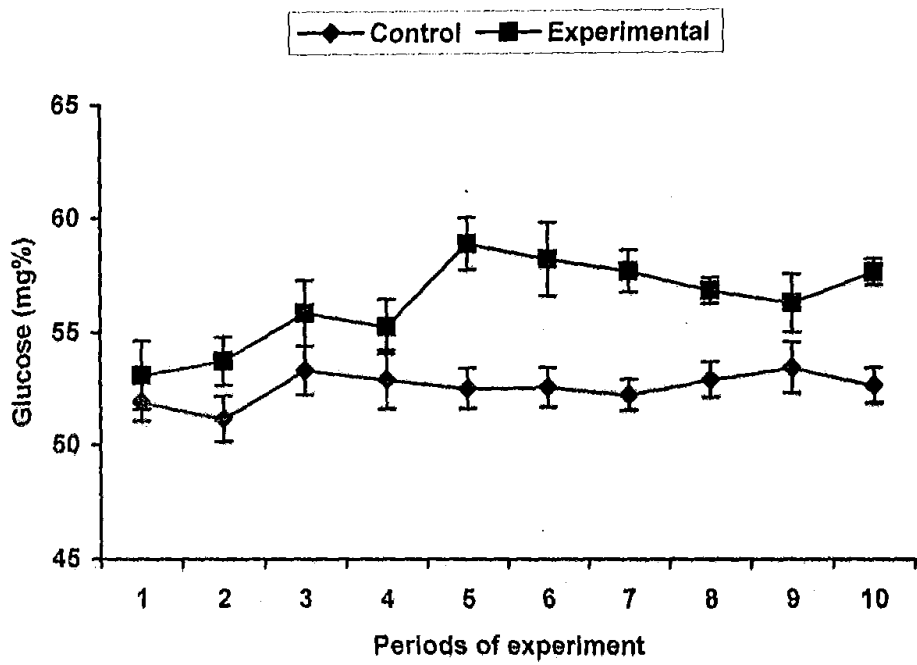
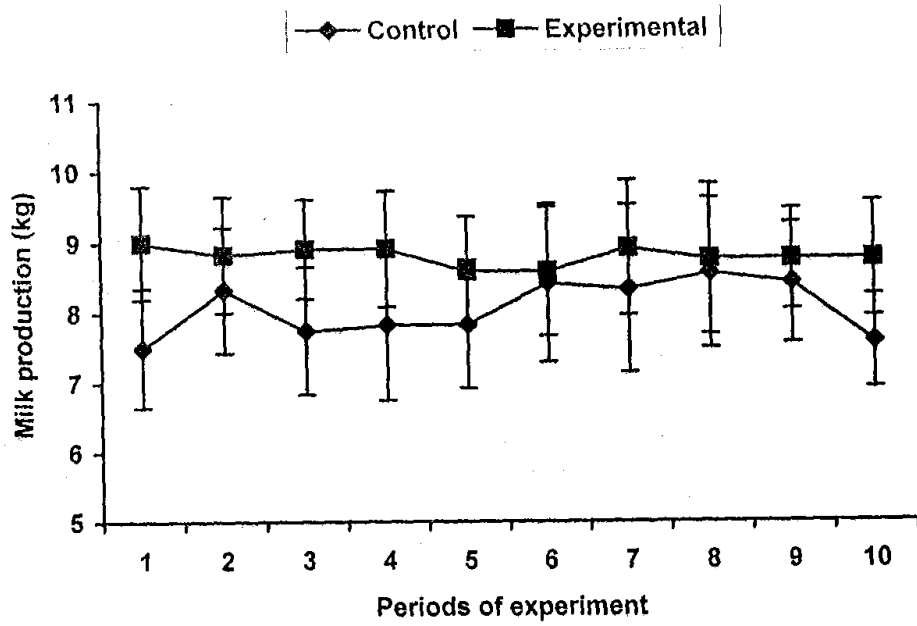
#### **4.7.2 Blood metabolites:**

Average plasma glucose was significantly (P<0.01) more in experimental than the control (53.14 vs. 51.96 mg%) during period one, which further increased to peak level of 57.52 to 58.83 mg% during different periods of the study in experimental group. But in control group glucose levels were maintained (Table 78 & 84; Figure 37). The overall plasma glucose averaged 56.29±0.41 mg% and 52.59±0.29 mg% in experimental and control groups, respectively. The variation in plasma glucose between the animals and periods was significant (P<0.01).

Average plasma NEFA concentration did not exhibit a set pattern of change in both the groups of buffaloes though the concentration was lower in experimental group (175.83 to 184.16 µmol/l) than the control (193.33 to 201.66 µmol/l) during different periods of the study. Overall plasma NEFA averaged 197.50±1.11 µmol/l and 180.58±1.00 µmol/l in control and experimental group, respectively (Table 78; Figure 24). NEFA concentration varied significantly between animals (P<0.01), however, between period effects was not significant. Average plasma urea concentration was significantly higher (P<0.01) in control (29.02±0.25 mg%) than experimental group of buffaloes (26.26±0.38 mg%). Further, between animal and between period variation in urea concentration was non-significant. Average plasma urea concentration was lower during all the periods of study in experimental buffaloes but there was no set pattern of change in urea concentration in both the groups of buffaloes.

#### **4.7.3 Physiological responses:**

The average RT was significantly (P<0.01) different during morning and evening between 2 groups of buffaloes. Morning average RT was low (99.96±0.09<sup>0</sup>F) in control group than the experimental buffaloes



**Figure 37. Milk production and glucose levels during winter season in buffaloes**

(100.30±0.08°F). During evening average RT in control group increased from morning values of 99.96 to 100.74°F in comparison to a change from 100.30 to 101.02°F in experimental buffaloes (Table 79 & 80a). Overall average value of RT was less by 0.34°F and 0.28°F in control buffaloes than in experimental buffaloes during morning and evening, respectively. Morning and evening value of RT significantly ( $P<0.01$ ) varied between animals and between periods. Average RR was significantly higher during morning ( $P<0.05$ ) and evening ( $P<0.01$ ) between 2 groups of buffaloes. The average morning RR increased from 12.20 to 14.83 breaths/min. and 11.46 to 16.45 breaths/min. during evening in control and experimental buffaloes (Table 79 & 80a). Average RR was more by 0.74 breaths/min. in control during morning, however, during evening average RR in control group decreased by 1.62 breaths/min. The RR changes between animals and between periods during morning and evening non-significantly varied.

Average HR during morning non-significantly varied in control and experimental buffaloes, the respective value was 45.21 vs. 44.98/min. However, between periods variation was significant ( $P<0.05$ ; Table 79 & 86). During evening average HR was significantly more ( $P<0.01$ ) in experimental than the control buffaloes (48.60 vs. 47.80/min.). Further, between period variation in HR was significant ( $P<0.01$ ) but between animal variation was found to be non-significant. In both control and experimental buffaloes HR increased from 45.21 to 47.80 and 44.98 to 48.60/min. during evening (Table 80a). Average ST non-significantly varied between the groups during morning and evening but between period variation ( $P<0.01$ ) was significant (Table 86). The overall average ST during morning and evening was 29.70 and 30.41°C, 29.81 and 30.54°C in control and experimental group, respectively (Figure 26 & 27). Further, between animal variation in ST during morning and evening was non-significant.

When the data of morning and evening was pooled RT, RR, HR and ST varied significantly ( $P<0.01$ ) between groups and between periods. However, RR non-significantly varied between periods. Further, changes in RT ( $P<0.01$ ), RR and HR ( $P<0.05$ ) were significant between the animals (Table 85).

The comparative analysis of physiological responses data of cows and buffaloes indicated that basal values of physiological responses were higher in cows during different seasons than the buffaloes (Table 92).

#### **4.7.4 Body weight changes, feed and water intake:**

The body weight of buffaloes of both the groups was significantly different ( $P<0.01$ ) due to the initial differences in the body weight of buffaloes (Table 80b).

Feed intake was similar in both the groups during 1<sup>st</sup> week and an increase was observed during 3<sup>rd</sup> and 4<sup>th</sup> week in experimental group but in case of control group feed intake declined during different weeks of the study. Average feed intake non-significantly varied between the groups (Table 80b).

Water intake also varied non-significantly between control and experimental group and did not exhibit any distinct pattern of change (Table 80b).

#### **4.7.5 Milk production:**

Average milk production changes between two groups of buffaloes significantly varied ( $P<0.01$ ) and overall average milk production was high ( $8.81\pm 0.22$  kg) in comparison to control ( $8.05\pm 0.29$  kg; Table 81). On percent basis the milk production increased by 9.44 (2.02 to 20.00%) in experimental buffaloes over the control group (Figure 16 & 37). The changes in milk production between the animals were found to be significant ( $P<0.01$ ) but between periods effect was non-significant (Table 87).

#### **4.7.6 Milk composition:**

Total solids of milk varied between groups, between periods ( $P < 0.01$ ) and the interaction of animal x group x period was significant ( $P < 0.01$ ). However, between animal variation in total solids was non-significant. Average total solids during period 1 of the study was similar in control and experimental group (17.98 vs. 17.96%) but increased in experimental group during the periods of study. The overall average total solids was higher in experimental than the control (18.23 vs. 17.99; Table 82).

Fat content of milk varied significantly between the animals ( $P < 0.01$ ) and between periods changes were non-significant. The value of fat increased in experimental group during different periods of the study and was significantly ( $P < 0.01$ ) higher in comparison to control (7.91 vs. 7.25%; Table 82 & 87). Average protein concentration was significantly higher ( $P < 0.05$ ) in experimental buffaloes ( $4.97 \pm 0.02\%$ ) in comparison to control ( $4.62 \pm 0.01\%$ ). In experimental buffaloes, average protein increased from initial value of 4.74 to 5.10% during different periods of study but in control protein concentration was maintained (Table 82). Further, protein varied between animals and between periods ( $P < 0.01$ ). The interaction of animal x group x periods was also significant ( $P < 0.01$ ).

Average values of lactose were significantly higher ( $P < 0.01$ ) in experimental buffaloes than the control, the respective values were 5.21 and 5.01%. Lactose content of milk increased gradually during different periods of study and was maximum ( $5.29 \pm 0.01\%$ ) during period 10 of the study (Table 82). However, in control, lactose was maintained. Further, lactose content of milk non-significantly varied between periods and between animals ( $P < 0.01$ ).

Average NEFA concentration was higher ( $P < 0.01$ ) in control in comparison to experimental buffaloes and was  $189.00 \pm 1.01$  and

180.66±0.97 µmol/l respectively (Table 81; Figure 28). Milk NEFA concentration increased in both the groups of buffaloes during the periods of the study but concentration was less in experimental than the control group. The changes in NEFA concentration between animals were significant ( $P<0.01$ ) but NEFA varied non-significantly between periods of the study (Table 87).

Milk urea concentration varied significantly ( $P<0.01$ ) between the groups and was high in control (27.25±0.30 mg%) than the experimental (24.56±0.44 mg%) buffaloes (Table 81). The variability in urea concentration between periods and between animals was found to be non-significant.

#### **4.7.7 Somatic cell count (SCC):**

Average SCC of milk non-significantly varied between animals and between periods. Further, there was no significant variation in SCC of milk between 2 groups of buffaloes. The overall average SCC was  $1.01\pm 0.02 \times 10^5$  cells/ml of milk and  $0.98\pm 0.01 \times 10^5$  cells/ml in control and experimental buffaloes, respectively (Table 83; Figure 29). The overall average epithelial cells were similar in control and experimental buffaloes. Average TLC significantly varied between 2 groups of the study but there was no significant difference in TLC between periods and between animals (Table 87). The overall average values of TLC was high in control ( $0.43\pm 0.01 \times 10^5$  cells/ml) than experimental buffaloes ( $0.40\pm 0.01 \times 10^5$  cells/ml). On percent basis the ratio of epithelial cells to TLC was 57:43 and 59:41 in control and experimental buffaloes (Table 83).

SCC in buffaloes during different seasons varied and was low during hot-dry season in comparison to hot-humid and winter. TLC of milk increased significantly ( $P<0.01$ ) during winter and was low during hot-dry and hot-humid season (Figure 29).

The comparative analysis of SCC data of cows and buffaloes indicated that basal values of SCC were higher in cows during different seasons than the buffaloes (Table 93).

#### **4.7.8 Correlation coefficients:**

The correlation data of physiological responses and minimum temperature (Table 89) revealed a positive correlation of RT with RR, HR and ST ( $P < 0.01$ ) in both control and experimental group. RR was also found to be positively correlated with HR and ST ( $P < 0.01$ ) in control and experimental group. Further, ST was positively correlated ( $P < 0.01$ ) to HR in both the groups. Milk production was not found to have any correlation with RT, RR, HR and ST. Minimum temperature during the study was positively correlated to HR and ST ( $P < 0.01$ ) in control group, however, in experimental group minimum temperature was positively correlated with RT ( $P < 0.05$ ) and ST ( $P < 0.01$ ) only.

The pooled analysis of data (Table 88) indicated a positive correlation of minimum temperature with RT, RR and ST ( $P < 0.01$ ), however, milk production was not correlated to RT, RR, HR and ST. Further, RT was positively correlated to RR, HR and ST ( $P < 0.01$ ) and the RR was positively correlated to HR and ST ( $P < 0.01$ ).

During winter season, minimum temperature was negatively correlated with plasma glucose and protein ( $P < 0.01$ ),  $T_4$  was positively correlated with glucose ( $P < 0.01$ ) and lactose content of milk ( $P < 0.05$ ) and negatively with milk NEFA concentration ( $P < 0.05$ ). Plasma concentration of  $T_3$  was negatively correlated with milk production ( $P < 0.05$ ), however, insulin was positively correlated with milk production ( $P < 0.05$ ). Fat ( $P < 0.05$ ), protein and lactose ( $P < 0.05$ ) was positively correlated with insulin. During winter season, plasma concentration of cortisol was not correlated to any of the parameters studied. Plasma glucose was negatively correlated with plasma and milk NEFA ( $P < 0.01$ ) and with milk production, protein and lactose content of milk ( $P < 0.01$ ). Milk production

in buffaloes was negatively correlated with TLC, milk NEFA and urea ( $P<0.01$ ) and positively with fat, protein and lactose ( $P<0.01$ ). SCC was positively correlated with TLC and epithelial cells ( $P<0.01$ ) while epithelial cells were positively correlated to TLC ( $P<0.01$ ). Fat content of milk was found to be positively correlated to lactose ( $P<0.01$ ) and negatively with milk NEFA ( $P<0.01$ ). Lactose was negatively correlated with milk NEFA and urea ( $P<0.01$ ; Table 90).

#### **4.8 Economics of modified management:**

Considering the market cost of various inputs at the time of experiment, economics of the modified management was calculated. Following variables were considered for calculation;

1. Cost of infrastructure required for mister system.
2. Cost of feed offered to the animal.
3. Cost of electricity and water charges.
4. Cost of treatment of animals of both groups during different seasons.
5. Cost of additional milk produced by experimental animals in different experiments and seasons.
6. Cost of maintenance of infrastructure for experimental group i.e. mister system.
7. Cost of labor for bedding/removal of bedding during winter.

The cost of following variables was taken as constant and not included as the input cost was similar for both the groups.

1. Number of labor and labor charges for fodder distribution, cleaning and milking.
2. Cost of loose housing establishment.





### **Buffaloes (Hot-dry season)**

#### **Input cost:**

- (i) Extra fodder consumed @ 4.57 kg/day/animal x 6 animals  
= 27.42 kg/day  
Cost of 27.42 kg fodder = Rs. 6.85 /day x 30 days = Rs. 205.50

#### **Output cost:**

- (i) Increase in milk production @ 1.02 kg/day/animal x 6 animals  
= 6.12 kg milk/day  
6.12 kg x Rs. 14 (Cost of milk/kg) = Rs. 85.68 x 30 days = Rs. 2570.40

**Net profit = Rs. 2570.40 – Rs. 205.50 = Rs. 2364.90**

### **Buffaloes (Hot-humid season)**

#### **Input cost:**

- (i) Extra fodder consumed @ 4.15 kg/day/animal x 6 animals  
= 24.90 kg/day  
Cost of 24.90 kg fodder = Rs. 6.21 /day x 30 days = Rs. 186.30

#### **Output cost:**

- (i) Increase in milk production @ 0.92 kg/day/animal x 6 animals  
= 5.52 kg milk/day  
5.52 kg x Rs. 14 (Cost of milk/kg) = Rs. 77.28 x 30 days = Rs. 2318.40

**Net profit = Rs. 2318.40 – Rs. 186.30 = Rs. 2132.10**

### **Buffaloes (Winter season)**

#### **Input cost:**

- (i) Extra fodder consumed @ 3.04 kg/day/animal x 6 animals  
= 18.24 kg/day  
Cost of 18.24 kg fodder = Rs. 4.56 /day x 30 days = Rs. 136.80

- (ii) Paddy straw bedding (10 kg) on alternate days

Rs.10/day x 15 days = Rs. 150.00

- (iii) Cost of labor (to remove paddy straw bedding/disposal)

0.25 man months/day @ Rs. 20/day = Rs. 600.00

Total

Rs. 886.80

**Output cost:**

(i) Increase in milk production @ 0.76 kg/day/animal x 6 animals  
= 4.56 kg milk/day

4.56 kg x Rs. 14 (Cost of milk/kg) = Rs. 63.84 x 30 days = Rs.1915.20

**Net profit = Rs. 1915.20 - Rs. 886.80 = Rs. 1028.40**

# CHAPTER 5

## DISCUSSION

## 5. DISCUSSION

During hot-dry season, mister cooling of cows resulted in non-significant increase in plasma T<sub>4</sub> level, though feed intake was increased significantly by 13.14%. The increase in T<sub>3</sub> level in control group was due to high ambient temperature as reported by Aceves *et al.* (1985). The increased level of cortisol in control group of cows and buffaloes were attributed to the high maximum temperature conditions which resulted in decline in milk production and change in milk composition as reported earlier (Abilay *et al.* 1975; Roman-Ponce *et al.* 1981). This fact was further supported by negative correlation of cortisol with milk production and milk composition. The significant changes in cortisol levels in cows indicated that crossbred cows are more sensitive to high ambient temperature in comparison to buffaloes in which between animal variation in control was non-significant.

In the present study, higher T<sub>4</sub> level (P<0.01) in wallowing buffaloes (experimental) in comparison to control was due to increase in milk production (by 13.45%) and feed intake (by 11.62%). High ambient temperature (39.20 to 43.40°C) during the experiment could be the reason of decreased thyroid gland activity in control animals. Khurana (1983) reported decreased plasma T<sub>4</sub> concentration (39.10 ng/ml) in hot-dry season compared to 41.44 ng/ml during hot-humid season. Further, during hot conditions the animals in early lactation have low levels of T<sub>4</sub> and T<sub>3</sub> (Aceves *et al.*, 1985). Contrary to this, in the present study plasma T<sub>3</sub> level increased in control group. The increased feed intake concomitant to an increase in milk production suggests a major role for thyroid hormone metabolism in regulating homeorhetic responses involved in maintenance of high priority functions. Further, this fact was also evident from higher insulin level in wallowing buffaloes in comparison to control. The higher magnitude of reduction in thyroid activity in summer heat in buffalo than cows appeared to be mainly due

to a difference in their capacity to adapt to subtropical conditions (Hassan and El-Nouty, 1985).

The improvement in feed intake in cooled experimental cows led to more milk production and glucose in comparison to control. Since plasma NEFA level of the cooled cows was low, the lower levels of NEFA also appeared in milk. Contrary to this, Flamenbaum *et al.* (1995) reported no effect of cooling on NEFA concentration. The elevated glucose levels in experimental cows resulted in low plasma NEFA and urea levels inspite of significant increase in milk production in cooled cows. Aboulnaga *et al.* (1989) also found increase in glucose levels of cows sprinkled with water, however, Flamenbaum *et al.* (1995) reported low levels of glucose in cooled cows. The significantly higher levels of glucose in wallowing buffaloes could probably led to increase in milk production and a decline in plasma NEFA and urea levels. Yash Pal (1996) reported plasma NEFA levels around 534 to 299  $\mu\text{mol/l}$  upto day 19 of lactation which subsequently declined to 256  $\mu\text{mol/l}$  by day 54 of lactation in Murrah buffaloes. However, higher level of free fatty acids (47.38 mg/l) during early lactation in Murrah buffaloes was reported by Setia *et al.* (1992). Jindal (1998) reported average plasma NEFA in lactating buffaloes, which were similar to the level as observed in this study.

The negative correlation of plasma NEFA and glucose with milk composition further revealed the importance of these energy metabolites in milk synthesis. Moreover, energy balance of the animal is also responsible for the changes in glucose levels (Blum *et al.*, 1973). The increase in blood glucose observed in this study in experimental cows was due to stress relieving effect of cooling (Aboulnaga *et al.*, 1989) as value of  $\text{THI} > 71$  decreased blood glucose (Abeni *et al.*, 1993; Itoh *et al.*, 1997). The positive correlation of milk yield with blood glucose found in this study was reported earlier in cows (Chla'dek and Machal, 2002). Basal levels of glucose observed in this study were within normal range

(Sharma *et al.*, 2002) but, differences in glucose levels could probably be due to differences in milk production.

During hot-dry season, the increased RT in control group cows resulted in increased RR, HR and ST (Itoh *et al.*, 1997). Due to high ambient temperature, the most affected parameter of the study was RT and ST. However, in experimental animals subjected to mister and fan, RT and ST did not change during morning and evening and therefore the changes in RR and HR was less pronounced which led to increase in milk production by 10.23%. However, in control group cows RT and ST significantly varied during morning and evening indicating thereby adverse effect of temperature leading to decrease in feed intake and adversely affected milk production of cows (Korsun, 1993; Frazzi *et al.*, 1997). Cooling by mist and fan in hot-humid season decreased not only ST but resulted in low values of RT, RR and HR in experimental cows. However, decline in RR was more which further gives evidence that RR declined due to significant decrease in ST. The average RT, RR and HR in control group of animals was within normal range as reported earlier (Morrison *et al.*, 1973; Lall *et al.*, 1987; Prasanpanich *et al.*, 2002).

The availability of showers/ water sprinklers over the lactating animals reduces heat stress and milk production can either be sustained (Spain *et al.*, 1998) or enhanced (Singh and Mehla, 1999; Singh *et al.*, 2003). Further, use of sprinkling and ventilation cooling system improves milk production by 1 to 5 kg/day during summer (Flamenbaum *et al.*, 1995) due to water acting as a cooling agent by effective evaporation on animal skin and reducing heat stress considerably (Lin *et al.*, 1996; Frazzi *et al.*, 1997; Rossi *et al.*, 1998; Anjuli and Singh, 2003). The beneficial effect of cooling was evident from a decline in RT by 1.62<sup>o</sup>F, RR by 5.23 breaths/min., HR by 4.67/min. and ST by 2.74<sup>o</sup>C in experimental cows which was reported in cows by various workers earlier (Gomila *et al.*, 1976; Amakiri and Funsho, 1979; Huang *et al.*, 1986;

Flamenbaum *et al.*, 1995). Omar *et al.* (1996) reported a significant decline in RT (0.7°C), RR (18.7 breaths/min.) and HR (8.3/min.) by cooling the cows with sprinklers and ventilators. Cooling of skin by water and fan in sheds sustained body temperature of crossbred cows between 38.4 to 38.6°C and increased daily milk yield by 2.66 and 2.26 kg/day in hot-dry and hot-humid season (Singh and Mehla, 1999). The effects of cooling on milk yield probably could be due to change in rectal temperature diurnal rhythm (Singh *et al.*, 2003) or by direct influence on milk secretion from the mammary gland and by restoring feed intake which can be marked by energy metabolites like plasma glucose and NEFA (Abeni *et al.*, 1993; Armstrong, 1994).

The average RT, RR, HR and ST in both the groups of buffaloes during hot-dry season were similar due to similar set of management as animals received showers (control) or wallowing (experimental) only from 11.00 A.M. to 4.00 P.M. The more decline in values of RT, RR, HR and ST in experimental group indicated that wallowing was more effective in reducing heat stress in comparison to water showers. Gangwar (1985) reported a decline by 1°F through showers and 2 to 3°F by wallowing. Verma *et al.* (2000) displayed a significant linear increase (upto 38.5°C) in RT and RR and both were positively correlated with ambient temperature during summer.

The lower range of changes in RR in buffaloes indicated that role of respiration in regulating body temperature in this species was weaker than in cows (Chikamune, 1986). The hairless hide of buffaloes may contribute to heat dissipation and consequently, when buffaloes are transferred to shade, they can cool off more readily than cows. The values of regression coefficients indicated better efficiency of wallowing in lowering body temperature and respiration rate of buffaloes than showering or shading (Chikamune, 1986). In this study also maximum temperature was positively correlated to RT, however no correlation was

found with ST. Das *et al.* (1997) found a positive correlation of ST with RT in buffaloes. The negative correlation of milk production with RT, RR, HR and ST further indicates that adverse effect of hot-dry season on milk production of buffaloes can be checked by improving the physiological responses, which are important tool for comfort of the animal in a particular climate (Das *et al.*, 1997). The increased milk production of wallowing buffaloes was therefore, due to more improvement in physiological responses in comparison to control (shower group). The decline in plasma NEFA resulted in a decreased milk NEFA level in wallowing buffaloes as found in cows also. Milk urea level in both the group of buffaloes in this study was within normal range (Bhavadasan *et al.*, 1982). However, in buffalo milk lower levels of urea (30.4 mg%) was reported by Khan *et al.* (1986) and urea levels during summer are higher than autumn and winter season in Nili Ravi buffaloes (Qureshi *et al.*, 1999).

Sprinkling of water and provision of shed during summer results in faster body weight gain in Murrah buffaloes (Tripathi *et al.*, 1972), however, in this study the body weight changes were non-significant. The body weight changes in wallowing buffalo could be due to more feed intake in comparison to control. Sastry *et al.* (1981) reported 16% higher weekly gains in live weight of young buffaloes by additional shelter and sprinkling of water. However, more comfort due to wallowing resulted in a decline in water intake by 2.92%. The water intake of buffaloes increase by 25% during summer as lactating buffaloes require 1 kg extra water for every kg milk production/100 gm metabolic body size (Satya Pal *et al.*, 1973). The restriction of water therefore impairs the milk synthesis of mammary gland during summer.

The basal values of fat, protein and lactose in control group of cows were in agreement with the earlier reports in cows (Kitchen, 1981). The significantly high fat, protein and lactose values were attributed to

more comfort due to cooling, more feed intake and a decline in water intake of experimental animals. The increased fat, protein and lactose in experimental group cows due to cooling have been attributed to increased feed intake by dairy animals (Flamenbaum *et al.*, 1995; Anjuli and Singh, 2004). However, in control group of animals steady increase in fat% observed during different periods of the study was due to decline in milk production. The earlier studies (Gangwar, 1985) also found similar increase in fat content of milk of buffaloes provided with showers or wallowing during hot-dry season. In this study, fat, protein and lactose increased by 7.23, 20.32 and 3.61% in wallowing buffaloes. Verma and Hussain (1988) in Murrah buffaloes found similar increases in fat, protein and lactose in milk of buffaloes subjected to water application for 15 and 20 min. The values of fat, protein and lactose found in this study was within normal range and in agreement to earlier reports in buffaloes (Rao and Ranganathan, 2000).

The non-significant changes in epithelial cells of milk in wallowing or shower group indicate that both the treatments were equally effective from the hygienic point of view of milk production. However, detailed information for comparison on this aspect is lacking in buffaloes. The fact that SCC was within normal range in cows kept with or without mist and fan indicated no adverse effect of modified management on udder health of cows. Reduction of thermal stress by air-conditioning (Thatcher *et al.*, 1974) or shelter management (Roman-Ponce *et al.*, 1981) results in a lower incidence of clinical mastitis than for cows exposed to their natural environment. Apparently either heat stress impairs defense mechanisms or else cows in attempting to cool themselves are exposed to greater contact with pathogens (Roman-Ponce *et al.*, 1981). Cooling by automated system reduced SCC of milk in cows. Lee-ShanNan *et al.*, (1999) reported that SCC in cooled cows remains within normal range ( $3.5 \times 10^5$ /ml).

SCC of milk which are essential index of quality of milk and health of udder (Schukken *et al.*, 1992) were found to be within normal range in both the groups of animals (Singh and Ludri, 2001). The decline in average SCC in experimental group indicated more comfort to the animal leading to less secretion of epithelial cells and TLC in milk in comparison to control. SCC of milk are influenced by many factors like parity, stage of lactation, managemental conditions and seasons (Sethar *et al.*, 1979; Kennedy *et al.*, 1982; Roman-Ponce *et al.*, 1981; Singh and Ludri, 2001). Irrespective of stage of lactation average SCC in KF cows have been reported to be  $1.61 \times 10^5$  cells/ml of milk (Singh and Ludri, 2001). The availability of mist and fan lowers SCC of milk indicating thereby that animals and the floor became cleaner due to mister water and thus improved hygienic conditions of the animals. In the present study, maximum temperature did not influence SCC of milk due to non-significant correlations, however, hot-humid conditions are more vulnerable to udder health and affect total SCC of milk as evidenced from negative correlation of maximum temperature with SCC during hot-humid season.

The mist and fan cooling during hot-humid season decreased secretion of somatic cells in milk due to more comfort and cleaned surface of the animal and the floor as observed in hot-dry season. Since TLC of 2 groups of animals was non-significantly different and the epithelial cells increased in control group of cows further indicates that hot-humid conditions had an adverse effect on mammary health. However, such stress can be minimized on animals and mammary health (Thatcher *et al.*, 1974; Roman-Ponce *et al.*, 1981).

During hot-humid season, low T<sub>4</sub> concentration in control group was due to decreased feed intake and high ambient temperature associated with high humidity (30.0 to 39.0°C and 71 to 92% RH). However, in experimental cows the beneficial effect of mist and fan led to

improvement in feed intake an increase in T<sub>4</sub> was found. Valtorta *et al.*, (1980) reported a decline in T<sub>4</sub> and T<sub>3</sub> concentration at 30°C heat exposure in lactating dairy cows. In this study, decline in T<sub>4</sub>, T<sub>3</sub> and insulin concentration in control animals was due to high ambient temperature and high humidity in comparison to the experimental animals where the maximum temperature varied from 29.7 to 38.5°C and THI from 79.26 to 86.17. The differences in the maximum temperature and THI indicated more comfort as a result of mist and fan cooling which subsequently increased plasma T<sub>4</sub>, T<sub>3</sub> and insulin in experimental animals. Aceves *et al.* (1985) found that during extreme conditions plasma T<sub>4</sub> and T<sub>3</sub> declines in lactating cows. They further suggested that heat acclimation in dairy cattle does not depress thyroid gland activity and that adaptive thermoregulatory mechanisms involves peripheral mono-deiodinative pathways of thyroid hormones (Aceves *et al.*, 1987).

Aboulnaga *et al.* (1989) reported increased T<sub>4</sub> concentration in cows cooled with sprinkling of water at 35°C and RH (40 to 77%). In the present study, T<sub>4</sub>, T<sub>3</sub> and insulin concentration were increased by 4.31, 1.5 and 32.27% in experimental animals due to mist and fan cooling effect. Such changes were reported and correlated earlier also in cows (Liang-SunYi *et al.*, 1996).

The decreased insulin level during hot-humid season observed in this study was also reported by Itoh *et al.* (1987) due to protein catabolism during heat exposure. However, in lactating cows plasma insulin concentration does not correlate with utilization of glucose by mammary gland (McGuire *et al.*, 1995) and the whole body utilization of glucose mostly occurs through non-insulin dependent pathway (Rose *et al.*, 1997).

During hot-humid season, the increase in milk protein was due to improved feed intake of experimental animals in comparison to control.

The increases in the milk production were attributed to increase in feed intake and more glucose in blood. Since higher basal values of glucose is required for synthesis of lactose, the improvement in milk production could probably be attributed to higher glucose in experimental animals (Itoh *et al.*, 1998). The increased milk production is generally associated with an increase in plasma NEFA concentrations due to mobilization of body reserves which does not commensurate with increased demand of nutrients for milk production (Radloff *et al.*, 1966). Availability of the mist and fan improved the physiological responses which subsequently led to increase in feed intake (Flamenbaum *et al.*, 1995; Lee-ShanNan *et al.*, 1999; Anjuli and Singh, 2004) and therefore, a decline in milk NEFA concentration was observed during different periods of the study in comparison to control. In control group cows, the feed intake decreased due to heat stress induced effect on hypothalamus feed intake centers, increased gut fill due to reduced rate of passage and increase in RR (McDowell *et al.*, 1976). Such improvement in feed intake of experimental animals and a decline in water intake was reported earlier in cows subjected to sprinkling of water (Fulsoundar and Radadia, 1994). Flamenbaum *et al.* (1995) reported that cooled cows by sequential sprinkling consumed 1.6 kg more DM/day and 9 litres less of water/day than uncooled cows. Further, experimental cows produced more milk (8.05%) during the experiment as availability of spray and fans (Igono *et al.*, 1987) or mist particles and fan (Aii *et al.*, 1988; Anjuli and Aggarwal, 2004) result in more milk production. In the present study increase in milk yield was from 2.27 to 16.85% which indicated a differential response of cows to mist and fan cooling due to changes in climatic variables during different periods of the study. Further, heat relieving by combination of shades, feed and water offered under shed, ventilation, sprinklers and fan cooling improves not only cow comfort but also increased milk production in hot-humid season (Bucklin *et al.*, 1991;

Abdel-Samee and Ibrahim, 1992; Ryan *et al.*, 1992; Flamenbaum *et al.*, 1995). Armstrong (1994) reported that effect of cooling by spray and fan was more in high producing cows in comparison to medium and low producers and the increases in milk production per day was 4.0, 3.5 and 3.2 kg/day, respectively.

The average fat, protein and lactose content of milk in this study was found to be within normal range as reported by various workers (Kitchen, 1981; Foltys *et al.*, 1994; Itoh *et al.*, 1998; Baknik *et al.*, 2000). The increased protein values in experimental group was due to increased protein production as a result of more feed intake and hence in protein supply from heat stress relief (Flamenbaum *et al.*, 1995). The decreased milk urea concentration in experimental group in comparison to control further supports the above facts. The higher concentration of milk urea in control group cows reflect enhanced tissue catabolism to supply the nutritional demands of milk production due to decreased feed intake during heat stress (Flamenbaum *et al.*, 1995). Milk urea level has been found to be positively correlated with milk yield (Carlsson *et al.*, 1995), whereas, a negative relationship has also been reported (Broderick and Clayton, 1997). Flamenbaum *et al.* (1995) found decreased urea levels in cooled cows than in uncooled cows. The higher level of plasma urea observed in uncooled cows probably reflect enhanced tissue catabolism to supply the nutritional demands for milk production due to suppression of feed intake during heat stress (Flamenbaum *et al.*, 1995). The association between milk urea and season could be confounded by stage of lactation and nutritional effects. Carlsson *et al.* (1995) attributed variation in milk urea to seasonal changes in pasture protein and energy components. The conversion of ammonia to urea by the liver has been estimated to cost the animal 12 Kcal/g of excess nitrogen excreted (Van Soest, 1994). It has been suggested that energy tax associated with the conversion of excess amounts of ammonia to urea in the liver may

contribute to reduced milk production (Nelson, 1996). Thus overall improved performance of experimental cows than the control ones suggests beneficial effect of mister cooling by maintaining the animals close to normal thermal state and thereby improving the milk production during heat stress.

The study indicated that wallowing was more effective in comparison to water showers to alleviate the stress of hot-humid conditions when average maximum temperature was 33.65°C (ranged from 30.0 to 39.0°C) and relative humidity was 81.1% (ranged from 71 to 92%). It was further evident by more feed intake (13.25%) which resulted in increased milk production (by 13.27%) and indicated wallowing being more effective as evidenced by skin temperature difference of two groups. Various reports also indicate that cooling of lactating cows result in increase in milk production due to more feed intake (Armstrong, 1994; Flamenbaum *et al.*, 1995). In high producing dairy cows exposed to a moderately hot and humid environment, direct evaporative body cooling by sequential sprinkling and forced ventilation increased DM intake by 1.6 kg/day (Flamenbaum *et al.*, 1995). Cooling by automated system caused a 2.8 kg/day increase in feed intake of cooled cows than the control (Lee-ShanNan *et al.*, 1999) and during heat stress reduced feed intake is related to the heat increment associated with feed intake (McDowell *et al.*, 1976).

The values of fat, protein and lactose in control group buffaloes were within normal range and were reported earlier also (Rao and Rangnathan, 2000; Sebastian *et al.*, 1972; Sud and Singh, 1974). Gangwar (1985) found a significant increase in milk production in wallowing buffaloes as found in this study also. Further, blood glucose levels in three groups were 59.29, 60.31 and 66.80 mg% respectively as reported by Gangwar (1985). Glucose is the most important substrate required to maintain milk production and the energy balance of the

animal is also responsible for the changes in the levels (Blum *et al.*, 1973). In present experiments, blood glucose level was 18.3% higher in wallowing group in comparison to shower group which resulted in more milk production. Increased glucose levels could be ascribed to more feed intake of wallowing buffaloes which led to maintain milk production (Gangwar, 1985). Verma and Hussain (1988) reported an increase in total feed intake by 12% in three groups of Murrah buffaloes kept as control, water application for 15 (group 2) and 20 (group 3) minutes before milking and the milk production was 4.27, 5.26 and 6.06 kg, respectively.

In wallowing buffaloes milk fat and total solids increased probably due to more feed intake. The high protein percent in wallowing buffaloes may result from an induced increase in feed intake and hence in protein supply from heat stress relief, or from the prevention of a hyperthermia-induced impairment of protein synthesis in mammary gland (Flamenbaum *et al.*, 1995). As the milk protein percent increased, milk urea decreased in Holstein cows (Johnson and Young, 2003). Also SCC showed a negative relationship with milk urea. The low milk NEFA concentration in wallowing buffaloes than the control group were due to improvement in energy balance by cooling (Flamenbaum *et al.*, 1995). The higher plasma NEFA levels subsequently appear in milk and indicate negative energy balance of animal (De Boer *et al.*, 1985). The basal NEFA levels found in this study were within normal range (Jyotsna, 2000). The higher milk urea levels in control group was reflected due to enhanced tissue catabolism to supply the nutritional demands of milk production owing to suppression of feed intake during heat stress (Kunz *et al.*, 1985). Godden *et al.* (2001) found a positive association between milk urea and milk yield. Contrary to this, in the present study milk urea and milk production were negatively correlated. Although milk urea indicated the relative balance between protein and energy (protein: energy ratio), it

does not indicate which of these two nutrients is in absolute excess or deficiency. Milk urea appears to be moderate when the levels of protein and energy are balanced relative to one another, whether both fed in excess, both are underfed or both are fed to recommended requirements (Oltner and Wiktorsson, 1983). Clearly variations in these nutrients could result in different levels of production among different studies. Thus, variability in the quantity and quality of feedstuffs could explain differences in the association between milk urea and milk production. The overall improved performance of experimental buffaloes than the control ones suggests more beneficial effect of wallowing in maintaining a normal thermal state and improvement in milk production during stress.

The non-significant differences in physiological responses (RT, RR and HR) during morning were due to similar condition of management. But significantly ( $P < 0.01$ ) low values of physiological responses in afternoon indicated more stress relieving effect of wallowing than showers, due to complete merging of buffaloes in water pond. Buffaloes exposed to direct sunlight had more respiration rate followed by a rise in body temperature and heart rate (Mishra *et al.*, 1963). However, when moved to shed, a sudden decline in respiration rate followed by a reduction in body temperature and heart rate occurs. Gangwar (1985) observed a decrease in rectal temperature by showers ( $1^{\circ}\text{F}$ ) and by wallowing ( $2-3^{\circ}\text{F}$ ) and decrease in respiration rate by 2/min by showers and 3-4/min by wallowing. Cooling by spraying water, wallowing and mud plaster on buffalo for 30 days in summer (June-July) significantly improved physiological responses. Therefore, wallowing was found to be the best system for alleviation of heat stress (Balakrishnan and Nagarcenkar, 1988) and in sustaining the physiological responses close to normal range as observed in this study also.

Introduction of water cooling and fan cooling helps in sustaining the body temperature of crossbred cows at normal body temperature level ( $38.4$  to  $38.6^{\circ}\text{C}$ ) and average body temperature can be reduced

from 39.9 to 38.4°C in hot-humid season. The process of sustaining body temperature close to normal range increased average daily milk productivity of crossbred cows to 2.26 kg of milk (Singh and Mehla, 1999). The increase in fat and other constituents of milk and a decline in NEFA and urea in wallowing buffaloes indicated more comfort in conjunction with the improvement in physiological reactions and thus wallowing being more beneficial as compared to showers.

In the present study, in-house keeping of buffaloes resulted in increased milk production due to protection from cold stress. The buffaloes kept in open loose housing system with inadequate shelter showed a decline in milk yield. Further, increased milk production was attributed to significant increase in feed intake in experimental group of buffaloes. During winter when ambient temperature falls to <7°C, adequate protection from cold stress improved milk production of cows by 2.6 kg per day (Singh and Mehla, 1999). Animals kept outside in adverse winter consume more feed but produce less milk because less feed energy is available for production processes (Young, 1978). Lactating cows preferred bedding during winter when kept inside (Albright and Arave, 1997). Short-term experiments during cold decrease milk production by 5-13% in loose housing system as compared to barns (Andreae, 1973). Glucose is the most important substrate required to maintain milk production since ruminants depend on gluconeogenesis for their glucose requirements and the energy balance of the animal is also responsible for the changes in the levels (Blum *et al.*, 1973). In present experiment, high blood glucose level in experimental group resulted in more milk production than the control group. Such increases in glucose levels were observed in cows during cold exposure by Itoh *et al.* (1997). The increase in fat and total solids in experimental buffaloes was probably due to the more intake of feed. Increased NEFA level in control group of buffaloes was due to increased catabolism of fat to meet energy requirements (Nangia and Garg, 1992) and thereby improvement

in energy balance to protect the buffaloes from cold stress. It was further reflected by the higher level of milk urea in control group required to supply the nutritional demands of milk production (Kunz *et al.*, 1985; Anjuli and Singh, 2004). Thomas *et al.* (1978) reported higher values of rectal temperature, respiration rate and heart rate in buffaloes kept in the barn. However, differences between two housing condition were marginal in their studies and not significant enough due to mild winter conditions. The beneficial effects of in-house shelter on milk production were due to low values of maximum temperature and high values of minimum temperature compared to loose housing. In the present study, rectal temperature was high in experimental group (by 0.34<sup>0</sup>F) in the morning and also in the evening (by 0.72<sup>0</sup>F). The physiological responses were significantly higher and close to normal values in experimental buffaloes, thereby indicating more comfort to the animals kept in-houses.

Increased plasma T<sub>4</sub> and insulin levels in buffaloes kept in-houses with floor bedding could be due to improvement in basal metabolic rate associated with an increase in feed intake (by 7.00%). Such increases in T<sub>4</sub> concentration during winter season were reported earlier in cows and buffaloes (Khurana, 1983), however, in this study T<sub>3</sub> concentration non-significantly varied. The increase in milk production of experimental group was concomitant to an increase in feed intake which in this study also had higher plasma glucose and lower NEFA concentration. The elevated NEFA concentration in control group indicated more mobilization of body reserves to sustain the milk production when average maximum and minimum temperatures were 16.17 and 4.72<sup>0</sup>C, respectively. Increased NEFA concentration during early lactation suggests negative energy balance status and more mobilization of free fatty acids from adipose tissue to provide energy (De Boer *et al.*, 1985). However, magnitude of rise depends on the degree of energy deficiencies in cows (Kunz *et al.*, 1985). The increased concentration of NEFA in both cows and buffaloes of control group during winter observed in this study

was also reported earlier by Itoh *et al.* (1997). The higher concentration of plasma NEFA was in cold season (201.0  $\mu$  eq/l) than in hot (97.6  $\mu$  eq/l) environment in lactating cows (Itoh *et al.*, 1997). Urea level in cow's milk differ widely and is influenced by feed intake. Since an increase in feed intake was observed in cows (10.74%) and buffaloes (7.00%) kept in-houses in comparison to control, reflects the balance between dietary carbohydrate and protein and therefore plasma urea indicated a better feed efficiency indicator (Roth *et al.*, 1996). The high urea level in control group of cows and buffaloes during the winter season probably indicate deficiency of energy at surplus of crude protein in diet (Zieminski and Juszczak, 1997). Lower urea in milk during winter as observed in present studies was also reported by others (Hwang *et al.*, 2000). This indicated that lactating cows had excess protein and/or inadequate energy intake during summer.

The comparative data of cows during winter season indicated similar trend of  $T_4$ ,  $T_3$  and insulin. However, basal levels were lower in cows in comparison to buffaloes, which probably were due to the higher milk production and species difference in cows than the buffaloes (Khurana, 1983; YashPal, 1996). Further in this study, the increase in  $T_4$  level was associated with significant higher glucose levels due to more feed intake by cows kept in-houses (by 5.24%) as compared to control. Similar result was also observed in buffaloes. Better-feed conversion efficiency was found in bedded loose housing system for cows (Konggaard, 1977) and also the cows with shelter tended to convert feed more efficiently (Leu *et al.*, 1977).

The lower plasma  $T_3$  level in experimental cows is indicative of increased metabolism which leads to decrease in  $T_3$ . However, insulin level because of their least important role in metabolism of the animal was not affected by winter season. The non-significant variation between animals further supports beneficial effect of in-house keeping of animals

which protected the animal from severe cold leading to improvement in basal metabolism. The low levels of glucose in control group kept in loose housing probably indicated diversion of energy to maintain the body temperature. RT of control and experimental cows during morning and evening remained similar and RT of buffaloes during morning and evening was more in-house conditions. This fact was further supported by wider difference in RR of control and experimental cows during evening in comparison to control and experimental buffaloes. The positive correlation of minimum temperature with HR and ST in control group of buffaloes indicated that buffaloes are more sensitive to lower temperature in comparison to cows in which minimum temperature was not positively correlated with either ST or HR. Further, negative correlation of milk production with RT in cows indicated RT being an important determinant of milk production in cows during winter season. Since RT of experimental group kept in-houses was increased in comparison to control the milk production was not correlated to RT. However, a positive correlation of milk production with RR and HR indicated that the improvement in milk production of cows was directly related to improvement in RR and higher values of RR and HR in experimental cows. However, in buffaloes such correlation of milk production was not observed but minimum temperature influenced ST in both control and experimental buffaloes and was positively correlated. The fact that milk production of buffaloes and cows kept in-houses was more (by 9.44 and 7.41%) further indicated that adequate shelter ensures improvement in milk production commensurate with increase in feed intake of lactating animals in comparison to control group kept under loose housing system. Further, milk production, fat, protein and lactose content also increased in cows and buffaloes kept in-houses than the control group. Seasons of the study also influenced fat and total solids of milk and were highest during winter season. Seasonal variation in total solids was due primarily to variation in protein content of milk

(Bauman and Davis, 1974; Larson, 1985). However, the values of fat, protein and lactose were within normal range (Sebastian *et al.*, 1972; Sud and Singh, 1974).

The SCC of milk in control and experimental buffaloes was similar during winter season and indicated no adverse effect on mammary health by two sets of management. In cows, SCC of milk during winter season was significantly higher in control due to variation in epithelial and TLC between the animals. The model of multiple analyses of variance revealed that results of milk analyses (fat, protein, lactose, urea, somatic cell count) were statistically significant and influenced particularly by the year of investigation, farm, season (winter-summer feed intake) and to a lesser extent by health status of the udder (SCC x 1000/ml) (Zadnik *et al.*, 1995).

The modified managerial practices followed in this study during all the seasons was remunerative and economical. The farmers may use these without any adverse effect on health of the animal. Further, the cost of mister system can be compensated within a period of two months. Earlier reports in cattle suggested use of mister systems or use of sprinklers as highly economical (Thatcher *et al.* 1974; Martens *et al.*, 1980). However, cooling by air conditioning did not compensate for the cost of equipment by improvement in milk yield and reproduction (Vermeulen, 1988). The economic analysis of comparison between performance of cooled and uncooled cows in hot-dry and hot-humid climate (Strickland *et al.*, 1989) and mist and fan system was economical when daily milk yield gain per cow was 0.81 kg (Aii *et al.*, 1988; Lin *et al.*, 1996).

# **CHAPTER 6**

## **SUMMARY AND CONCLUSIONS**

## 6. SUMMARY AND CONCLUSIONS

### 6.1 Summary:

1. The importance of research problem on which present study has been carried out and reported in this thesis has been clearly defined and the need of carrying out such research is explained in chapter "Introduction".
2. An up-to-date review of literature covering hormonal profile, blood metabolites, milk production and composition in association with physiological responses during early lactation has been presented in "Review" chapter.
3. The study was conducted on lactating crossbred KF cows and Murrah buffaloes during hot-dry, hot-humid and winter seasons.
4. During hot-dry season (May-June), 12 lactating crossbred cows were selected and were divided in 2 groups. Group-I was kept as per the routine management practices followed at the Institute's herd while group-II cows were provided with fan and mist from 11.00 A.M. to 4.00 P.M.
5. During hot-humid season (August-September), 12 crossbred KF cows were selected during early lactation of 50-80 days and the experimental group of cows were provided with fan and mist from 11.00 A.M. to 4.00 P.M. while cows of group-I served as control.
6. During winter season (December-January), 12 crossbred KF cows were selected and divided in 2 groups. Group-I (control) was kept under loose housing system while group-II was kept in houses with paddy straw bedding.
7. During hot-dry season, 12 lactating Murrah buffaloes were selected and divided in 2 groups of 6 each. Group-I buffaloes were provided with mist system from 11.00 A.M. to 4.00 P.M. and served as control. Group-II buffaloes were allowed to wallow in a water pond during the same interval.

8. During hot-humid season, 12 lactating Murrah buffaloes were selected and divided in 2 groups of 6 each. Group-I buffaloes were subjected to showers from 11.00 A.M. to 4.00 P.M. and served as control while group-II buffaloes were allowed to wallow in a water pond.
9. During winter season also 12 lactating Murrah buffaloes were selected and divided in 2 groups of 6 each. Group-I buffaloes were kept under loose housing open system (control) while group-II was kept in houses with paddy straw bedding.
10. The study was conducted when the maximum temperatures during hot-dry and hot-humid seasons were  $>36^{\circ}\text{C}$  and  $>33^{\circ}\text{C}$  with RH values of 25-40% and  $>90\%$ . During winter the experiment was started when minimum temperature was  $<8^{\circ}\text{C}$  and RH was 35-55%.
11. During the experimental period of 3 seasons, maximum and minimum temperatures, dry and wet bulb temperatures were recorded and the RH was calculated.
12. Jugular blood samples were collected in heparinized vacutainer tubes on periods 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (at 3 day interval) of the experiment from animals of both the groups during each season of study as mentioned above.
13. Milk samples were collected from cows and buffaloes on days similar to the blood sampling intervals. The aliquots of milk from A.M., noon and P.M. in case of cows and A.M. and P.M. milkings in buffaloes were composited in proportion to yield.
14. Physiological responses viz. RT, RR, HR and ST were recorded from both the groups of cows and buffaloes during each season at 8.00 A.M. during morning before subjecting the cow/buffalo to treatment. The physiological responses were also recorded at 4.00 P.M. after the treatment was over.

15. Milk yield of individual cows and buffaloes were recorded daily for the different sets of experiments. Body weight, feed and water intake was recorded at weekly intervals during different sets of experiments conducted.
16. Hormones viz. T<sub>4</sub>, T<sub>3</sub>, insulin and cortisol levels were measured using RIA methods.
17. Blood metabolites viz. plasma glucose, NEFA and urea were analyzed in plasma samples collected on the aforesaid periods.
18. Milk samples were analyzed for fat, protein, lactose, NEFA, urea, SCC (epithelial cells and TLC) and TS.
19. Standard methods of analysis/estimations were used.
20. Statistical analysis of data was carried out using Least Square Analysis of Variance (ANOVA) with interactions. Mean and S.E. were calculated and correlations were found out. The averages were compared by Duncan's Multiple Range Test.

The following results were obtained:

- i. During hot-dry season plasma level of T<sub>3</sub> and cortisol significantly decreased and insulin increased in experimental cows in comparison to control, however, there was no effect of fan and mist on plasma T<sub>4</sub> level.
- ii. Feed intake of the cooled cows increased significantly by 13.14% and water intake decreased by 2.92% in comparison to control.
- iii. Plasma glucose significantly increased in experimental cows in comparison to control and also had lower levels of plasma NEFA and urea during the experiment.
- iv. Values of morning RT, RR, HR and ST non-significantly varied during the experiment, however, in the evening RT, RR, HR and ST were significantly higher in control group in comparison to experimental group.
- v. Milk production in experimental cows was significantly higher (10.23%) than the control.

- vi. Milk NEFA and urea levels were significantly lower in experimental cows than the control.
- vii. Availability of mist and fan cooling increased fat, protein, lactose and TS of milk in experimental cows than the control.
- viii. There was no effect of fan and mist cooling on SCC of milk of both the groups, however, TLC decreased significantly in experimental cows.
- ix. RT and ST were negatively correlated to milk production indicating thereby that both RT and ST are critical determinants of physiological responses and milk production.
- x. During hot-humid season plasma insulin level was higher in experimental group of cows and there was no effect of mist and fan cooling on plasma T<sub>4</sub> and T<sub>3</sub> levels, however, plasma cortisol decreased in comparison to control.
- xi. Feed intake of experimental cows increased significantly by 11.82% and water intake decreased by 8.15%.
- xii. Level of plasma glucose was higher and NEFA and urea levels decreased in experimental group of cows in comparison to control. The values of physiological responses (RR, HR and ST) were significantly low in experimental group during morning and evening than the control. RT during morning was similar in both the groups but declined in experimental cows in the evening.
- xiii. Milk production of experimental cows subjected to mist and fan cooling was higher (8.03%) and the levels of NEFA and urea of milk were lower.
- xiv. Milk composition values for TS, fat, protein, lactose were higher in experimental group than the control during the periods of study.
- xv. SCC of milk decreased in experimental cows due to decrease in epithelial cells of milk. However, mist and fan cooling during hot-humid season did not influence TLC of milk.

- xvi. RT was positively correlated to RR, HR and ST. Maximum temperature influenced ST of control group of cows but in experimental cows with mist cooling such association of maximum temperature with ST was not found.
- xvii. During winter season plasma T<sub>4</sub> and T<sub>3</sub> levels declined in cows kept inside the houses without any adverse effect on insulin and cortisol levels.
- xviii. Feed and water intake of experimental cows kept inside the houses increased by 10.74 and 8.38% in comparison to control.
- xix. Values of plasma glucose increased and plasma NEFA and urea decreased in experimental cows than the control.
- xx. RT was similar during morning and evening in both the group of cows. RR, HR and ST were more during evening in experimental cows than the control. ST was found to be significantly higher during morning and evening in experimental cows.
- xxi. In-house keeping of lactating cows during winter increased milk production by 7.41% and levels of NEFA and urea were lower.
- xxii. TS, fat, protein and lactose content of milk was higher in experimental group of cows in comparison to control by 0.23, 3.02, 5.35 and 1.20%, respectively.
- xxiii. SCC of milk was lower in experimental cows but epithelial and TLC was not affected due to the treatment.
- xxiv. Milk production was positively associated with RR and HR in experimental group kept inside the houses but such association was not found in control group.
- xxv. Plasma levels of T<sub>4</sub> and insulin were more and cortisol levels were less in wallowing buffaloes (experimental) in comparison to control group of buffaloes provided with mist. However, plasma T<sub>3</sub> level was similar in both the groups.
- xxvi. Feed intake increased by 11.62% than the control and water intake decreased by 8.20% in wallowing buffaloes.

- xxvii. Plasma glucose level was high and values of NEFA and urea were lower in wallowing group of buffaloes.
- xxviii. The values of physiological responses (RT, HR and ST) were similar during morning in both the groups but RR was lower in wallowing buffaloes. Further, RT, RR, HR and ST values of wallowing buffaloes were lower during evening in comparison to control group of buffaloes.
- xxix. Wallowing buffaloes produced more milk (13.45%) and the values of milk urea and NEFA were decreased in comparison to control.
- xxx. TS, fat, protein and lactose content of milk was also higher by 1.83, 7.23, 20.31 and 3.61%, respectively in wallowing buffaloes than control group with mist system.
- xxxi. SCC and epithelial cells of milk was similar in both the groups, however, TLC values declined due to wallowing.
- xxxii. Milk production was associated negatively with values of physiological responses (RT, RR, HR and ST).
- xxxiii. During hot-humid season, plasma levels of  $T_4$ ,  $T_3$  and insulin were higher and cortisol was lower in experimental buffaloes (wallowing) in comparison to showers (control).
- xxxiv. Blood metabolites levels were significantly influenced due to wallowing. Plasma glucose was more and NEFA and urea levels were low in wallowing buffaloes than the control.
- xxxv. Values of RT, RR, HR and ST declined after the wallowing during evening in comparison to control, however, morning values of RT and ST were similar in both the groups.
- xxxvi. Milk production in wallowing buffaloes increased by 13.27% over the control as was also found during hot-dry (13.45%) season.
- xxxvii. Feed intake of wallowing buffaloes was more (10.15%) and water intake declined by 7.64%. Further, plasma NEFA and urea levels were low in experimental buffaloes than the control.

- xxxviii. The values of TS, fat, protein and lactose were more in wallowing buffaloes in comparison to buffaloes kept under showers.
- xxxix. SCC, epithelial cells and TLC values were similar in both the group of buffaloes during the experimental period.
- xl. Milk production was negatively associated with RT, RR, HR and ST which indicated that later are important determinants of stress.
- xli. Plasma levels of T<sub>4</sub> and insulin in buffaloes kept inside the houses were higher in comparison to loose housing (control) during winter season. There was no effect of wallowing on T<sub>3</sub> and cortisol.
- xlii. Feed and water intake of experimental buffaloes was more by 7.00 and 4.09% in comparison to control group. Further, glucose level was higher and NEFA and urea were lower in experimental buffaloes than the control.
- xliii. RT, RR, HR and ST values were more during evening in experimental buffaloes. Further, RT was more and RR was less during morning in experimental buffaloes. The HR and ST during morning were similar in both the groups. The values of RT, RR, HR and ST were more in evening than morning in both the group of buffaloes.
- xliv. Milk production was higher (9.44%) and milk NEFA and urea were lower in experimental buffaloes than the control.
- xlv. Milk composition values for TS, fat, protein and lactose were higher in experimental buffaloes in comparison to control.
- xlvi. SCC and epithelial cells of milk were similar in both the group of buffaloes, however, TLC was lower in buffaloes kept inside the houses in comparison to buffaloes kept under loose housing system.
- xlvii. Basal average SCC of milk during different seasons in cows in comparison to buffaloes was higher and indicated species variation. Further, SCC of milk also varied during different seasons of experiment in both cows and buffaloes.

xlvi. During winter season minimum temperature was associated positively with HR, ST and RT.

## **6.2 CONCLUSIONS:**

1. During hot-dry season availability of mist and fan did not influence  $T_4$  level, however,  $T_3$  decreased and insulin increased due to significant increase in feed intake. A decline in cortisol level in experimental group indicated less stress as also evidenced from the decreased values of physiological responses i.e. RT, RR, HR and ST. Blood glucose level increased in experimental cows due to increased feed intake which also resulted in decreased NEFA and urea level. Availability of mist and fan results in more milk yield and more TS, fat, protein, lactose and low milk NEFA and urea levels concomitant to decline in plasma NEFA and urea concentration. Further, SCC of milk was not influenced indicating thereby no adverse effect of mist cooling on udder health. Increased values of physiological responses (RT, RR, HR and ST) in control group indicate that RT and ST were more influenced in comparison to other parameters (RR and PR) and have adverse effect on milk production.
2. During hot-humid season, fan and mist availability increased plasma insulin and decreased cortisol levels but there was no effect on plasma thyroid hormone. The increase in milk production in experimental cows was concomitant to increased feed intake, glucose level and a decline in plasma NEFA and urea levels which result in low milk NEFA and urea. Fan and mist cooling decreased RT, RR, HR and ST in evening. Decreased SCC of milk in cooled cows was attributed to decreased epithelial cells of milk. Non-significant changes in TLC of both groups indicate no adverse effect of mist and fan cooling on udder health.

3. During winter season in-house keeping of cows increased  $T_4$ , cortisol and decreased  $T_3$  level without any effect on insulin. Similar values of physiological responses (RT, RR and HR) in control and experimental cows indicated stress on cows of both the groups due to minimum average temperature ( $6.44^{\circ}\text{C}$ ) during the experiment, however, ST was found to be significantly influenced by in-house keeping of animals. The milk production in experimental cows was more due to more feed and water intake and higher glucose and lower levels of plasma and milk urea and NEFA concentration than the control. A decline in SCC in milk of experimental cows indicates less stress on mammary health. However, SCC was within normal physiological limits in control cows also.
4. Wallowing of buffaloes resulted in increase in  $T_4$ , insulin and decrease in cortisol without any effect on  $T_3$  concentration. Milk production of wallowing buffaloes increased due to more feed intake which resulted in significant increases in blood glucose and decreases in plasma NEFA and urea levels. The lower levels of milk NEFA and urea in wallowing buffaloes over the control were due to low plasma NEFA and urea levels. Wallowing of buffaloes significantly influenced milk composition by increase in TS, fat, protein and lactose in comparison to control. There was no adverse effect of wallowing on SCC and udder health. The RT and ST were found to be an important index for determining the comfort of animal.
5. During hot-humid season, wallowing resulted in increase in plasma insulin,  $T_4$ ,  $T_3$  and decrease in cortisol level. Feed intake in wallowing buffaloes significantly increased concomitant to increases in milk production and plasma glucose. Further, NEFA and urea levels of milk declined due to decrease in their plasma levels. The low values of physiological responses in wallowing

buffaloes indicate that wallowing was an effective method for amelioration of stress which was also reflected by low cortisol level. The significant increases in fat, protein, lactose and TS of milk in wallowing buffaloes were due to the combined effect of improvement in physiological responses and increase in feed and water intakes. The similar values of SCC, epithelial cells and TLC indicate that wallowing and showers availability has no adverse effect on udder health.

6. During winter season in-house keeping of buffaloes resulted in significant increases in plasma  $T_4$  and insulin due to increase in feed intake which resulted in significant increase in milk production than the control. The significant increase in plasma glucose and decreases in NEFA and urea levels, as a result of more feed intake which lead to low milk NEFA and urea levels. The in-house keeping of buffaloes during winter results in significant changes in milk composition and improvement in physiological responses (RT, RR and HR). There was no effect of modified management on SCC and epithelial cell of milk in both the groups of buffaloes.
7. The economics of the modified management provided through mister/shower and wallowing or provision of houses in cows and buffaloes indicate that a farmer can use these practices for being economical.

# **TABLES**

Table 1. Average values during hot-dry (May- June) season.

Periods of experiment	Max. (°C)	Min. (°C)	D <sub>b</sub> (°C)		W <sub>b</sub> (°C)		RH%		Sunshine hrs of prev. day	Wind speed (km/hr)	THI
			I	II	I	II	I	II			
1.	40.50	24.00	28.20	39.50	20.40	23.20	46.00	21.00	9.90	5.80	80.26
2.	39.60	26.40	24.00	40.40	22.80	24.40	58.00	13.00	9.40	1.80	80.37
3.	40.30	24.50	26.40	40.60	23.40	25.40	46.00	10.00	9.50	2.20	81.88
4.	40.10	22.60	25.80	38.20	19.60	22.50	47.00	14.00	10.50	4.30	78.39
5.	42.20	21.60	26.40	36.40	20.20	26.20	46.00	17.00	8.50	8.60	79.51
6.	43.10	24.60	28.80	39.20	22.60	26.50	47.00	14.00	10.50	4.30	82.35
7.	39.60	19.40	24.00	39.00	18.60	25.40	57.00	23.00	9.40	1.80	78.72
8.	39.00	24.60	26.60	31.80	24.60	27.00	31.00	34.00	10.60	3.00	79.80
9.	40.60	24.80	28.60	40.20	22.60	23.80	54.00	32.00	9.70	2.90	81.67
10.	43.80	26.20	30.20	40.10	24.20	27.90	56.00	35.00	9.50	3.70	84.26
<b>Average</b>	<b>40.88</b>	<b>23.87</b>	<b>26.90</b>	<b>38.54</b>	<b>21.90</b>	<b>25.23</b>	<b>48.80</b>	<b>21.30</b>	<b>9.75</b>	<b>3.84</b>	<b>80.72</b>

Max. = Maximum temperature, Min. = Minimum temperature, D<sub>b</sub> = Dry bulb temperature, W<sub>b</sub> = Wet bulb temperature, RH = Relative humidity, THI = Temperature humidity index, I and II = observation at 8.00 A.M. and 2.00 P.M.

Table 2. Average values of macroclimatic variables during hot-humid (August-September) season.

Periods of experiment	Max. (°C)	Min. (°C)	D <sub>b</sub> (°C)		W <sub>b</sub> (°C)		RH%		Sunshine hrs of prev. day	Wind speed (km/hr)	THI
			I	II	I	II	I	II			
1.	34.60	22.80	27.20	35.80	24.50	28.40	78.00	69.00	7.20	4.30	81.92
2.	38.50	20.20	27.40	35.20	22.60	26.40	75.00	73.00	8.20	3.50	80.37
3.	33.20	21.80	31.40	36.20	27.40	30.20	66.00	69.00	4.60	3.30	85.27
4.	33.40	26.90	32.20	34.50	28.20	29.40	78.00	74.00	7.90	5.10	84.94
5.	30.80	27.80	30.80	33.80	27.40	30.60	79.00	88.00	8.90	4.60	84.33
6.	30.40	25.60	28.70	32.20	26.80	29.60	86.00	84.00	6.90	5.10	82.42
7.	34.60	29.20	30.10	33.60	28.90	31.80	89.00	80.00	7.00	3.60	84.98
8.	31.60	31.40	27.40	34.50	28.40	29.40	84.00	82.00	7.50	4.20	83.29
9.	31.20	25.00	30.90	31.20	27.60	27.90	91.00	83.00	6.90	3.60	82.53
10.	35.40	27.80	27.40	39.40	28.20	32.80	87.00	81.00	7.60	4.20	86.20
<b>Average</b>	<b>33.37</b>	<b>25.85</b>	<b>29.35</b>	<b>34.64</b>	<b>27.00</b>	<b>29.65</b>	<b>81.30</b>	<b>78.30</b>	<b>7.27</b>	<b>4.15</b>	<b>83.62</b>

Max. = Maximum temperature, Min. = Minimum temperature, D<sub>b</sub> = Dry bulb temperature, W<sub>b</sub> = Wet bulb temperature, RH = Relative humidity, THI = Temperature humidity index, I and II = observation at 8.00 A.M. and 2.00 P.M.

**Table 3. Average values of macroclimatic variables during winter (December-January) season.**

Periods of experiment	Max. (°C)	Min. (°C)	D <sub>b</sub> (°C)		W <sub>b</sub> (°C)		RH %		Sunshine hrs of prev. day	Wind speed (km/hr)	THI
			I	II	I	II	I	II			
1.	11.30	6.20	6.60	12.00	6.60	11.60	100.00	95.00	0.00	3.40	53.44
2.	12.00	6.70	7.90	12.20	7.50	10.80	94.00	83.00	0.80	2.60	54.02
3.	12.50	5.40	6.40	10.00	6.40	9.00	100.00	87.00	0.00	1.90	51.64
4.	11.50	1.70	4.20	7.20	4.20	7.00	100.00	97.00	4.30	2.00	48.33
5.	12.80	3.40	5.00	15.20	5.00	12.20	100.00	68.00	2.80	1.80	53.66
6.	14.20	4.90	6.20	13.00	5.60	11.00	94.00	77.00	4.00	2.20	53.08
7.	14.00	3.30	3.60	17.00	3.60	13.30	100.00	63.00	0.00	1.70	53.70
8.	19.70	4.10	7.00	20.90	7.00	15.40	100.00	53.00	5.00	1.10	58.30
9.	22.30	5.70	7.40	22.50	7.40	16.00	100.00	47.00	6.70	1.50	59.38
10.	12.00	6.70	7.90	12.20	7.50	10.80	94.00	83.00	0.80	2.60	54.02
<b>Average</b>	<b>14.23</b>	<b>4.81</b>	<b>6.22</b>	<b>14.22</b>	<b>6.08</b>	<b>11.71</b>	<b>98.20</b>	<b>75.30</b>	<b>2.44</b>	<b>2.08</b>	<b>53.95</b>

Max. = Maximum temperature, Min. = Minimum temperature, D<sub>b</sub> = Dry bulb temperature, W<sub>b</sub> = Wet bulb temperature, RH = Relative humidity, THI = Temperature humidity index, I and II = observation at 8.00 A.M. and 2.00 P.M.

**Table 4. Average values of microclimatic variables in control and experimental shed during hot-dry (May-June) season.**

Periods of experiment	Max. (°C)		Min. (°C)		D <sub>b</sub> (°C)		W <sub>b</sub> (°C)		RH %			THI				
	I	II	I	II	I	II	I	II	I	II	III	I	II			
1.	40.40	40.00	26.20	26.00	30.00	38.90	37.50	24.20	26.20	26.00	26.00	53.00	27.00	28.00	83.14	82.57
2.	39.80	39.20	27.40	27.00	29.40	38.60	37.50	24.00	27.40	27.00	27.00	63.00	20.00	20.00	83.18	82.64
3.	40.20	39.30	24.90	24.50	29.80	38.80	37.60	24.60	27.20	27.00	27.00	56.00	26.00	27.00	84.59	83.04
4.	40.00	39.50	23.70	23.50	29.60	38.40	36.20	25.10	27.00	26.90	26.90	52.00	36.00	37.00	83.43	82.60
5.	42.00	41.60	21.00	20.60	30.20	39.00	37.80	25.40	27.40	27.20	27.20	49.00	28.00	28.00	84.52	83.61
6.	43.60	43.50	24.50	24.00	30.80	38.60	36.60	25.80	27.80	27.60	27.60	54.00	29.00	30.00	84.48	83.68
7.	39.40	39.20	20.20	20.00	29.20	38.20	36.00	24.60	25.80	24.40	24.40	62.00	30.00	31.00	82.60	81.31
8.	39.20	39.00	23.20	23.00	29.40	38.40	37.20	24.20	24.80	24.60	24.60	46.00	42.00	43.00	82.24	81.74
9.	40.40	40.00	23.80	23.50	30.00	39.00	37.80	24.80	26.40	26.00	26.00	54.00	42.00	43.00	83.87	82.89
10.	43.40	43.00	25.80	25.70	30.60	39.40	37.30	25.80	27.40	27.20	27.20	62.00	40.00	40.00	84.55	83.72
<b>Average</b>	<b>40.84</b>	<b>40.43</b>	<b>24.07</b>	<b>23.78</b>	<b>29.90</b>	<b>38.73</b>	<b>37.15</b>	<b>24.85</b>	<b>26.74</b>	<b>26.39</b>	<b>26.39</b>	<b>55.10</b>	<b>32.00</b>	<b>32.70</b>	<b>84.11</b>	<b>82.78</b>

Max. = Maximum temperature, Min. = Minimum temperature, I = observation in control shed, II = observation in experimental shed

D<sub>b</sub> = Dry bulb temperature, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

W<sub>b</sub> = Wet bulb temperature, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

RH = Relative humidity, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

THI = Temperature Humidity Index, I = observation in control shed, II = observation in experimental shed

**Table 5. Average values of microclimatic variables in control and experimental shed during hot-humid (August-September) season.**

Periods of experiment	Max. (°C)		Min. (°C)		D <sub>b</sub> (°C)			W <sub>b</sub> (°C)			RH %			THI	
	I	II	I	II	I	II	III	I	II	III	I	II	III	I	II
1.	35.00	34.80	23.00	23.00	28.00	55.00	34.50	24.00	29.00	29.00	80.00	71.00	72.00	81.96	81.78
2.	39.00	38.50	21.00	20.80	27.00	34.00	33.50	23.00	25.00	25.00	79.00	72.00	73.00	79.44	79.26
3.	34.50	34.50	22.00	21.70	32.00	35.00	33.40	28.00	31.00	31.00	71.00	72.00	73.00	85.56	84.98
4.	33.50	33.30	26.00	25.50	32.00	33.50	33.00	29.00	31.00	30.60	79.00	71.00	73.00	85.38	85.05
5.	31.50	31.00	28.00	28.00	31.00	32.50	31.00	29.00	30.00	29.60	80.00	87.00	92.00	84.30	83.76
6.	30.00	29.70	25.00	24.60	29.00	30.00	29.50	27.50	29.00	28.60	92.00	88.00	90.00	81.78	81.45
7.	35.00	34.80	30.00	29.50	32.00	33.00	31.50	28.00	30.50	30.00	92.00	84.00	85.00	84.66	83.94
8.	32.00	32.00	28.00	28.10	29.00	33.50	32.00	29.00	31.00	30.60	89.00	88.00	89.00	84.66	83.61
9.	30.00	29.80	26.00	25.60	31.00	30.50	30.00	27.00	28.00	27.60	92.00	81.00	80.00	82.86	81.81
10.	36.00	35.50	29.00	28.50	29.00	38.00	36.50	29.00	34.00	33.20	86.00	83.00	84.00	87.90	86.17
<b>Average</b>	<b>33.65</b>	<b>33.39</b>	<b>25.80</b>	<b>25.53</b>	<b>30.00</b>	<b>33.50</b>	<b>32.49</b>	<b>27.35</b>	<b>29.85</b>	<b>29.52</b>	<b>84.00</b>	<b>79.70</b>	<b>81.10</b>	<b>83.85</b>	<b>83.18</b>

Max. = Maximum temperature, Min. = Minimum temperature, I = observation in control shed, II = observation in experimental shed

D<sub>b</sub> = Dry bulb temperature, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

W<sub>b</sub> = Wet bulb temperature, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

RH = Relative humidity, I = observation at 8.00 A.M. in control and experimental shed, II and III = observation at 2.00 P.M. in control and experimental shed

THI = Temperature Humidity Index, I = observation in control shed, II = observation in experimental shed

Table 6. Average values of microclimatic variables in control shed and in -houses during winter (December- January) season.

Periods of experiment	Max. ( $^{\circ}\text{C}$ )		Min. ( $^{\circ}\text{C}$ )		$D_b$ ( $^{\circ}\text{C}$ )		$W_b$ ( $^{\circ}\text{C}$ )		RH %		THI							
	I	II	I	II	I <sup>a</sup>	I <sup>b</sup>	II <sup>a</sup>	II <sup>b</sup>	I <sup>a</sup>	I <sup>b</sup>	II <sup>a</sup>	II <sup>b</sup>	I	II				
1	12.90	14.40	5.90	7.90	8.60	11.00	20.80	21.00	7.80	8.50	14.60	15.80	88.00	86.00	41.00	40.00	58.84	60.46
2	14.80	16.90	5.50	8.50	8.40	11.50	21.00	21.60	7.60	8.70	15.20	16.60	87.00	86.00	44.00	44.00	58.99	61.22
3	16.40	18.50	5.80	7.50	8.40	11.70	20.60	21.10	7.80	9.00	14.80	16.80	90.00	89.00	59.00	58.00	58.77	61.29
4	12.00	15.00	4.60	8.60	8.20	11.30	20.40	20.80	7.40	8.60	14.60	16.50	88.00	86.00	62.00	60.00	58.41	60.79
5	17.40	20.60	4.40	9.40	8.00	11.90	20.00	20.90	7.20	8.50	13.60	15.60	86.00	85.00	55.00	54.00	57.76	60.68
6	16.80	19.30	4.80	8.60	8.40	12.00	19.80	20.70	7.80	9.40	13.00	15.80	92.00	90.00	58.00	57.00	57.84	61.04
7	18.00	21.40	3.80	6.90	7.80	11.60	16.80	19.60	6.90	7.60	12.80	14.60	89.00	88.00	49.00	47.00	56.14	59.42
8	18.20	20.20	3.60	7.60	7.60	11.50	16.40	20.00	6.80	8.30	12.60	14.80	87.00	87.00	47.00	46.00	55.82	59.85
9	17.40	20.20	4.00	8.70	8.20	12.30	17.00	19.80	7.20	8.60	13.60	15.60	86.00	83.00	49.00	48.00	56.76	60.46
10	17.80	20.90	4.80	7.90	8.60	13.20	17.40	20.40	7.40	8.40	14.80	15.20	84.00	85.00	54.00	50.00	57.55	60.79
<b>Average</b>	<b>16.17</b>	<b>18.74</b>	<b>4.72</b>	<b>8.16</b>	<b>8.22</b>	<b>11.80</b>	<b>19.02</b>	<b>20.59</b>	<b>7.39</b>	<b>8.56</b>	<b>13.96</b>	<b>15.73</b>	<b>87.70</b>	<b>86.50</b>	<b>51.80</b>	<b>50.40</b>	<b>57.69</b>	<b>60.60</b>

Max. = Maximum temperature, Min. = Minimum temperature, I = observation in control shed, II = observation in experimental shed

$D_b$  = Dry bulb temperature, I<sup>a</sup> and I<sup>b</sup> = observation at 8.00 A.M. in control and experimental shed, II<sup>a</sup> and II<sup>b</sup> = observation at 2.00 P.M. in control and experimental shed

$W_b$  = Wet bulb temperature, I<sup>a</sup> and I<sup>b</sup> = observation at 8.00 A.M. in control and experimental shed, II<sup>a</sup> and II<sup>b</sup> = observation at 2.00 P.M. in control and experimental shed

RH = Relative humidity, I<sup>a</sup> and I<sup>b</sup> = observation at 8.00 A.M. in control and experimental shed, II<sup>a</sup> and II<sup>b</sup> = observation at 2.00 P.M. in control and experimental shed

THI = Temperature Humidity Index, I = observation in control shed, II = observation in experimental shed

**Table 7. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental cows during hot-dry season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	48.50	47.35	48.03	45.21	46.56	43.33	43.93	46.55	44.01	44.43	45.79
	$\pm 1.41$	$\pm 1.59$	$\pm 1.67$	$\pm 0.99$	$\pm 2.39$	$\pm 0.39$	$\pm 0.97$	$\pm 2.05$	$\pm 1.84$	$\pm 2.55$	
Experimental	42.85	46.61	43.78	46.21	48.68	47.80	47.45	46.66	47.25	45.36	46.26
	$\pm 1.11$	$\pm 1.32$	$\pm 1.38$	$\pm 1.90$	$\pm 0.86$	$\pm 1.69$	$\pm 0.95$	$\pm 1.98$	$\pm 1.22$	$\pm 1.53$	
<b>Thyroxine (ng/ml)</b>											
Control	1.64	1.74	1.89	1.46	1.57	1.46	1.42	1.44	1.31	1.29	1.52 <sup>a</sup>
	$\pm 0.11$	$\pm 0.18$	$\pm 0.15$	$\pm 0.25$	$\pm 0.16$	$\pm 0.07$	$\pm 0.12$	$\pm 0.13$	$\pm 0.11$	$\pm 0.16$	
Experimental	1.37	1.26	1.37	1.22	1.39	1.40	1.49	1.28	1.27	1.43	1.35 <sup>b</sup>
	$\pm 0.12$	$\pm 0.16$	$\pm 0.14$	$\pm 0.06$	$\pm 0.12$	$\pm 0.13$	$\pm 0.11$	$\pm 0.16$	$\pm 0.15$	$\pm 0.10$	
<b>Triiodothyronine (ng/ml)</b>											
Control	9.42	7.63	10.29	9.19	8.06	10.29	10.98	9.35	7.18	8.39	9.08
	$\pm 1.61$	$\pm 1.32$	$\pm 1.16$	$\pm 1.10$	$\pm 0.73$	$\pm 0.89$	$\pm 1.85$	$\pm 1.43$	$\pm 0.41$	$\pm 1.57$	
Experimental	7.04	8.79	10.11	10.44	11.14	10.78	10.86	10.60	11.33	10.44	10.15
	$\pm 0.51$	$\pm 0.38$	$\pm 0.60$	$\pm 0.99$	$\pm 1.37$	$\pm 1.13$	$\pm 0.79$	$\pm 0.94$	$\pm 0.82$	$\pm 0.99$	
<b>Insulin (<math>\mu</math>U/ml)</b>											
Control	4.13	4.03	4.35	3.77	4.44	4.30	3.85	4.35	3.92	4.59	4.17 <sup>a</sup>
	$\pm 0.39$	$\pm 0.32$	$\pm 0.35$	$\pm 0.38$	$\pm 0.70$	$\pm 0.44$	$\pm 0.74$	$\pm 0.44$	$\pm 0.40$	$\pm 0.48$	
Experimental	2.13	2.48	2.55	2.50	2.52	2.36	2.93	2.38	2.35	2.13	2.38 <sup>b</sup>
	$\pm 0.15$	$\pm 0.17$	$\pm 0.23$	$\pm 0.17$	$\pm 0.13$	$\pm 0.30$	$\pm 0.25$	$\pm 0.18$	$\pm 0.25$	$\pm 0.27$	
<b>Cortisol (ng/ml)</b>											
Control	4.13	4.03	4.35	3.77	4.44	4.30	3.85	4.35	3.92	4.59	4.17 <sup>a</sup>
	$\pm 0.39$	$\pm 0.32$	$\pm 0.35$	$\pm 0.38$	$\pm 0.70$	$\pm 0.44$	$\pm 0.74$	$\pm 0.44$	$\pm 0.40$	$\pm 0.48$	
Experimental	2.13	2.48	2.55	2.50	2.52	2.36	2.93	2.38	2.35	2.13	2.38 <sup>b</sup>
	$\pm 0.15$	$\pm 0.17$	$\pm 0.23$	$\pm 0.17$	$\pm 0.13$	$\pm 0.30$	$\pm 0.25$	$\pm 0.18$	$\pm 0.25$	$\pm 0.27$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 8. Least Square Mean ( $\pm$ S.E.) of plasma glucose, NEFA and urea concentration in control and experimental cows during hot-dry season.

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	49.04	48.82	49.21	49.64	48.65	49.65	48.38	48.84	49.59	49.32	49.11 <sup>a</sup>
	$\pm 0.50$	$\pm 0.86$	$\pm 0.87$	$\pm 0.64$	$\pm 0.67$	$\pm 0.64$	$\pm 0.69$	$\pm 0.44$	$\pm 0.50$	$\pm 0.97$	$\pm 0.21$
Experimental	54.78	54.34	56.74	57.59	58.31	61.68	61.91	62.62	60.23	63.37	59.16 <sup>b</sup>
	$\pm 0.93$	$\pm 1.16$	$\pm 1.20$	$\pm 0.97$	$\pm 1.14$	$\pm 0.87$	$\pm 0.74$	$\pm 0.61$	$\pm 1.18$	$\pm 0.86$	$\pm 0.49$
											2.35
Control	270.00	276.66	281.66	281.66	287.50	288.33	286.66	280.00	288.33	280.83	282.16 <sup>a</sup>
	$\pm 5.77$	$\pm 4.40$	$\pm 5.10$	$\pm 4.59$	$\pm 4.95$	$\pm 7.60$	$\pm 5.42$	$\pm 5.32$	$\pm 3.57$	$\pm 4.16$	$\pm 1.67$
Experimental	249.16	236.66	238.33	229.16	230.00	219.16	232.50	230.00	229.16	225.83	232.00 <sup>b</sup>
	$\pm 5.38$	$\pm 2.58$	$\pm 6.37$	$\pm 5.16$	$\pm 5.32$	$\pm 4.59$	$\pm 4.94$	$\pm 5.28$	$\pm 5.47$	$\pm 6.24$	$\pm 1.78$
											13.06
Control	64.22	66.43	66.00	69.06	68.72	69.41	66.58	67.24	69.12	68.70	67.55 <sup>a</sup>
	$\pm 2.25$	$\pm 1.78$	$\pm 1.54$	$\pm 2.16$	$\pm 1.79$	$\pm 2.14$	$\pm 2.56$	$\pm 1.72$	$\pm 1.06$	$\pm 2.34$	$\pm 0.61$
Experimental	59.74	57.82	56.66	56.08	56.70	54.71	52.94	53.04	52.09	51.82	55.16 <sup>b</sup>
	$\pm 2.31$	$\pm 2.36$	$\pm 2.10$	$\pm 1.98$	$\pm 2.11$	$\pm 1.44$	$\pm 1.16$	$\pm 1.76$	$\pm 1.25$	$\pm 1.01$	$\pm 0.62$
											4.54

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 9. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during hot-dry season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		
Control	101.91	101.76	102.96	102.26	102.20	102.46	102.83	102.60	102.20	102.20	102.20	102.34
	$\pm 0.30$	$\pm 0.18$	$\pm 0.23$	$\pm 0.25$	$\pm 0.10$	$\pm 0.31$	$\pm 0.28$	$\pm 0.26$	$\pm 0.24$	$\pm 0.10$	$\pm 0.10$	$\pm 0.08$
Experimental	101.96	102.66	102.76	102.46	102.16	102.86	102.70	102.63	102.33	102.33	102.33	102.49
	$\pm 0.10$	$\pm 0.36$	$\pm 0.25$	$\pm 0.24$	$\pm 0.15$	$\pm 0.48$	$\pm 0.23$	$\pm 0.18$	$\pm 0.15$	$\pm 0.13$	$\pm 0.13$	$\pm 0.08$
Control	29.66	31.33	29.66	32.00	32.33	32.33	36.00	34.33	34.33	34.33	34.33	32.70
	$\pm 0.80$	$\pm 1.22$	$\pm 0.80$	$\pm 1.15$	$\pm 1.08$	$\pm 1.49$	$\pm 1.03$	$\pm 0.80$	$\pm 0.80$	$\pm 0.80$	$\pm 0.80$	$\pm 0.40$
Experimental	28.66	32.00	30.00	31.33	31.33	34.33	32.00	32.66	33.33	33.33	33.33	32.06
	$\pm 0.84$	$\pm 1.46$	$\pm 0.89$	$\pm 1.33$	$\pm 1.33$	$\pm 0.61$	$\pm 1.46$	$\pm 0.84$	$\pm 0.84$	$\pm 0.84$	$\pm 0.84$	$\pm 0.38$
Control	60.00	63.66	58.66	61.66	64.33	65.00	67.00	63.00	61.00	62.66	62.66	62.70
	$\pm 0.73$	$\pm 1.08$	$\pm 0.84$	$\pm 0.95$	$\pm 0.95$	$\pm 0.85$	$\pm 0.85$	$\pm 1.52$	$\pm 0.85$	$\pm 0.42$	$\pm 0.42$	$\pm 0.41$
Experimental	60.00	61.00	60.66	59.00	63.33	62.33	66.00	65.66	62.33	63.66	63.66	62.40
	$\pm 0.73$	$\pm 0.85$	$\pm 0.66$	$\pm 1.12$	$\pm 0.98$	$\pm 1.20$	$\pm 0.73$	$\pm 0.95$	$\pm 1.20$	$\pm 0.61$	$\pm 0.61$	$\pm 0.39$
Control	37.13	37.48	36.68	37.61	38.20	38.11	37.28	37.65	37.13	37.98	37.98	37.52
	$\pm 0.55$	$\pm 0.34$	$\pm 0.39$	$\pm 0.50$	$\pm 0.51$	$\pm 0.17$	$\pm 0.56$	$\pm 0.42$	$\pm 0.24$	$\pm 0.32$	$\pm 0.32$	$\pm 0.13$
Experimental	37.36	38.36	37.01	38.26	37.65	37.75	37.06	38.85	37.40	37.83	37.83	37.75
	$\pm 0.49$	$\pm 0.68$	$\pm 0.64$	$\pm 0.46$	$\pm 0.60$	$\pm 0.33$	$\pm 0.46$	$\pm 0.55$	$\pm 0.52$	$\pm 0.28$	$\pm 0.28$	$\pm 0.16$

**Table 10. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during hot-dry season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	103.96	103.76	104.53	103.93	103.36	104.26	103.70	103.93	103.56	103.43	103.84 <sup>a</sup>	
	$\pm 0.16$	$\pm 0.09$	$\pm 0.39$	$\pm 0.40$	$\pm 0.17$	$\pm 0.33$	$\pm 0.27$	$\pm 0.15$	$\pm 0.17$	$\pm 0.23$		$\pm 0.08$
Experimental	102.06	102.36	102.40	102.36	102.10	102.23	102.26	102.26	102.00	102.20	102.22 <sup>b</sup>	
	$\pm 0.06$	$\pm 0.24$	$\pm 0.26$	$\pm 0.18$	$\pm 0.08$	$\pm 0.15$	$\pm 0.16$	$\pm 0.12$	$\pm 0.05$	$\pm 0.08$		$\pm 0.04$
<b>Respiration Rate (breaths per min.)</b>												
Control	42.33	45.00	42.33	45.33	48.00	47.33	48.00	46.67	44.67	44.67	45.43 <sup>a</sup>	
	$\pm 1.20$	$\pm 0.85$	$\pm 1.89$	$\pm 1.60$	$\pm 0.51$	$\pm 0.84$	$\pm 0.51$	$\pm 0.67$	$\pm 1.11$	$\pm 0.67$		$\pm 0.40$
Experimental	38.33	39.33	37.00	40.67	44.33	40.33	41.67	41.33	40.33	38.67	40.20 <sup>b</sup>	
	$\pm 0.95$	$\pm 0.67$	$\pm 0.85$	$\pm 0.98$	$\pm 0.61$	$\pm 1.20$	$\pm 0.95$	$\pm 0.98$	$\pm 0.95$	$\pm 0.84$		$\pm 0.36$
<b>Heart rate (per min.)</b>												
Control	74.00	75.67	74.67	77.00	80.33	79.00	78.00	78.33	75.00	72.33	76.43 <sup>a</sup>	
	$\pm 1.15$	$\pm 1.74$	$\pm 1.52$	$\pm 0.85$	$\pm 0.95$	$\pm 0.85$	$\pm 0.51$	$\pm 0.95$	$\pm 0.85$	$\pm 1.20$		$\pm 0.44$
Experimental	69.33	70.33	70.67	73.33	77.67	75.67	71.67	71.33	69.00	68.67	71.76 <sup>b</sup>	
	$\pm 1.11$	$\pm 1.20$	$\pm 1.33$	$\pm 1.22$	$\pm 0.61$	$\pm 0.61$	$\pm 0.95$	$\pm 1.33$	$\pm 0.85$	$\pm 0.42$		$\pm 0.47$
<b>Skin temperature (<math>^{\circ}</math>C)</b>												
Control	41.03	40.01	40.05	39.98	40.06	40.06	39.56	39.65	39.13	39.13	39.87 <sup>a</sup>	
	$\pm 0.76$	$\pm 0.59$	$\pm 0.29$	$\pm 0.27$	$\pm 0.26$	$\pm 0.40$	$\pm 0.37$	$\pm 0.18$	$\pm 0.28$	$\pm 0.24$		$\pm 0.13$
Experimental	37.50	37.58	36.93	36.98	37.15	36.98	36.46	37.26	37.63	36.88	37.13 <sup>b</sup>	
	$\pm 0.26$	$\pm 0.52$	$\pm 0.43$	$\pm 0.31$	$\pm 0.40$	$\pm 0.18$	$\pm 0.18$	$\pm 0.20$	$\pm 0.34$	$\pm 0.63$		$\pm 0.12$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 11. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental cows during hot-dry season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	21.83	20.41	20.66	21.00	20.00	20.08	21.00	21.25	20.75	20.25	20.75 <sup>a</sup>	20.72 <sup>a</sup>
	$\pm 1.65$	$\pm 1.87$	$\pm 1.66$	$\pm 1.52$	$\pm 1.81$	$\pm 1.49$	$\pm 1.86$	$\pm 1.96$	$\pm 1.48$	$\pm 1.30$		
Experimental	21.08	21.33	22.41	21.83	22.83	24.83	23.67	23.75	23.16	23.50	3.09	22.84 <sup>b</sup>
	$\pm 1.45$	$\pm 1.08$	$\pm 1.47$	$\pm 1.30$	$\pm 1.44$	$\pm 0.88$	$\pm 1.45$	$\pm 0.94$	$\pm 1.49$	$\pm 1.57$		
Control	260.83	267.50	266.66	272.50	277.66	279.16	277.50	271.66	266.66	276.66	14.03	271.58 <sup>a</sup>
	$\pm 6.50$	$\pm 5.59$	$\pm 3.33$	$\pm 5.43$	$\pm 5.27$	$\pm 5.54$	$\pm 6.15$	$\pm 5.42$	$\pm 5.27$	$\pm 5.27$		
Experimental	235.83	225.00	224.16	215.00	220.00	211.66	223.33	217.50	215.00	210.83	14.03	169.58 <sup>b</sup>
	$\pm 5.38$	$\pm 2.58$	$\pm 6.37$	$\pm 5.16$	$\pm 5.32$	$\pm 4.59$	$\pm 4.94$	$\pm 5.28$	$\pm 5.47$	$\pm 6.24$		
Control	61.72	64.26	63.67	66.38	66.39	67.58	64.58	64.91	66.95	66.37	65.28 <sup>a</sup>	65.28 <sup>a</sup>
	$\pm 2.06$	$\pm 1.61$	$\pm 1.68$	$\pm 2.04$	$\pm 1.71$	$\pm 2.19$	$\pm 2.56$	$\pm 1.65$	$\pm 1.11$	$\pm 2.21$		
Experimental	57.24	55.98	53.83	52.58	53.69	52.88	50.77	49.65	49.60	49.32	4.34	52.55 <sup>b</sup>
	$\pm 2.23$	$\pm 1.73$	$\pm 2.34$	$\pm 2.00$	$\pm 1.86$	$\pm 1.41$	$\pm 1.36$	$\pm 1.99$	$\pm 1.46$	$\pm 1.22$		

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 12. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental cows during hot-dry season.

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	11.64	11.67	11.48	11.53	11.48	11.45	11.47	11.40	11.32	11.35	11.48 <sup>a</sup>	$\pm 0.02$
	$\pm 0.10$	$\pm 0.06$	$\pm 0.07$	$\pm 0.05$	$\pm 0.06$	$\pm 0.07$	$\pm 0.07$	$\pm 0.06$	$\pm 0.02$	$\pm 0.04$		
Experimental	12.13	12.28	12.39	12.37	12.47	12.44	12.53	12.59	12.68	12.69	12.45 <sup>b</sup>	$\pm 0.03$
	$\pm 0.09$	$\pm 0.07$	$\pm 0.04$	$\pm 0.05$	$\pm 0.03$	$\pm 0.05$	$\pm 0.03$	$\pm 0.05$	$\pm 0.04$	$\pm 0.03$		
Control	3.76	3.53	3.53	3.62	3.57	3.57	3.66	3.53	3.71	3.74	3.62 <sup>a</sup>	$\pm 0.04$
	$\pm 0.14$	$\pm 0.15$	$\pm 0.17$	$\pm 0.12$	$\pm 0.13$	$\pm 0.13$	$\pm 0.08$	$\pm 0.10$	$\pm 0.08$	$\pm 0.07$		
Experimental	4.12	4.10	4.05	4.15	4.17	4.19	4.16	4.33	4.32	4.36	4.20 <sup>b</sup>	$\pm 0.28$
	$\pm 0.04$	$\pm 0.14$	$\pm 0.15$	$\pm 0.14$	$\pm 0.15$	$\pm 0.15$	$\pm 0.17$	$\pm 0.16$	$\pm 0.29$	$\pm 0.29$		
Control	3.99	4.10	3.99	4.19	4.13	4.10	4.05	4.16	4.02	4.08	4.08 <sup>a</sup>	$\pm 0.04$
	$\pm 0.07$	$\pm 0.05$	$\pm 0.09$	$\pm 0.12$	$\pm 0.20$	$\pm 0.09$	$\pm 0.11$	$\pm 0.11$	$\pm 0.15$	$\pm 0.12$		
Experimental	4.27	4.53	4.42	4.50	4.51	4.62	4.81	4.76	4.82	5.10	4.63 <sup>b</sup>	$\pm 0.06$
	$\pm 0.06$	$\pm 0.05$	$\pm 0.05$	$\pm 0.03$	$\pm 0.06$	$\pm 0.03$	$\pm 0.05$	$\pm 0.06$	$\pm 0.03$	$\pm 0.05$		
Control	5.06	5.01	5.02	4.90	4.92	4.94	4.85	4.91	4.90	4.87	4.94 <sup>a</sup>	$\pm 0.01$
	$\pm 0.04$	$\pm 0.02$	$\pm 0.03$	$\pm 0.04$	$\pm 0.04$	$\pm 0.03$	$\pm 0.03$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$		
Experimental	4.97	5.00	5.06	5.08	5.09	5.17	5.00	5.19	5.15	5.20	5.21 <sup>b</sup>	$\pm 0.01$
	$\pm 0.06$	$\pm 0.05$	$\pm 0.03$	$\pm 0.04$	$\pm 0.03$	$\pm 0.03$	$\pm 0.03$	$\pm 0.03$	$\pm 0.05$	$\pm 0.05$		

Values with different superscripts <sup>a, b</sup> in a column differ (P<0.05)

**Table 13. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental cows during hot-dry season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
	<b>Somatic cell count (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	1.56 $\pm 0.07$	1.68 $\pm 0.03$	1.51 $\pm 0.05$	1.53 $\pm 0.03$	1.50 $\pm 0.04$	1.53 $\pm 0.03$	1.52 $\pm 0.03$	1.58 $\pm 0.04$	1.58 $\pm 0.04$	1.58 $\pm 0.04$	1.48 $\pm 0.04$	1.55 $\pm 0.21$
Experimental	1.61 $\pm 0.93$	1.53 $\pm 1.16$	1.55 $\pm 1.20$	1.52 $\pm 0.97$	1.46 $\pm 1.14$	1.44 $\pm 0.87$	1.49 $\pm 0.74$	1.55 $\pm 0.61$	1.53 $\pm 1.18$	1.53 $\pm 0.86$	-	1.52 $\pm 0.49$
	<b>Epithelial cells (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	0.95 $\pm 0.03$	0.93 $\pm 0.01$	0.86 $\pm 0.03$	0.86 $\pm 0.02$	0.84 $\pm 0.03$	0.87 $\pm 0.03$	0.84 $\pm 0.03$	0.89 $\pm 0.02$	0.89 $\pm 0.02$	0.89 $\pm 0.02$	0.82 $\pm 0.03$	0.87 $\pm 0.01$
Experimental	0.92 $\pm 0.04$	0.88 $\pm 0.03$	0.95 $\pm 0.06$	0.88 $\pm 0.03$	0.87 $\pm 0.02$	0.86 $\pm 0.01$	0.83 $\pm 0.02$	0.92 $\pm 0.03$	0.92 $\pm 0.03$	0.87 $\pm 0.02$	-	0.88 $\pm 0.01$
	<b>TLC (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	0.61 $\pm 0.04$	0.75 $\pm 0.04$	0.64 $\pm 0.03$	0.67 $\pm 0.02$	0.66 $\pm 0.02$	0.66 $\pm 0.02$	0.67 $\pm 0.02$	0.69 $\pm 0.03$	0.69 $\pm 0.02$	0.69 $\pm 0.02$	0.66 $\pm 0.02$	0.67 $\pm 0.01$
Experimental	0.68 $\pm 0.01$	0.65 $\pm 0.02$	0.60 $\pm 0.04$	0.63 $\pm 0.02$	0.59 $\pm 0.02$	0.57 $\pm 0.03$	0.65 $\pm 0.02$	0.63 $\pm 0.03$	0.66 $\pm 0.02$	0.65 $\pm 0.02$	-	0.63 $\pm 0.01$

**Table 14. Analysis of Variance (ANOVA) of complete data on plasma thyroxine, triiodothyronine, insulin, cortisol, glucose, NEFA and urea of cows during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	34.83*	0.049	9.69	1.73*	8.13	521.83**	135.20**
Between Groups	1	6.76	0.90**	34.39**	145.17**	3027.11**	75500.84**	4605.15**
Between Periods	9	7.71	0.12	9.50	0.86	33.17**	78.70	14.11
All effect interaction	9	32.46	0.13	9.93	0.98	32.12**	517.03**	46.20**
Error	95	14.04	0.13	7.24	0.75	4.22	130.07	15.76

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

**Table 15. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of cows during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	0.94	4.80	8.87	3.61**
Between Groups	3	34.22**	2454.97**	2886.72**	89.97**
Between Periods	9	1.33	67.17	99.83**	1.97
All effect interaction	27	0.30	11.31	19.87	1.38
Error	195	0.31	6.31	5.98	1.07

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

\* P<0.05, \*\* P<0.01

**Table 16. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration rate, heart rate and skin temperature of cows during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares											
		RT		RR		HR		ST					
		M	E	M	E	M	E	M	E				
Between Animals	5	0.74*	0.29	3.95	9.39	7.17	5.84	2.22	2.93**				
Between Groups	1	0.66	78.73**	12.03	821.63**	2.70	653.33**	1.56	223.85**				
Between Periods	9	1.06**	0.60**	42.52**	47.58**	56.81**	84.31**	2.55*	1.57*				
Periods x Groups	9	0.29	0.29	7.58	3.41	12.10*	6.22	0.95	1.03				
Animals x Groups	5	0.0057	0.0016	0.053	0.013	0.0021	0.0047	0.011	0.011				
Error	90	0.27	0.23	6.13	5.99	5.44	7.01	1.22	0.66				

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P<0.05, \*\* P<0.01

**Table 17. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of cows during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	136.02**	0.065	0.021	0.0096	0.013*	0.71	0.73*	0.046	565.70	144.12
Between Groups	1	134.40**	28.61**	0.022	0.0032	0.042**	9.89**	9.16**	0.90**	80341.88**	4859.20**
Between Periods	9	3.42	0.019	0.022	0.012	0.0078	0.080	0.19	0.0048	92.15	14.51
All effect interaction	9	6.10	0.24	0.011	0.0050	0.0074	0.038	0.17	0.065	467.33	49.62
Error	95	7.31	0.020	1.012	0.0065	0.0045	0.14	0.13	0.0084	150.09	14.40

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

Table 18. Correlation coefficients (r) # of pooled data on maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in cows during hot-dry season.

	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0285	1.0000				
RT	-0.0445	-0.1491**	1.0000			
RR	-0.0574	-0.0557	0.4537**	1.0000		
HR	-0.0446	-0.0315	0.4619**	0.8392**	1.0000	
ST	-0.0195	-0.2826**	0.5382**	0.4129**	0.3964**	1.0000

# = No. of observations (240)

\* = P<0.05

\*\* = P<0.01

**Table 19. Correlation coefficients (r) # of maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of cows during hot-dry season**

<b>Control group:</b>						
	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0777	1.0000				
RT	-0.0494	-0.3038**	1.0000			
RR	0.0297	-0.2265*	0.6503**	1.0000		
HR	0.0206	-0.2422*	0.6871**	0.8654**	1.0000	
ST	0.0784	-0.3161**	0.6108**	0.6444**	0.6938**	1.0000
<b>Experimental group:</b>						
	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	0.0214	1.0000				
RT	-0.0518	0.1546	1.0000			
RR	0.1030	0.2147*	-0.2410*	1.0000		
HR	0.0794	0.2722**	-0.1828*	0.7744**	1.0000	
ST	-0.0534	-0.2526**	0.0305	-0.1924*	-0.2417**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 20. Correlation coefficients (r) # between plasma hormones, metabolites, milk production and composition in control and experimental group of cows during hot-dry season.**

Variables	Significance	Value of 'r'
<b>T<sub>4</sub> vs:</b>		
Lactose	P<0.05	0.176
<b>T<sub>3</sub> vs:</b>		
Cortisol	P<0.01	0.243
Plasma glucose	P<0.05	-0.199
TS	P<0.05	-0.215
<b>Insulin vs:</b>		
Plasma glucose	P<0.01	0.258
Plasma NEFA	P<0.05	- 0.178
Plasma urea	P<0.01	- 0.233
TS	P<0.05	0.219
TLC	P<0.05	- 0.181
Lactose	P<0.01	0.261
Milk NEFA	P<0.05	- 0.187
Milk urea	P<0.01	- 0.235
<b>Cortisol vs:</b>		
Plasma glucose	P<0.01	- 0.654
Plasma NEFA	P<0.01	0.654
Plasma urea	P<0.01	0.635
MP	P<0.05	- 0.218
TS	P<0.01	- 0.740
Fat	P<0.01	- 0.474
Protein	P<0.01	- 0.481
Lactose	P<0.01	- 0.412
Milk NEFA	P<0.01	0.638
Milk urea	P<0.01	0.638
<b>Plasma glucose vs:</b>		
Plasma NEFA	P<0.01	- 0.814
Plasma urea	P<0.01	- 0.706
MP	P<0.01	0.308
TS	P<0.01	0.876
TLC	P<0.01	- 0.235
Fat	P<0.01	0.592
Protein	P<0.01	0.554
Lactose	P<0.01	0.638
Milk NEFA	P<0.01	- 0.808
Milk urea	P<0.01	- 0.714
<b>Plasma NEFA vs:</b>		
Plasma urea	P<0.01	0.791
MP	P<0.01	- 0.332
TS	P<0.01	- 0.862

Contd...

Variables	Significance	Value of 'r'
TLC	P<0.01	0.252
Fat	P<0.01	- 0.453
Protein	P<0.01	- 0.480
Lactose	P<0.01	- 0.602
Milk NEFA	P<0.01	0.962
Milk urea	P<0.01	0.804
<b>Plasma urea vs:</b>		
MP	P<0.01	- 0.241
TS	P<0.01	- 0.810
TLC	P<0.01	0.248
Fat	P<0.01	- 0.451
Protein	P<0.01	- 0.574
Lactose	P<0.01	- 0.568
Milk NEFA	P<0.01	0.789
Milk urea	P<0.01	0.989
<b>MP vs:</b>		
TS	P<0.01	0.314
Lactose	P<0.01	0.282
Milk NEFA	P<0.01	- 0.341
Milk urea	P<0.01	- 0.238
<b>TS vs:</b>		
TLC	P<0.05	- 0.215
Fat	P<0.01	0.530
Protein	P<0.01	0.571
Lactose	P<0.01	0.624
Milk NEFA	P<0.01	- 0.855
Milk urea	P<0.01	- 0.813
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.756
TLC	P<0.01	0.689
<b>TLC vs:</b>		
Fat	P<0.05	- 0.201
Lactose	P<0.01	- 0.256
Milk NEFA	P<0.01	0.251
Milk urea	P<0.01	0.232
<b>Fat vs:</b>		
Protein	P<0.01	0.368
Lactose	P<0.01	0.473
Milk NEFA	P<0.01	- 0.438
Milk urea	P<0.01	- 0.453
<b>Protein vs:</b>		
Lactose	P<0.01	0.395
Milk NEFA	P<0.01	- 0.501
Milk urea	P<0.01	- 0.582
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	- 0.590
Milk urea	P<0.01	0.576
<b>Milk NEFA vs:</b>		
Milk urea	P<0.01	0.807

**Table 21. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental cows during hot-humid season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	43.43	43.65	44.53	44.70	46.56	44.75	45.15	43.76	46.66	41.70	44.49	-
	$\pm 0.63$	$\pm 0.86$	$\pm 1.17$	$\pm 1.69$	$\pm 1.63$	$\pm 1.66$	$\pm 2.76$	$\pm 2.28$	$\pm 1.54$	$\pm 1.02$	$\pm 0.51$	
Experimental	46.65	47.60	46.41	45.36	48.41	44.96	42.88	45.23	49.88	44.73	46.21	-
	$\pm 1.56$	$\pm 1.13$	$\pm 1.72$	$\pm 1.18$	$\pm 0.89$	$\pm 0.99$	$\pm 1.63$	$\pm 1.04$	$\pm 1.93$	$\pm 2.24$	$\pm 0.94$	
Control	1.13	1.26	1.11	1.65	1.19	1.47	1.46	1.30	1.29	1.44	1.33	-
	$\pm 0.05$	$\pm 0.08$	$\pm 1.12$	$\pm 0.34$	$\pm 0.09$	$\pm 0.14$	$\pm 0.16$	$\pm 0.10$	$\pm 0.09$	$\pm 0.09$	$\pm 0.33$	
Experimental	1.39	1.49	1.43	1.40	1.31	1.24	1.14	1.56	1.23	1.34	1.35	-
	$\pm 0.16$	$\pm 0.07$	$\pm 0.06$	$\pm 0.12$	$\pm 0.10$	$\pm 0.12$	$\pm 0.11$	$\pm 0.05$	$\pm 0.10$	$\pm 0.11$	$\pm 0.03$	
Control	6.47	5.13	7.05	6.08	6.22	4.84	6.44	6.28	6.63	4.89	6.00 <sup>a</sup>	-
	$\pm 0.95$	$\pm 0.58$	$\pm 1.59$	$\pm 0.94$	$\pm 0.91$	$\pm 0.78$	$\pm 0.97$	$\pm 0.70$	$\pm 0.85$	$\pm 0.86$	$\pm 0.29$	
Experimental	9.56	9.66	8.55	8.39	7.96	8.50	9.04	9.37	9.01	8.58	8.86	2.41
	$\pm 0.93$	$\pm 0.44$	$\pm 1.10$	$\pm 0.59$	$\pm 0.45$	$\pm 1.02$	$\pm 0.96$	$\pm 0.24$	$\pm 0.55$	$\pm 0.75$	$\pm 0.23^b$	
Control	4.05	3.83	5.02	4.01	4.89	5.31	3.97	4.54	3.85	3.99	4.35 <sup>a</sup>	-
	$\pm 0.34$	$\pm 0.46$	$\pm 0.37$	$\pm 0.42$	$\pm 0.34$	$\pm 0.36$	$\pm 0.44$	$\pm 0.64$	$\pm 0.56$	$\pm 0.52$	$\pm 0.25$	
Experimental	2.47	2.83	2.77	2.78	2.67	3.16	2.82	2.89	2.93	2.96	2.83 <sup>b</sup>	1.26
	$\pm 0.24$	$\pm 0.26$	$\pm 0.35$	$\pm 0.27$	$\pm 0.35$	$\pm 0.22$	$\pm 0.24$	$\pm 0.36$	$\pm 0.31$	$\pm 0.27$	0.04	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )



**Table 23. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during hot-humid season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	101.56	101.66	101.75	101.36	103.10	101.83	101.70	102.03	101.30	101.79		101.79
	$\pm 0.14$	$\pm 0.16$	$\pm 0.08$	$\pm 0.26$	$\pm 0.23$	$\pm 0.10$	$\pm 0.22$	$\pm 0.03$	$\pm 0.11$	$\pm 0.11$		$\pm 0.08$
Experimental	101.50	101.70	101.48	102.00	103.40	101.56	101.90	102.00	101.23	101.93	-	101.87
	$\pm 0.09$	$\pm 0.21$	$\pm 0.16$	$\pm 0.17$	$\pm 0.37$	$\pm 0.18$	$\pm 0.11$	$\pm 0.13$	$\pm 0.07$	$\pm 0.08$		$\pm 0.09$
Control	26.33	27.00	26.08	30.00	29.66	26.66	26.33	28.00	24.66	26.33		27.10
	$\pm 0.55$	$\pm 0.68$	$\pm 0.66$	$\pm 0.89$	$\pm 0.80$	$\pm 0.66$	$\pm 0.80$	$\pm 0.30$	$\pm 0.42$	$\pm 0.61$		$\pm 0.28$
Experimental	25.00	25.66	23.91	28.00	27.66	25.66	26.00	25.33	24.33	23.33	-	25.49
	$\pm 0.44$	$\pm 0.80$	$\pm 0.45$	$\pm 1.03$	$\pm 1.30$	$\pm 0.61$	$\pm 0.89$	$\pm 0.84$	$\pm 0.80$	$\pm 0.42$		$\pm 0.29$
Control	54.00	56.00	57.33	54.33	58.00	54.00	52.33	53.00	53.00	53.33		54.53
	$\pm 1.71$	$\pm 2.06$	$\pm 0.66$	$\pm 1.30$	$\pm 0.89$	$\pm 1.78$	$\pm 1.08$	$\pm 1.12$	$\pm 0.68$	$\pm 0.84$		$\pm 0.44$
Experimental	51.33	56.00	50.00	54.00	53.66	50.66	52.66	52.33	50.00	49.33	-	52.00
	$\pm 1.11$	$\pm 1.03$	$\pm 0.89$	$\pm 0.89$	$\pm 0.33$	$\pm 1.76$	$\pm 1.33$	$\pm 0.33$	$\pm 0.89$	$\pm 0.84$		$\pm 0.39$
Control	32.50	32.58	32.25	33.66	31.25	31.33	33.25	29.75	31.83	32.16		32.05
	$\pm 0.22$	$\pm 0.32$	$\pm 0.47$	$\pm 0.47$	$\pm 0.11$	$\pm 0.10$	$\pm 0.11$	$\pm 0.11$	$\pm 0.10$	$\pm 0.10$		$\pm 0.15$
Experimental	31.66	32.16	31.91	33.41	31.16	31.33	32.75	29.66	31.41	32.00	-	31.75
	$\pm 0.27$	$\pm 0.27$	$\pm 0.30$	$\pm 0.30$	$\pm 0.10$	$\pm 0.16$	$\pm 0.11$	$\pm 0.10$	$\pm 0.08$	$\pm 0.18$		$\pm 0.14$

**Table 24. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during hot-humid season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	102.03	102.06	102.15	102.06	103.46	102.10	102.30	102.56	101.86	102.10	102.27 <sup>a</sup>
	$\pm 0.07$	$\pm 0.13$	$\pm 0.12$	$\pm 0.36$	$\pm 0.25$	$\pm 0.19$	$\pm 0.16$	$\pm 0.06$	$\pm 0.04$	$\pm 0.16$	
Experimental	101.20	101.33	101.00	101.66	103.63	101.26	101.43	101.66	101.00	101.73	101.59 <sup>b</sup>
	$\pm 0.08$	$\pm 0.22$	$\pm 0.14$	$\pm 0.20$	$\pm 0.32$	$\pm 0.17$	$\pm 0.14$	$\pm 0.13$	$\pm 0.05$	$\pm 0.12$	
	<b>Respiration Rate (breaths per min.)</b>										
Control	34.66	34.33	36.33	38.33	36.66	33.00	32.66	34.66	36.33	35.66	35.26 <sup>a</sup>
	$\pm 0.98$	$\pm 1.20$	$\pm 0.61$	$\pm 0.61$	$\pm 0.66$	$\pm 1.98$	$\pm 1.68$	$\pm 0.84$	$\pm 0.95$	$\pm 0.95$	
Experimental	22.66	23.50	22.16	25.33	25.00	23.33	22.33	22.66	22.66	21.33	23.10 <sup>b</sup>
	$\pm 0.33$	$\pm 0.61$	$\pm 0.16$	$\pm 0.42$	$\pm 0.68$	$\pm 0.42$	$\pm 0.61$	$\pm 0.42$	$\pm 0.84$	$\pm 0.42$	
	<b>Heart rate (per min.)</b>										
Control	61.33	67.33	68.33	63.66	67.33	66.33	56.66	59.33	62.66	65.66	63.66 <sup>a</sup>
	$\pm 1.68$	$\pm 1.22$	$\pm 0.61$	$\pm 1.66$	$\pm 0.84$	$\pm 1.30$	$\pm 0.66$	$\pm 0.66$	$\pm 1.22$	$\pm 1.30$	
Experimental	49.66	51.00	47.50	47.66	50.00	48.33	48.33	49.00	47.00	47.00	48.55 <sup>b</sup>
	$\pm 0.80$	$\pm 1.00$	$\pm 0.71$	$\pm 1.08$	$\pm 1.03$	$\pm 0.80$	$\pm 0.80$	$\pm 1.00$	$\pm 0.68$	$\pm 0.68$	
	<b>Skin temperature (<math>^{\circ}</math>C)</b>										
Control	34.41	33.58	34.18	35.25	32.68	32.58	35.00	31.75	33.25	33.58	33.62 <sup>a</sup>
	$\pm 0.23$	$\pm 0.37$	$\pm 0.61$	$\pm 0.21$	$\pm 0.10$	$\pm 0.08$	$\pm 0.18$	$\pm 0.35$	$\pm 0.11$	$\pm 0.08$	
Experimental	30.50	30.33	29.91	31.25	29.83	29.83	30.00	30.08	29.16	30.08	30.10 <sup>b</sup>
	$\pm 0.34$	$\pm 0.10$	$\pm 0.23$	$\pm 0.51$	$\pm 0.35$	$\pm 0.35$	$\pm 0.59$	$\pm 0.49$	$\pm 0.45$	$\pm 0.30$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 25. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental cows during hot-humid season.

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	9.50	9.58	11.00	11.00	10.08	10.16	9.91	10.33	10.75	11.08		10.34
	$\pm 0.99$	$\pm 0.91$	$\pm 1.05$	$\pm 1.02$	$\pm 1.12$	$\pm 1.10$	$\pm 0.95$	$\pm 0.79$	$\pm 0.87$	$\pm 0.91$		$\pm 0.29$
Experimental	9.91	10.50	11.25	11.41	11.41	11.16	11.58	11.33	11.50	11.66		11.17
	$\pm 0.49$	$\pm 0.56$	$\pm 0.44$	$\pm 0.55$	$\pm 0.15$	$\pm 0.38$	$\pm 0.50$	$\pm 0.24$	$\pm 0.61$	$\pm 0.21$		$\pm 0.14$
	NEFA ( $\mu$ mol/l)											
Control	178.33	180.00	188.33	181.66	185.83	176.66	180.83	183.33	177.50	186.66		181.91 <sup>a</sup>
	$\pm 4.40$	$\pm 4.08$	$\pm 2.78$	$\pm 5.27$	$\pm 2.71$	$\pm 6.28$	$\pm 7.23$	$\pm 4.77$	$\pm 3.81$	$\pm 2.10$		$\pm 1.43$
Experimental	163.33	168.33	180.00	175.00	170.00	162.50	175.00	167.50	170.00	164.16		169.58 <sup>b</sup>
	$\pm 3.57$	$\pm 4.40$	$\pm 2.88$	$\pm 4.28$	$\pm 4.83$	$\pm 3.09$	$\pm 2.88$	$\pm 2.81$	$\pm 4.83$	$\pm 3.96$		$\pm 1.31$
	Urea (mg%)											
Control	51.47	52.22	53.63	52.60	53.73	53.71	54.39	53.28	52.29	53.96		53.14 <sup>a</sup>
	$\pm 0.79$	$\pm 0.83$	$\pm 0.74$	$\pm 0.74$	$\pm 0.85$	$\pm 0.77$	$\pm 0.63$	$\pm 0.88$	$\pm 0.49$	$\pm 0.95$		$\pm 0.25$
Experimental	48.93	48.47	48.88	49.48	49.40	48.64	48.14	47.39	47.95	47.74		48.50 <sup>b</sup>
	$\pm 0.82$	$\pm 0.78$	$\pm 0.92$	$\pm 0.77$	$\pm 0.68$	$\pm 0.40$	$\pm 0.88$	$\pm 0.70$	$\pm 0.66$	$\pm 1.02$		$\pm 0.24$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 26. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental cows during hot-humid season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
	<b>Total solids (%)</b>											
Control	12.64 $\pm 0.11$	12.46 $\pm 0.18$	12.74 $\pm 0.13$	12.58 $\pm 0.06$	12.64 $\pm 0.09$	12.62 $\pm 0.12$	12.90 $\pm 0.07$	12.66 $\pm 0.06$	12.80 $\pm 0.06$	12.93 $\pm 0.02$	12.70 <sup>a</sup> $\pm 0.04$	
Experimental	13.10 $\pm 0.05$	13.18 $\pm 0.09$	13.01 $\pm 0.03$	13.03 $\pm 0.02$	13.08 $\pm 0.01$	13.04 $\pm 0.02$	13.03 $\pm 0.01$	13.04 $\pm 0.02$	13.05 $\pm 0.02$	13.06 $\pm 0.01$	13.06 <sup>b</sup> $\pm 0.01$	
	<b>Fat (%)</b>											
Control	3.31 $\pm 0.11$	3.39 $\pm 0.11$	3.39 $\pm 0.11$	3.43 $\pm 0.09$	3.45 $\pm 0.07$	3.51 $\pm 0.08$	3.53 $\pm 0.12$	3.58 $\pm 0.11$	3.54 $\pm 0.12$	3.53 $\pm 0.10$	3.47 $\pm 0.03$	
Experimental	3.54 $\pm 0.08$	3.57 $\pm 0.09$	3.57 $\pm 0.09$	3.61 $\pm 0.08$	3.74 $\pm 0.08$	3.76 $\pm 0.12$	3.77 $\pm 0.10$	3.84 $\pm 0.05$	3.86 $\pm 0.08$	4.02 $\pm 0.04$	3.73 $\pm 0.03$	
	<b>Protein (%)</b>											
Control	3.74 $\pm 0.06$	3.68 $\pm 0.05$	3.71 $\pm 0.06$	3.82 $\pm 0.03$	3.88 $\pm 0.05$	3.88 $\pm 0.05$	3.93 $\pm 0.05$	3.99 $\pm 0.05$	3.82 $\pm 0.05$	3.82 $\pm 0.03$	3.83 <sup>a</sup> $\pm 0.02$	
Experimental	3.74 $\pm 0.06$	3.96 $\pm 0.05$	4.13 $\pm 0.05$	4.13 $\pm 0.03$	4.08 $\pm 0.06$	4.16 $\pm 0.03$	4.16 $\pm 0.05$	4.08 $\pm 0.06$	4.13 $\pm 0.03$	4.10 $\pm 0.05$	4.07 <sup>b</sup> $\pm 0.02$	
	<b>Lactose (%)</b>											
Control	4.94 $\pm 0.05$	4.94 $\pm 0.04$	4.92 $\pm 0.03$	4.94 $\pm 0.03$	4.94 $\pm 0.04$	4.96 $\pm 0.03$	4.87 $\pm 0.03$	4.90 $\pm 0.02$	4.92 $\pm 0.03$	4.89 $\pm 0.02$	4.92 $\pm 0.01$	
Experimental	4.97 $\pm 0.01$	4.98 $\pm 0.03$	4.99 $\pm 0.03$	4.98 $\pm 0.02$	4.95 $\pm 0.03$	4.98 $\pm 0.03$	5.00 $\pm 0.02$	4.94 $\pm 0.03$	4.92 $\pm 0.05$	4.99 $\pm 0.02$	4.97 $\pm 0.01$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 27. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental cows during hot-humid season.

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
	Somatic cell count ( $\times 10^5$ cells/ml of milk)										
Control	1.50 $\pm 0.04$	1.52 $\pm 0.01$	1.48 $\pm 0.03$	1.52 $\pm 0.02$	1.50 $\pm 0.03$	1.59 $\pm 0.02$	1.57 $\pm 0.03$	1.54 $\pm 0.03$	1.57 $\pm 0.03$	1.62 $\pm 0.02$	1.54 $\pm 0.01$
Experimental	1.50 $\pm 0.04$	1.47 $\pm 0.03$	1.47 $\pm 0.01$	1.46 $\pm 0.03$	1.55 $\pm 0.05$	1.61 $\pm 0.02$	1.46 $\pm 0.03$	1.51 $\pm 0.05$	1.58 $\pm 0.02$	1.51 $\pm 0.01$	1.51 $\pm 0.03$
	Epithelial cells ( $\times 10^5$ cells/ml of milk)										
Control	0.87 $\pm 0.02$	0.81 $\pm 0.03$	0.83 $\pm 0.02$	0.86 $\pm 0.03$	0.81 $\pm 0.02$	0.86 $\pm 0.03$	0.86 $\pm 0.03$	0.94 $\pm 0.03$	0.95 $\pm 0.03$	1.00 $\pm 0.03$	0.88 $\pm 0.01$
Experimental	0.84 $\pm 0.03$	0.82 $\pm 0.03$	0.83 $\pm 0.02$	0.82 $\pm 0.03$	0.80 $\pm 0.04$	0.87 $\pm 0.02$	0.82 $\pm 0.02$	0.87 $\pm 0.02$	0.88 $\pm 0.02$	0.93 $\pm 0.03$	0.85 $\pm 0.01$
	TLC ( $\times 10^5$ cells/ml of milk)										
Control	0.63 $\pm 0.04$	0.71 $\pm 0.03$	0.65 $\pm 0.03$	0.66 $\pm 0.04$	0.68 $\pm 0.02$	0.72 $\pm 0.03$	0.70 $\pm 0.04$	0.59 $\pm 0.02$	0.62 $\pm 0.04$	0.62 $\pm 0.02$	0.66 $\pm 0.01$
Experimental	0.66 $\pm 0.03$	0.64 $\pm 0.02$	0.63 $\pm 0.03$	0.63 $\pm 0.02$	0.75 $\pm 0.03$	0.74 $\pm 0.04$	0.64 $\pm 0.04$	0.64 $\pm 0.04$	0.69 $\pm 0.02$	0.57 $\pm 0.02$	0.66 $\pm 0.01$

**Table 28. Analysis of Variance (ANOVA) of complete data on plasma thyroxine, triiodothyronine, cortisol, glucose, NEFA and urea of cows during hot-humid season.**

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	638.06	2.52	4.41	2.89*	8.13	10.33	2.90
Between Groups	1	1356.11	2.28	244.44**	69.22**	3027.11**	18007.50**	848.63**
Between Periods	9	756.29	3.54	2.77	1.15	33.17	221.66**	3.52
All effect interaction	9	724.95	3.42	2.68	0.84	32.12	157.03*	3.88
Error	95	745.60	3.48	4.44	1.21	4.22	65.07	4.41

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

Table 29. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of cows during hot-humid season.

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	1.05**	5.44	16.21	1.36*
Between Groups	3	4.88**	1672.68**	2583.24**	125.47**
Between Periods	9	7.69**	39.41**	69.40**	18.11**
All effect interaction	27	0.23	5.98	26.68	1.47
Error	195	0.16	3.94	7.15	0.51

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

\*  $P < 0.05$ , \*\*  $P < 0.01$

**Table 30. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration rate, heart rate and skin temperature of cows during hot-humid season.**

Source effect	d.f.	Mean Sum of Squares													
		RT		RR		HR		ST							
		M	E	M	E	M	E	M	E	M	E	M	E		
Between Animals	5	0.55**	0.49*	2.66	7.83	9.25	14.00*	0.65	0.98						
Between Groups	1	0.16	13.80**	78.40**	4440.83**	192.53**	7038.00**	2.85**	373.47**						
Between Periods	9	3.41**	4.33**	27.30**	19.71**	32.08**	52.80**	13.21**	6.46**						
Periods x Groups	9	0.23	0.42*	2.48	7.77	17.42*	47.15**	0.18	2.66**						
Animals x Groups	5	0.0012	0.0012	0.0066	0.027	0.032	0.065	0.0002	0.0018						
Error	90	0.14	0.17	3.37	4.08	7.86	5.56	0.33	0.72						

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P<0.05, \*\* P<0.01

Table 31. Analysis of Variance of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of cows during hot-humid season.

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	84.39**	0.094*	0.014	0.016**	0.011	0.037	0.052**	0.0018	139.00	3.42
Between Groups	1	43.80**	3.94**	0.027**	0.028**	0.0000	2.08**	1.74**	0.069**	4563.33**	644.36**
Between Periods	9	2.48	0.051	0.019**	0.030**	0.020**	0.16**	0.11**	0.0038	207.77	3.18
All effect interaction	9	0.88	0.10	0.0089	0.0031	0.0083	0.026	0.044	0.0051	84.16	4.83
Error	95	1.97	0.035	0.0063	0.0044	0.0067	0.059	0.015	0.0076	106.28	3.70

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P<0.05, \*\* P<0.01

**Table 32. Correlation coefficients (r) # of maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in cows during hot-humid season.**

	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0645	1.0000				
RT	-0.1370	-0.0671	1.0000			
RR	-0.0395	0.0157	0.4175**	1.0000		
HR	0.0684	-0.0030	0.3518**	0.8160**	1.0000	
ST	0.2470**	-0.0658	0.1342	0.6416**	0.5985**	1.0000

# = No. of observations (240)

\* =  $P < 0.05$

\*\* =  $P < 0.01$

Table 33. Correlation coefficients (r) # of maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of cows during hot-humid season.

Control group:						
	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0436	1.0000				
RT	-0.1311	-0.0509	1.0000			
RR	-0.0698	-0.0220	0.3597**	1.0000		
HR	0.1464	0.0890	0.2055*	0.3411**	1.0000	
ST	0.4150**	-0.0792	-0.2599**	0.0667	0.0241	1.0000
Experimental group:						
	Max temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0948	1.0000				
RT	-0.1461	-0.1332	1.0000			
RR	-0.0367	-0.1166	0.4682**	1.0000		
HR	0.0469	-0.1660	0.4169**	0.8756**	1.0000	
ST	0.1716	-0.1579	0.3142**	0.8007**	0.7543**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 34. Correlation coefficients (r) # of maximum temperature, plasma hormones, metabolites, milk production and composition in control and experimental group of cows during hot-humid season.**

Variables	Significance	Value of 'r'
<b>Max temp. vs:</b>		
SCC	P<0.01	- 0.248
Protein	P<0.01	- 0.255
<b>T<sub>4</sub> vs:</b>		
SCC	P<0.05	- 0.197
Epithelial cells	P<0.05	- 0.180
<b>T<sub>3</sub> vs:</b>		
TS	P<0.05	0.203
<b>Insulin vs:</b>		
Cortisol	P<0.01	- 0.304
Plasma glucose	P<0.01	0.465
Plasma NEFA	P<0.01	- 0.403
Plasma urea	P<0.01	- 0.374
MP	P<0.05	0.200
TS	P<0.01	0.385
Fat	P<0.01	0.290
Protein	P<0.05	0.205
Lactose	P<0.05	0.185
Milk NEFA	P<0.01	- 0.292
Milk urea	P<0.01	- 0.405
<b>Cortisol vs:</b>		
Plasma glucose	P<0.01	- 0.515
Plasma NEFA	P<0.01	0.481
Plasma urea	P<0.01	0.433
MP	P<0.05	- 0.219
TS	P<0.01	- 0.353
Epithelial cells	P<0.05	0.176
Fat	P<0.01	- 0.250
Protein	P<0.01	- 0.327
Milk NEFA	P<0.05	0.203
Milk urea	P<0.01	0.444
<b>Plasma glucose vs:</b>		
Plasma NEFA	P<0.01	- 0.773
Plasma urea	P<0.01	- 0.757
MP	P<0.01	0.259
TS	P<0.01	0.571
Fat	P<0.01	0.466
Protein	P<0.01	0.631
Lactose	P<0.05	0.191
Milk NEFA	P<0.01	- 0.497
Milk urea	P<0.01	- 0.722
<b>Plasma NEFA vs:</b>		
Plasma urea	P<0.01	0.722
MP	P<0.05	- 0.191
TS	P<0.01	- 0.557
Fat	P<0.01	- 0.334
Protein	P<0.01	- 0.443

Contd...

Variables	Significance	Value of 'r'
Lactose	P<0.01	- 0.299
Milk NEFA	P<0.01	0.665
Milk urea	P<0.01	0.690
<b>Plasma urea vs:</b>		
TS	P<0.01	- 0.464
Fat	P<0.01	- 0.397
Protein	P<0.01	- 0.500
Lactose	P<0.05	- 0.179
Milk NEFA	P<0.01	0.523
Milk urea	P<0.01	0.961
<b>MP vs:</b>		
TS	P<0.01	0.298
Fat	P<0.01	0.398
Protein	P<0.01	0.249
Lactose	P<0.01	0.243
<b>TS vs:</b>		
Fat	P<0.01	0.348
Protein	P<0.01	0.389
Lactose	P<0.05	0.177
Milk NEFA	P<0.01	-0.352
Milk urea	P<0.01	-0.451
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.465
TLC	P<0.01	0.563
<b>Epithelial cells vs:</b>		
TLC	P<0.01	- 0.469
<b>Fat vs:</b>		
Protein	P<0.01	0.380
Milk urea	P<0.01	- 0.363
<b>Protein vs:</b>		
Lactose	P<0.05	0.191
Milk NEFA	P<0.01	- 0.344
Milk urea	P<0.01	- 0.427
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	- 0.241
<b>Milk NEFA vs:</b>		
Milk urea	P<0.01	0.533

**Table 35. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental cows during winter season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	50.70	50.50	46.50	54.08	48.20	53.68	54.28	53.93	56.58	58.20		52.66
	$\pm 2.35$	$\pm 1.34$	$\pm 1.71$	$\pm 3.50$	$\pm 2.15$	$\pm 1.80$	$\pm 2.27$	$\pm 1.93$	$\pm 3.18$	$\pm 3.67$		$\pm 0.85$
Experimental	53.40	53.50	55.61	55.33	54.72	52.11	53.28	56.50	60.85	58.95	-	55.42
	$\pm 1.99$	$\pm 3.07$	$\pm 2.56$	$\pm 1.66$	$\pm 2.82$	$\pm 1.35$	$\pm 1.86$	$\pm 3.00$	$\pm 1.62$	$\pm 2.97$		$\pm 0.77$
Control	1.51	1.56	1.62	1.77	1.65	1.45	1.46	1.68	1.71	1.77		1.62
	$\pm 0.10$	$\pm 0.15$	$\pm 0.12$	$\pm 0.13$	$\pm 0.12$	$\pm 0.09$	$\pm 0.06$	$\pm 0.09$	$\pm 0.14$	$\pm 0.08$		$\pm 0.04$
Experimental	1.32	1.28	1.40	1.23	1.52	1.44	1.28	1.36	1.31	1.33	-	1.35
	$\pm 0.09$	$\pm 0.05$	$\pm 0.11$	$\pm 0.15$	$\pm 0.16$	$\pm 0.28$	$\pm 0.09$	$\pm 0.16$	$\pm 0.15$	$\pm 0.06$		$\pm 0.04$
Control	6.43	7.14	5.95	7.29	5.49	5.83	8.36	10.63	7.51	6.27		7.09
	$\pm 1.12$	$\pm 1.13$	$\pm 0.78$	$\pm 1.37$	$\pm 0.83$	$\pm 0.74$	$\pm 1.26$	$\pm 1.27$	$\pm 1.30$	$\pm 1.46$		$\pm 0.38$
Experimental	7.18	6.90	6.95	6.87	7.48	7.04	8.65	6.69	8.08	6.42	-	7.23
	$\pm 0.64$	$\pm 0.53$	$\pm 0.49$	$\pm 0.62$	$\pm 0.91$	$\pm 1.05$	$\pm 1.07$	$\pm 0.60$	$\pm 0.46$	$\pm 0.80$		$\pm 0.23$
Control	1.85	2.04	2.00	2.11	2.20	2.01	2.01	2.12	2.24	1.82		2.09
	$\pm 0.23$	$\pm 0.15$	$\pm 0.05$	$\pm 0.05$	$\pm 0.07$	$\pm 0.04$	$\pm 0.20$	$\pm 0.11$	$\pm 0.17$	$\pm 0.10$		$\pm 0.05$
Experimental	2.55	2.10	2.02	2.08	2.08	2.42	2.21	2.53	2.33	2.16	-	2.25
	$\pm 0.11$	$\pm 0.08$	$\pm 0.09$	$\pm 0.12$	$\pm 0.07$	$\pm 0.28$	$\pm 0.14$	$\pm 0.16$	$\pm 0.17$	$\pm 0.20$		$\pm 0.06$



**Table 37. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during winter season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	100.90	101.06	101.00	100.90	100.90	100.93	101.03	100.90	100.90	101.30		100.98
	$\pm 0.52$	$\pm 0.23$	$\pm 0.14$	$\pm 0.15$	$\pm 0.03$	$\pm 0.07$	$\pm 0.09$	$\pm 0.04$	$\pm 0.19$	$\pm 0.33$		$\pm 0.07$
Experimental	100.56	100.90	100.83	100.60	100.86	100.96	100.90	101.16	100.96	101.00	-	100.87
	$\pm 0.49$	$\pm 0.22$	$\pm 0.18$	$\pm 0.14$	$\pm 0.04$	$\pm 0.06$	$\pm 0.05$	$\pm 0.06$	$\pm 0.06$	$\pm 0.25$		$\pm 0.06$
	<b>Respiration Rate (breaths per min.)</b>											
Control	22.33	23.33	22.67	20.33	20.33	19.33	20.00	19.33	22.33	21.00		21.10
	$\pm 0.61$	$\pm 0.42$	$\pm 0.66$	$\pm 0.61$	$\pm 0.61$	$\pm 0.42$	$\pm 0.51$	$\pm 0.66$	$\pm 0.61$	$\pm 0.85$		$\pm 0.25$
Experimental	22.58	22.33	22.00	20.67	19.33	20.33	20.67	20.00	21.00	21.33	-	21.02
	$\pm 0.65$	$\pm 0.61$	$\pm 0.73$	$\pm 0.66$	$\pm 0.66$	$\pm 0.61$	$\pm 0.66$	$\pm 0.45$	$\pm 0.45$	$\pm 0.45$		$\pm 0.26$
	<b>Heart rate (per min.)</b>											
Control	51.00	51.33	52.33	49.00	50.00	51.67	51.00	50.33	51.00	52.67		51.03
	$\pm 0.85$	$\pm 0.84$	$\pm 0.95$	$\pm 0.68$	$\pm 0.73$	$\pm 0.33$	$\pm 0.85$	$\pm 0.61$	$\pm 0.68$	$\pm 0.42$		$\pm 0.25$
Experimental	51.67	51.67	51.67	49.33	50.67	51.00	51.00	51.00	51.33	52.33	-	51.16
	$\pm 0.61$	$\pm 0.61$	$\pm 0.61$	$\pm 0.66$	$\pm 0.66$	$\pm 0.68$	$\pm 0.85$	$\pm 0.85$	$\pm 0.66$	$\pm 0.80$		$\pm 0.23$
	<b>Skin temperature (<math>^{\circ}</math>C)</b>											
Control	29.91	28.75	27.16	27.91	28.25	28.83	28.58	29.41	28.75	29.91		28.75
	$\pm 0.08$	$\pm 0.11$	$\pm 1.44$	$\pm 0.15$	$\pm 0.17$	$\pm 0.11$	$\pm 0.15$	$\pm 0.30$	$\pm 0.21$	$\pm 0.08$		$\pm 0.17$
Experimental	30.00	28.91	28.66	27.83	28.58	29.08	29.16	29.41	29.41	29.83	-	29.09
	$\pm 0.01$	$\pm 0.08$	$\pm 0.16$	$\pm 0.10$	$\pm 0.23$	$\pm 0.23$	$\pm 0.21$	$\pm 0.20$	$\pm 0.23$	$\pm 0.27$		$\pm 0.08$

Table 38a. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental cows during winter season.

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	101.36	101.30	101.20	101.30	101.20	101.20	101.26	101.16	101.16	101.50	101.26	1.76
	$\pm 0.29$	$\pm 0.15$	$\pm 0.14$	$\pm 0.13$	$\pm 0.09$	$\pm 0.07$	$\pm 0.07$	$\pm 0.08$	$\pm 0.17$	$\pm 0.27$	$\pm 0.05$	
Experimental	101.20	101.33	101.33	100.96	101.30	101.36	101.50	101.53	101.56	101.50	101.36	0.51
	$\pm 0.28$	$\pm 0.15$	$\pm 0.12$	$\pm 0.11$	$\pm 0.04$	$\pm 0.09$	$\pm 0.08$	$\pm 0.11$	$\pm 0.17$	$\pm 0.17$	$\pm 0.05$	
Control	25.00	26.00	26.33	24.00	24.67	24.00	24.33	25.00	26.00	25.00	25.03 <sup>a</sup>	1.76
Experimental	$\pm 0.61$	$\pm 0.42$	$\pm 0.66$	$\pm 0.61$	$\pm 0.61$	$\pm 0.42$	$\pm 0.51$	$\pm 0.67$	$\pm 0.61$	$\pm 0.85$	$\pm 0.25$	
Control	26.33	27.33	27.33	26.33	26.00	26.67	26.67	28.00	26.00	27.33	26.80 <sup>b</sup>	1.87
Experimental	$\pm 0.61$	$\pm 0.42$	$\pm 0.42$	$\pm 0.61$	$\pm 0.73$	$\pm 0.67$	$\pm 0.67$	$\pm 0.51$	$\pm 0.73$	$\pm 0.67$	$\pm 0.20$	
Control	54.33	55.33	55.33	53.67	54.00	55.67	56.67	55.67	54.33	56.33	55.13 <sup>a</sup>	1.76
Experimental	$\pm 0.61$	$\pm 0.84$	$\pm 0.98$	$\pm 0.80$	$\pm 0.73$	$\pm 0.95$	$\pm 0.66$	$\pm 0.80$	$\pm 0.61$	$\pm 0.33$	$\pm 0.25$	
Control	56.33	58.00	56.33	57.33	57.33	58.00	58.67	57.67	57.00	57.00	57.36 <sup>b</sup>	1.87
Experimental	$\pm 0.61$	$\pm 0.52$	$\pm 0.61$	$\pm 0.42$	$\pm 0.42$	$\pm 0.51$	$\pm 0.42$	$\pm 0.61$	$\pm 0.68$	$\pm 0.85$	$\pm 0.19$	
Control	30.50	29.58	29.58	28.91	29.00	29.75	29.50	30.33	29.41	30.67	29.72 <sup>a</sup>	1.76
Experimental	$\pm 0.13$	$\pm 0.15$	$\pm 0.15$	$\pm 0.15$	$\pm 0.22$	$\pm 0.11$	$\pm 0.18$	$\pm 0.16$	$\pm 0.23$	$\pm 0.16$	$\pm 0.09$	
Control	30.67	29.75	29.75	29.33	29.41	29.91	30.08	30.25	30.67	30.75	30.06 <sup>b</sup>	1.76
Experimental	$\pm 0.10$	$\pm 0.11$	$\pm 0.11$	$\pm 0.27$	$\pm 0.08$	$\pm 0.24$	$\pm 0.15$	$\pm 0.21$	$\pm 0.17$	$\pm 0.21$	$\pm 0.08$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 38b. Body weight, feed and water intake of cows during hot-dry, hot-humid and winter season.**

	Weeks of experiment				Overall average
	1	2	3	4	
<b>Body weight (kg)</b>					
<b>Hot-dry</b>					
Control	418.65	417.75	417.58	418.16	418.03±12.28
Experimental	433.83	435.41	437.16	437.75	436.03±14.92
<b>Hot-humid</b>					
Control	362.66	362.00	361.50	361.00	361.79±13.86
Experimental	355.33	357.33	359.16	360.16	357.99±21.61
<b>Winter</b>					
Control	370.41	370.66	371.58	372.66	371.32±24.97
Experimental	375.75	376.91	378.75	381.08	378.12±8.13
<b>Feed intake (kg/day)</b>					
<b>Hot-dry</b>					
Control	38.80	37.40	33.34	37.50	36.76±1.18 <sup>a</sup>
Experimental	39.24	41.61	43.00	42.50	41.59±0.83 <sup>b</sup>
<b>Hot-humid</b>					
Control	36.00	32.29	32.10	34.27	33.67±0.92 <sup>a</sup>
Experimental	35.48	37.49	39.47	38.15	37.65±0.83 <sup>b</sup>
<b>Winter</b>					
Control	40.40	35.47	38.90	37.50	38.07±1.04 <sup>a</sup>
Experimental	39.80	41.44	44.90	42.50	42.16±1.06 <sup>b</sup>
<b>Water intake (litres/day)</b>					
<b>Hot-dry</b>					
Control	60.50	55.28	59.25	57.75	58.20±0.56
Experimental	61.27	54.39	56.58	53.75	56.50±0.85
<b>Hot-humid</b>					
Control	56.40	60.57	58.29	53.25	57.13±0.77 <sup>a</sup>
Experimental	59.40	52.48	48.22	49.79	52.47±1.23 <sup>b</sup>
<b>Winter</b>					
Control	38.29	32.47	36.88	35.00	35.66±0.63
Experimental	40.29	36.34	39.98	38.00	38.65±0.46

Values with different superscripts <sup>a, b</sup> in a column differ (P<0.05)

**Table 39. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental cows during winter season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	16.16	16.41	17.00	18.16	17.00	17.75	16.50	17.25	16.50	17.25	17.00	17.00
	$\pm 0.88$	$\pm 0.73$	$\pm 0.99$	$\pm 0.70$	$\pm 0.88$	$\pm 0.65$	$\pm 0.64$	$\pm 0.71$	$\pm 0.76$	$\pm 0.86$	$\pm 0.49$	$\pm 0.49$
Experimental	18.33	18.50	18.33	18.00	18.66	18.25	17.91	18.66	17.66	18.33	18.26	18.26
	$\pm 0.50$	$\pm 0.61$	$\pm 0.54$	$\pm 0.63$	$\pm 0.60$	$\pm 0.60$	$\pm 0.47$	$\pm 0.54$	$\pm 0.53$	$\pm 0.52$	$\pm 0.40$	$\pm 0.40$
Control	162.50	162.50	160.83	166.67	163.33	162.50	163.33	160.83	164.16	165.83	163.25 <sup>a</sup>	163.25 <sup>a</sup>
	$\pm 3.82$	$\pm 3.82$	$\pm 3.51$	$\pm 1.05$	$\pm 4.21$	$\pm 2.81$	$\pm 2.79$	$\pm 3.00$	$\pm 3.96$	$\pm 3.51$	$\pm 1.00$	$\pm 1.00$
Experimental	149.16	144.16	147.50	146.67	149.16	150.83	153.33	148.33	152.50	150.83	149.25 <sup>b</sup>	149.25 <sup>b</sup>
	$\pm 3.00$	$\pm 3.74$	$\pm 3.81$	$\pm 2.10$	$\pm 5.38$	$\pm 2.71$	$\pm 2.47$	$\pm 2.47$	$\pm 4.23$	$\pm 2.71$	$\pm 1.05$	$\pm 1.05$
Control	45.98	45.72	44.02	43.34	44.28	42.52	45.68	46.26	45.44	45.85	44.91	44.91
	$\pm 1.28$	$\pm 1.43$	$\pm 0.76$	$\pm 1.68$	$\pm 1.16$	$\pm 0.65$	$\pm 1.15$	$\pm 0.75$	$\pm 0.85$	$\pm 1.06$	$\pm 0.36$	$\pm 0.36$
Experimental	44.02	43.03	44.37	43.30	44.20	43.11	44.41	44.61	43.37	43.11	43.75	43.75
	$\pm 1.05$	$\pm 0.90$	$\pm 0.57$	$\pm 1.43$	$\pm 1.64$	$\pm 1.06$	$\pm 1.17$	$\pm 0.78$	$\pm 1.00$	$\pm 1.25$	$\pm 0.34$	$\pm 0.34$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 40. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental cows during winter season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	13.02	13.05	13.01	13.02	13.04	13.06	13.02	13.02	12.99	13.01	13.03
	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	
Experimental	13.43	13.27	13.39	13.32	13.35	13.38	13.37	13.39	13.34	13.35	13.36
	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	
Control	4.10	4.35	4.34	4.31	4.29	4.29	4.31	4.34	4.34	4.32	4.30
	$\pm 0.28$	$\pm 0.26$	$\pm 0.24$	$\pm 0.23$	$\pm 0.22$	$\pm 0.21$	$\pm 0.22$	$\pm 0.22$	$\pm 0.20$	$\pm 0.20$	
Experimental	4.41	4.14	4.29	4.38	4.45	4.50	4.51	4.53	4.52	4.56	4.43
	$\pm 0.16$	$\pm 0.17$	$\pm 0.17$	$\pm 0.18$	$\pm 0.17$	$\pm 0.17$	$\pm 0.17$	$\pm 0.17$	$\pm 0.17$	$\pm 0.18$	
Control	4.08	4.08	4.08	4.13	4.19	4.05	4.11	4.08	4.11	4.19	4.11 <sup>a</sup>
	$\pm 0.06$	$\pm 0.04$	$\pm 0.06$	$\pm 0.05$	$\pm 0.09$	$\pm 0.03$	$\pm 0.03$	$\pm 0.07$	$\pm 0.03$	$\pm 0.08$	
Experimental	4.02	4.05	4.22	4.22	4.30	4.42	4.44	4.39	4.59	4.67	4.33 <sup>b</sup>
	$\pm 0.06$	$\pm 0.05$	$\pm 0.11$	$\pm 0.05$	$\pm 0.05$	$\pm 0.08$	$\pm 0.03$	$\pm 0.08$	$\pm 0.04$	$\pm 0.04$	
Control	5.02	5.02	4.99	4.99	5.00	4.97	4.98	4.99	4.99	4.96	4.99 <sup>a</sup>
	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.03$	
Experimental	4.97	4.98	5.02	5.06	5.07	5.08	5.06	5.08	5.11	5.11	5.05 <sup>b</sup>
	$\pm 0.03$	$\pm 0.04$	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 41. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental cows during winter season.

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
	Somatic cell count ( $\times 10^5$ cells/ml of milk)											
Control	1.32 $\pm 0.03$	1.36 $\pm 0.01$	1.35 $\pm 0.04$	1.33 $\pm 0.04$	1.38 $\pm 0.04$	1.34 $\pm 0.05$	1.28 $\pm 0.04$	1.39 $\pm 0.04$	1.38 $\pm 0.05$	1.39 $\pm 0.03$		1.35 $\pm 0.01$
Experimental	1.30 $\pm 0.03$	1.41 $\pm 0.03$	1.35 $\pm 0.03$	1.27 $\pm 0.03$	1.26 $\pm 0.02$	1.33 $\pm 0.02$	1.35 $\pm 0.02$	1.36 $\pm 0.02$	1.29 $\pm 0.02$	1.29 $\pm 0.02$	-	1.32 $\pm 0.01$
	Epithelial cells ( $\times 10^5$ cells/ml of milk)											
Control	0.78 $\pm 0.01$	0.80 $\pm 0.01$	0.79 $\pm 0.02$	0.79 $\pm 0.04$	0.82 $\pm 0.03$	0.82 $\pm 0.04$	0.74 $\pm 0.02$	0.81 $\pm 0.03$	0.81 $\pm 0.04$	0.82 $\pm 0.01$		0.80 $\pm 0.01$
Experimental	0.78 $\pm 0.02$	0.81 $\pm 0.02$	0.80 $\pm 0.02$	0.72 $\pm 0.03$	0.74 $\pm 0.02$	0.79 $\pm 0.02$	0.82 $\pm 0.02$	0.80 $\pm 0.02$	0.78 $\pm 0.03$	0.77 $\pm 0.02$	-	0.78 $\pm 0.01$
	TLC ( $\times 10^5$ cells/ml of milk)											
Control	0.53 $\pm 0.02$	0.55 $\pm 0.02$	0.55 $\pm 0.02$	0.54 $\pm 0.01$	0.55 $\pm 0.02$	0.52 $\pm 0.03$	0.54 $\pm 0.03$	0.58 $\pm 0.01$	0.56 $\pm 0.02$	0.56 $\pm 0.02$		0.55 $\pm 0.01$
Experimental	0.51 $\pm 0.03$	0.59 $\pm 0.02$	0.54 $\pm 0.02$	0.55 $\pm 0.03$	0.51 $\pm 0.02$	0.53 $\pm 0.03$	0.53 $\pm 0.03$	0.56 $\pm 0.03$	0.50 $\pm 0.02$	0.52 $\pm 0.03$	-	0.53 $\pm 0.01$

**Table 42. Analysis of Variance (ANOVA) of complete data on plasma glucose, NEFA, urea, thyroxine, triiodothyronine, insulin and cortisol of cows during winter season.**

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	161.67**	0.091	10.54	2.89*	0.0013	101.70	5.326
Between Groups	1	228.62**	2.21**	0.56	69.22**	0.031**	12301.88**	83.33**
Between Periods	9	92.39**	0.053	9.00	1.15	0.0044*	72.24	21.00**
All effect interaction	9	32.03	0.073	7.65	0.84	0.0059**	85.20	8.25
Error	95	29.29	0.10	5.46	1.21	0.0017	82.76	7.07

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

**Table 43. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of cows during winter season.**

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	0.85**	127.62	4.15	0.12
Between Groups	3	3.11**	340.55*	580.50**	21.12**
Between Periods	9	0.25	204.99	10.90**	8.90**
All effect interaction	27	0.10	182.22	3.14	0.57
Error	195	0.20	165.41	2.87	0.50

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

**Table 44. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration rate, heart rate and skin temperature of cows during winter season.**

Source effect	d.f.	Mean Sum of Squares												
		RT			RR			HR			ST			
		M	E		M	E		M	E		M	E		
Between Animals	5	0.60*	0.32*	278.67	4.35	2.55	4.06	0.41	0.19					
Between Groups	1	0.34	0.26	313.63	93.63**	0.53	149.63**	3.50*	3.33**					
Between Periods	9	0.18	0.11	407.11	4.38	10.08**	6.83**	6.16**	3.32**					
Periods x Groups	9	0.10	0.15	337.63	2.52	0.82	2.59	0.69	0.42*					
Animals x Groups	5	0.0030	0.0014	1.17	0.0075	0.025	0.0044	0.0044	0.0001					
Error	90	0.26	0.13	3.29	2.38	2.91	2.66	0.82	0.20					

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P<0.05, \*\* P<0.01

Table 45. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of cows during winter season.

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	395.65**	2.80	0.013	0.013**	0.021**	2.78**	0.063*	0.013**	159.00*	1.20
Between Groups	1	89.68**	350.44**	0.030*	0.0086	0.0056	0.52*	1.50**	0.10**	5880.00**	40.05*
Between Periods	9	1.82	15.18**	0.0087	0.0029	0.0034	0.062	0.16**	0.0037	38.88	7.38
All effect interaction	9	1.29	11.03**	0.011	0.0065	0.0023	0.070	0.11**	0.012**	28.61	4.78
Error	95	4.32	2.34	0.0061	0.0035	0.0028	0.11	0.021	0.0036	64.00	7.95

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P<0.05, \*\* P<0.01

**Table 46. Correlation coefficients (r) # of pooled data on minimum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in cows during winter season.**

	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	0.0024	1.0000				
RT	-0.0629	-0.0226	1.0000			
RR	0.1363	-0.0822	-0.2778**	1.0000		
HR	0.0100	0.1228	0.3573**	0.0951	1.0000	
ST	0.0070	-0.0016	0.2803**	0.1271	0.4343**	1.0000

# = No. of observations (240)

\* = P<0.05

\*\* = P<0.01

Table 47. Correlation coefficients (r) # of minimum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of cows during winter season.

Control group:						
	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	0.0394	1.0000				
RT	0.0606	-0.3161	1.0000			
RR	0.2288**	0.0269	0.1765*	1.0000		
HR	0.0432	0.0731	0.2224	0.5708**	1.0000	
ST	0.0132	0.0082	0.2310**	0.2680**	0.3245**	1.0000
Experimental group:						
	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	-0.0049	1.0000				
RT	-0.1325	0.2167	1.0000			
RR	0.0907	0.2460**	0.4445**	1.0000		
HR	-0.0049	0.2516**	0.4829**	0.7960**	1.0000	
ST	0.1478	0.0854	0.3772**	0.5231**	0.5548**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 48. Correlation coefficients (r) # of minimum temperature, plasma hormones, metabolites, milk production and composition in control and experimental group of cows during winter season.**

Variables	Significance	Value of 'r'
<b>Min. temp. vs:</b>		
T <sub>4</sub>	P<0.05	- 0.215
Insulin	P<0.01	- 0.242
Plasma glucose	P<0.01	- 0.279
Protein	P<0.01	- 0.305
Lactose	P<0.05	- 0.177
<b>T<sub>4</sub> vs:</b>		
Plasma glucose	P<0.01	0.294
Protein	P<0.05	0.207
Milk NEFA	P<0.05	- 0.175
<b>T<sub>3</sub> vs:</b>		
Cortisol	P<0.01	- 0.225
Plasma glucose	P<0.01	- 0.297
Plasma NEFA	P<0.05	0.188
Epithelial cells	P<0.05	0.205
Milk urea	P<0.05	0.206
<b>Cortisol vs:</b>		
Plasma glucose	P<0.01	0.239
Protein	P<0.01	0.230
Milk NEFA	P<0.01	- 0.228
<b>Plasma glucose vs:</b>		
Plasma NEFA	P<0.01	- 0.549
TS	P<0.05	0.184
SCC	P<0.05	- 0.192
Epithelial cells	P<0.05	- 0.186
Protein	P<0.01	0.378
Milk NEFA	P<0.01	- 0.390
<b>Plasma NEFA vs:</b>		
Plasma urea	P<0.05	0.213
TS	P<0.01	- 0.369
Protein	P<0.01	- 0.353
Lactose	P<0.01	- 0.344
Milk NEFA	P<0.01	0.687
<b>Plasma urea vs:</b>		
TS	P<0.01	- 0.236
Protein	P<0.01	- 0.274
Lactose	P<0.05	- 0.182
Milk urea	P<0.01	0.301
<b>MP vs:</b>		
SCC	P<0.05	0.194
TLC	P<0.01	0.445
Fat	P<0.01	- 0.501
<b>TS vs:</b>		
Protein	P<0.01	0.387
Lactose	P<0.01	0.442

Contd...

Variables	Significance	Value of 'r'
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.707
TLC	P<0.01	0.651
<b>TLC vs:</b>		
Fat	P<0.05	- 0.216
<b>Fat vs:</b>		
Protein	P<0.05	0.215
<b>Protein vs:</b>		
Lactose	P<0.01	0.469
Milk NEFA	P<0.01	- 0.269
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	- 0.243

**Table 49. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental buffaloes during hot-dry season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	50.03	48.23	48.98	49.31	50.25	51.05	48.46	53.85	51.16	55.20		50.65
	$\pm 1.01$	$\pm 1.71$	$\pm 2.12$	$\pm 1.80$	$\pm 1.17$	$\pm 0.99$	$\pm 0.75$	$\pm 1.41$	$\pm 1.40$	$\pm 1.44$		$\pm 0.50$
Experimental	47.36	51.58	49.53	50.86	52.48	55.50	53.46	53.91	52.41	58.63	-	52.57
	$\pm 2.13$	$\pm 3.32$	$\pm 1.60$	$\pm 2.31$	$\pm 1.10$	$\pm 1.96$	$\pm 1.45$	$\pm 0.69$	$\pm 1.60$	$\pm 1.28$		$\pm 0.67$
Control	1.88	1.99	1.79	2.09	1.91	2.10	1.97	1.97	1.95	2.05		1.97
	$\pm 0.08$	$\pm 0.07$	$\pm 0.07$	$\pm 0.09$	$\pm 0.10$	$\pm 0.14$	$\pm 0.04$	$\pm 0.09$	$\pm 0.08$	$\pm 0.09$		$\pm 0.03$
Experimental	1.85	1.90	1.80	1.78	1.70	1.84	1.98	2.09	1.89	1.99	-	1.88
	$\pm 0.06$	$\pm 0.07$	$\pm 0.05$	$\pm 0.08$	$\pm 0.14$	$\pm 0.13$	$\pm 0.07$	$\pm 0.22$	$\pm 0.12$	$\pm 0.07$		$\pm 0.03$
Control	9.28	8.23	9.22	9.81	8.34	6.96	8.15	7.85	7.94	7.26		8.30
	$\pm 1.31$	$\pm 0.84$	$\pm 0.48$	$\pm 1.38$	$\pm 0.96$	$\pm 0.40$	$\pm 0.35$	$\pm 0.68$	$\pm 0.73$	$\pm 0.49$		$\pm 0.26$
Experimental	9.32	7.87	10.44	10.60	11.39	11.85	12.19	11.99	11.50	11.52	-	10.86
	$\pm 0.48$	$\pm 0.58$	$\pm 0.49$	$\pm 0.70$	$\pm 1.26$	$\pm 0.85$	$\pm 0.72$	$\pm 0.60$	$\pm 0.89$	$\pm 0.71$		$\pm 0.27$
Control	5.19	4.60	5.37	5.23	4.33	4.94	4.76	4.11	4.41	5.07		4.80 <sup>a</sup>
	$\pm 0.21$	$\pm 0.36$	$\pm 0.28$	$\pm 0.29$	$\pm 0.53$	$\pm 0.52$	$\pm 0.29$	$\pm 0.57$	$\pm 0.51$	$\pm 0.40$		$\pm 0.14$
Experimental	2.84	2.76	2.61	2.33	2.07	3.01	2.89	2.94	2.49	2.06	1.06	2.60 <sup>b</sup>
	$\pm 0.25$	$\pm 0.22$	$\pm 0.22$	$\pm 0.23$	$\pm 0.24$	$\pm 0.28$	$\pm 0.21$	$\pm 0.23$	$\pm 0.28$	$\pm 0.14$		$\pm 0.08$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

Table 50. Least Square Mean ( $\pm$ S.E.) of plasma glucose, NEFA and urea concentration in control and experimental buffaloes during hot-dry season.

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	56.46	55.28	54.48	53.29	52.68	52.79	52.45	51.66	51.77	51.07	53.19 <sup>a</sup>
	$\pm 0.66$	$\pm 0.98$	$\pm 0.84$	$\pm 1.06$	$\pm 1.24$	$\pm 1.50$	$\pm 1.06$	$\pm 1.17$	$\pm 0.83$	$\pm 0.52$	
Experimental	59.84	60.22	60.45	62.47	64.27	63.37	66.90	66.87	67.05	68.04	63.95 <sup>b</sup>
	$\pm 0.61$	$\pm 0.92$	$\pm 1.14$	$\pm 1.14$	$\pm 0.61$	$\pm 0.58$	$\pm 0.85$	$\pm 1.07$	$\pm 0.70$	$\pm 1.03$	
Control	276.66	285.83	285.83	295.83	290.00	295.00	294.16	295.00	304.16	302.50	292.50 <sup>a</sup>
	$\pm 5.42$	$\pm 5.97$	$\pm 6.88$	$\pm 7.12$	$\pm 6.19$	$\pm 3.41$	$\pm 6.50$	$\pm 7.52$	$\pm 3.27$	$\pm 4.60$	
Experimental	265.00	263.33	254.16	270.00	246.66	240.83	251.66	244.16	245.83	253.33	253.50 <sup>b</sup>
	$\pm 6.83$	$\pm 8.23$	$\pm 5.68$	$\pm 10.64$	$\pm 3.57$	$\pm 3.96$	$\pm 6.54$	$\pm 4.72$	$\pm 3.27$	$\pm 7.14$	
Control	40.01	41.37	40.98	40.75	43.92	43.22	41.85	42.45	43.66	43.68	42.19 <sup>a</sup>
	$\pm 0.90$	$\pm 0.85$	$\pm 0.47$	$\pm 0.89$	$\pm 0.53$	$\pm 0.59$	$\pm 0.95$	$\pm 0.78$	$\pm 1.24$	$\pm 0.86$	
Experimental	40.32	39.05	38.29	37.49	37.22	36.27	35.87	36.79	35.75	36.28	37.33 <sup>b</sup>
	$\pm 1.03$	$\pm 0.44$	$\pm 1.23$	$\pm 1.46$	$\pm 0.79$	$\pm 0.83$	$\pm 1.12$	$\pm 1.51$	$\pm 0.44$	$\pm 1.15$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 51. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during hot-dry season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	101.63	100.90	101.36	101.66	101.63	101.60	101.26	101.16	101.43	101.33		101.40
	$\pm 0.08$	$\pm 0.29$	$\pm 0.28$	$\pm 0.18$	$\pm 0.06$	$\pm 0.07$	$\pm 0.13$	$\pm 0.18$	$\pm 0.14$	$\pm 0.20$		$\pm 0.06$
Experimental	101.36	101.40	100.93	101.56	101.60	101.63	100.93	101.06	101.23	101.66	-	101.34
	$\pm 0.08$	$\pm 0.12$	$\pm 0.32$	$\pm 0.34$	$\pm 0.07$	$\pm 0.06$	$\pm 0.15$	$\pm 0.14$	$\pm 0.19$	$\pm 0.04$		$\pm 0.06$
	<b>Rectal Temperature (<math>^{\circ}</math>F)</b>											
Control	24.00	25.00	23.33	23.66	23.66	24.00	25.33	23.66	24.00	24.66		24.13
	$\pm 0.51$	$\pm 0.45$	$\pm 0.99$	$\pm 0.61$	$\pm 0.61$	$\pm 0.73$	$\pm 0.98$	$\pm 1.20$	$\pm 0.73$	$\pm 0.42$		$\pm 0.23$
Experimental	23.33	24.00	22.66	22.33	23.66	23.33	25.00	22.66	23.66	22.66	-	23.33
	$\pm 0.98$	$\pm 0.73$	$\pm 0.66$	$\pm 1.20$	$\pm 0.61$	$\pm 0.84$	$\pm 0.68$	$\pm 0.66$	$\pm 0.61$	$\pm 0.66$		$\pm 0.25$
	<b>Respiration Rate (breaths per min.)</b>											
Control	55.66	54.66	57.66	56.33	56.00	58.33	55.33	56.66	56.66	55.66		56.30
	$\pm 0.95$	$\pm 0.84$	$\pm 0.61$	$\pm 1.08$	$\pm 1.46$	$\pm 0.61$	$\pm 0.98$	$\pm 0.66$	$\pm 0.66$	$\pm 0.95$		$\pm 0.30$
Experimental	56.00	57.00	57.00	55.66	55.00	55.33	55.33	56.00	57.00	55.66	-	56.00
	$\pm 1.15$	$\pm 0.85$	$\pm 0.85$	$\pm 0.61$	$\pm 1.12$	$\pm 0.66$	$\pm 0.66$	$\pm 0.73$	$\pm 0.85$	$\pm 0.61$		$\pm 0.26$
	<b>Heart rate (per min.)</b>											
Control	36.31	34.95	34.75	36.41	36.21	36.55	34.75	35.48	35.45	36.16		35.70
	$\pm 0.25$	$\pm 0.40$	$\pm 0.59$	$\pm 0.55$	$\pm 0.63$	$\pm 0.21$	$\pm 0.40$	$\pm 0.36$	$\pm 0.35$	$\pm 0.18$		$\pm 0.15$
Experimental	36.51	34.38	35.55	36.01	35.31	36.23	35.85	35.48	35.63	35.83	-	35.68
	$\pm 0.54$	$\pm 0.40$	$\pm 0.34$	$\pm 0.49$	$\pm 0.55$	$\pm 0.32$	$\pm 0.29$	$\pm 0.19$	$\pm 0.54$	$\pm 0.35$		$\pm 0.14$
	<b>Skin temperature (<math>^{\circ}</math>C)</b>											

Table 52. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during hot-dry season.

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	101.50	100.86	101.06	101.30	101.26	101.46	101.16	101.16	101.26	101.36	101.24 <sup>a</sup>
	$\pm 0.07$	$\pm 0.19$	$\pm 0.20$	$\pm 0.12$	$\pm 0.08$	$\pm 0.06$	$\pm 0.08$	$\pm 0.10$	$\pm 0.06$	$\pm 0.11$	
Experimental	100.66	100.56	100.50	100.40	100.33	100.53	100.43	100.70	100.30	100.26	100.47 <sup>b</sup>
	$\pm 0.11$	$\pm 0.09$	$\pm 0.15$	$\pm 0.11$	$\pm 0.06$	$\pm 0.07$	$\pm 0.08$	$\pm 0.09$	$\pm 0.13$	$\pm 0.08$	
	Respiration Rate (breaths per min.)										
Control	21.33	23.00	19.00	20.00	22.00	21.33	23.00	21.00	19.66	21.66	21.20 <sup>a</sup>
	$\pm 0.84$	$\pm 1.23$	$\pm 0.85$	$\pm 0.73$	$\pm 0.73$	$\pm 0.84$	$\pm 0.85$	$\pm 0.45$	$\pm 0.61$	$\pm 0.95$	
Experimental	16.33	15.66	16.66	17.00	17.00	17.33	18.00	15.00	15.66	14.66	16.33 <sup>b</sup>
	$\pm 0.61$	$\pm 0.80$	$\pm 0.66$	$\pm 0.44$	$\pm 0.86$	$\pm 0.66$	$\pm 0.73$	$\pm 0.45$	$\pm 0.95$	$\pm 0.84$	
	Heart rate (per min.)										
Control	55.66	53.33	56.00	55.33	52.00	54.66	53.33	53.00	52.33	52.66	53.83 <sup>a</sup>
	$\pm 0.80$	$\pm 0.84$	$\pm 0.73$	$\pm 0.98$	$\pm 0.89$	$\pm 0.84$	$\pm 0.66$	$\pm 0.85$	$\pm 0.61$	$\pm 0.42$	
Experimental	49.00	48.00	46.33	47.33	48.33	44.33	44.33	45.00	44.33	43.00	46.00 <sup>b</sup>
	$\pm 0.85$	$\pm 1.15$	$\pm 0.61$	$\pm 0.84$	$\pm 0.95$	$\pm 0.61$	$\pm 0.61$	$\pm 0.85$	$\pm 0.61$	$\pm 0.44$	
	Skin temperature ( $^{\circ}$ C)										
Control	34.16	33.50	33.30	34.06	33.93	33.96	33.78	34.91	34.16	33.10	33.89 <sup>a</sup>
	$\pm 0.14$	$\pm 0.25$	$\pm 0.52$	$\pm 0.48$	$\pm 0.34$	$\pm 0.24$	$\pm 0.17$	$\pm 0.91$	$\pm 0.26$	$\pm 0.39$	
Experimental	31.40	31.45	31.50	31.03	31.75	31.33	31.45	31.21	31.43	31.66	31.42 <sup>b</sup>
	$\pm 0.26$	$\pm 0.52$	$\pm 0.43$	$\pm 0.31$	$\pm 0.40$	$\pm 0.18$	$\pm 0.18$	$\pm 0.20$	$\pm 0.34$	$\pm 0.63$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 53. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental buffaloes during hot-dry season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	7.41	7.67	7.67	7.75	7.58	7.33	7.50	7.67	7.67	7.58	7.58 <sup>a</sup>
	$\pm 0.35$	$\pm 0.38$	$\pm 0.40$	$\pm 0.52$	$\pm 0.43$	$\pm 0.33$	$\pm 0.54$	$\pm 0.40$	$\pm 0.51$	$\pm 0.39$	
Experimental	7.91	8.00	8.00	8.33	8.83	9.00	8.67	8.91	9.08	9.25	8.60 <sup>b</sup>
	$\pm 0.56$	$\pm 0.51$	$\pm 0.50$	$\pm 0.38$	$\pm 0.21$	$\pm 0.25$	$\pm 0.21$	$\pm 0.20$	$\pm 0.20$	$\pm 0.21$	
	NEFA ( $\mu$ mol/l)										
Control	265.00	272.50	272.50	279.16	276.67	281.67	286.67	280.83	292.50	287.50	279.50 <sup>a</sup>
	$\pm 7.18$	$\pm 6.15$	$\pm 4.95$	$\pm 5.38$	$\pm 4.94$	$\pm 3.57$	$\pm 7.71$	$\pm 5.54$	$\pm 5.28$	$\pm 3.35$	
Experimental	253.33	245.00	252.50	240.00	235.83	237.50	234.16	231.67	232.50	231.67	239.41 <sup>b</sup>
	$\pm 5.57$	$\pm 5.77$	$\pm 8.63$	$\pm 5.62$	$\pm 6.50$	$\pm 7.93$	$\pm 3.00$	$\pm 4.21$	$\pm 3.59$	$\pm 7.14$	
	Urea (mg%)										
Control	38.01	38.95	38.99	38.92	41.26	39.99	39.72	39.03	40.46	40.87	39.62
	$\pm 0.85$	$\pm 0.87$	$\pm 0.16$	$\pm 0.90$	$\pm 0.71$	$\pm 0.47$	$\pm 0.98$	$\pm 0.59$	$\pm 0.62$	$\pm 0.74$	
Experimental	37.74	36.81	35.97	34.62	35.07	40.86	33.34	33.67	34.02	33.84	35.59
	$\pm 1.07$	$\pm 0.63$	$\pm 1.45$	$\pm 1.43$	$\pm 0.90$	$\pm 7.04$	$\pm 1.00$	$\pm 1.06$	$\pm 1.40$	$\pm 1.08$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 54. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental buffaloes during hot-dry season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	17.45	17.54	17.44	17.42	17.53	17.44	17.33	17.28	17.30	17.40	17.41 <sup>a</sup>	0.26
	$\pm 0.15$	$\pm 0.12$	$\pm 0.10$	$\pm 0.09$	$\pm 0.10$	$\pm 0.12$	$\pm 0.06$	$\pm 0.07$	$\pm 0.06$	$\pm 0.08$	$\pm 0.03$	
Experimental	17.96	18.07	18.05	18.06	18.03	18.05	18.07	18.01	18.26	18.06	18.23 <sup>b</sup>	0.17
	$\pm 0.15$	$\pm 0.14$	$\pm 0.15$	$\pm 0.14$	$\pm 0.15$	$\pm 0.14$	$\pm 0.13$	$\pm 0.13$	$\pm 0.15$	$\pm 0.13$	$\pm 0.04$	
Control	6.97	6.96	6.94	6.92	6.93	6.93	6.91	6.91	6.89	6.89	6.92 <sup>a</sup>	0.17
	$\pm 0.17$	$\pm 0.17$	$\pm 0.17$	$\pm 0.16$	$\pm 0.16$	$\pm 0.17$	$\pm 0.10$	$\pm 0.16$	$\pm 0.17$	$\pm 0.17$	$\pm 0.05$	
Experimental	7.29	7.29	7.37	7.39	7.52	7.44	7.46	7.48	7.48	7.50	7.42 <sup>b</sup>	0.28
	$\pm 0.11$	$\pm 0.14$	$\pm 0.14$	$\pm 0.14$	$\pm 0.12$	$\pm 0.13$	$\pm 0.13$	$\pm 0.14$	$\pm 0.15$	$\pm 0.13$	$\pm 0.04$	
Control	4.74	4.50	4.37	4.36	4.42	4.34	4.29	4.25	4.25	4.33	4.38 <sup>a</sup>	0.17
	$\pm 0.07$	$\pm 0.11$	$\pm 0.05$	$\pm 0.08$	$\pm 0.15$	$\pm 0.14$	$\pm 0.08$	$\pm 0.12$	$\pm 0.12$	$\pm 0.12$	$\pm 0.04$	
Experimental	4.73	4.91	4.93	5.07	5.24	5.35	5.63	5.55	5.63	5.66	5.27 <sup>b</sup>	0.14
	$\pm 0.11$	$\pm 0.09$	$\pm 0.04$	$\pm 0.10$	$\pm 0.11$	$\pm 0.03$	$\pm 0.11$	$\pm 0.05$	$\pm 0.11$	$\pm 0.10$	$\pm 0.05$	
Control	5.05	5.02	5.07	5.02	4.96	4.96	4.95	4.96	4.92	4.90	4.98 <sup>a</sup>	0.14
	$\pm 0.06$	$\pm 0.04$	$\pm 0.05$	$\pm 0.04$	$\pm 0.04$	$\pm 0.06$	$\pm 0.05$	$\pm 0.04$	$\pm 0.01$	$\pm 0.04$	$\pm 0.01$	
Experimental	5.06	5.08	5.12	5.18	5.18	5.22	5.24	5.25	5.09	5.24	5.16 <sup>b</sup>	0.14
	$\pm 0.04$	$\pm 0.01$	$\pm 0.03$	$\pm 0.03$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.03$	$\pm 0.16$	$\pm 0.02$	$\pm 0.02$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 55. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental buffaloes during hot-dry season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	1.02	0.99	1.01	0.95	0.96	0.98	0.93	0.97	0.99	1.01	0.98
	$\pm 0.04$	$\pm 0.01$	$\pm 0.01$	$\pm 0.05$	$\pm 0.02$	$\pm 0.03$	$\pm 0.01$	$\pm 0.03$	$\pm 0.03$	$\pm 0.04$	
Experimental	1.00	0.96	0.97	0.95	1.01	0.92	0.97	0.94	0.94	0.93	0.96
	$\pm 0.01$	$\pm 0.04$	$\pm 0.04$	$\pm 0.05$	$\pm 0.01$	$\pm 0.04$	$\pm 0.03$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	
Control	0.71	0.60	0.63	0.63	0.69	0.69	0.53	0.68	0.68	0.66	0.65
	$\pm 0.04$	$\pm 0.02$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.03$	$\pm 0.02$	$\pm 0.04$	$\pm 0.03$	$\pm 0.04$	
Experimental	0.70	0.67	0.70	0.72	0.71	0.60	0.64	0.61	0.61	0.57	0.65
	$\pm 0.03$	$\pm 0.05$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.03$	$\pm 0.03$	$\pm 0.03$	$\pm 0.02$	$\pm 0.03$	
Control	0.30	0.38	0.37	0.31	0.27	0.29	0.40	0.29	0.31	0.35	0.33
	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	
Experimental	0.29	0.29	0.27	0.23	0.29	0.32	0.33	0.33	0.32	0.36	0.30
	$\pm 0.02$	$\pm 0.01$	$\pm 0.03$	$\pm 0.01$	$\pm 0.01$	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.03$	$\pm 0.02$	

Table 56. Analysis of Variance (ANOVA) of complete data on plasma glucose, NEFA, urea, thyroxine, triiodothyronine, insulin and cortisol of buffaloes during hot-dry season.

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	4.64	0.044	3.35	0.85	36.69**	538.50*	23.68**
Between Groups	1	110.80**	0.22	196.71**	96.17**	3470.69**	45630.00**	707.08**
Between Periods	9	74.40**	0.079	4.68	0.22	8.23	269.35	3.11
All effect interaction	9	15.75	0.055	11.36**	0.35	68.99	708.24**	22.00**
Error	95	17.49	0.067	3.90	0.86	3.94	211.57	4.56

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

**Table 57. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of buffaloes during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	0.22	2.52	2.30	1.06
Between Groups	3	11.29**	736.60**	1391.60**	245.27
Between Periods	9	0.48	13.00	15.93	2.51
All effect interaction	27	0.23	3.63	11.06	1.46
Error	195	0.13	3.68	4.20	0.96

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

\* P < 0.05, \*\* P < 0.01

**Table 58. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration rate, heart rate and skin temperature of buffaloes during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares												
		RT			RR			HR			ST			
		M	E		M	E		M	E		M	E		
Between Animals	5	0.25	0.082	1.25	3.09	3.74	0.51	0.95	0.56					
Between Groups	1	0.11	17.94**	19.20*	710.53**	2.70	1840.83**	0.016	182.53**					
Between Periods	9	0.60**	0.15*	5.42	9.71*	5.21	25.42**	3.94**	0.56					
Periods x Groups	9	0.25	0.19**	0.97	7.79*	5.36	13.12**	1.14	1.27					
Animals x Groups	5	0.0094	0.0005	0.014	0.0040	0.013	0.019	0.0023	0.0039					
Error	90	0.19	0.034	3.67	3.80	4.67	3.66	1.07	0.86					

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P < 0.05, \*\* P < 0.01

**Table 59. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of buffaloes during hot-dry season.**

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	19.98**	0.83**	0.0070	0.0055	0.0026	2.40**	0.15	0.029	204.70	37.04
Between Groups	1	23.81**	3.06**	0.015	0.0000	0.018**	7.45**	23.53**	1.02**	48200.21**	485.98**
Between Periods	9	0.65	0.019	0.0050	0.017*	0.010**	0.011	0.17	0.011	54.18	16.06
All effect interaction	9	0.49	0.033	0.0050	0.017*	0.0087**	0.035	0.67	0.041	756.22	23.18
Error	95	0.60	0.055	0.0073	0.0071	0.0032	0.024	0.059	0.016	202.86	19.17

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P<0.05, \*\* P<0.01

Table 60. Correlation coefficients (r) # of pooled data on maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in buffaloes during hot-dry season.

	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	0.0548	1.0000				
RT	0.1506*	-0.2718**	1.0000			
RR	-0.0089	-0.4366**	0.5202**	1.0000		
HR	-0.0414	-0.4344**	0.5858**	0.6915**	1.0000	
ST	0.0589	-0.3511**	0.5951**	0.6907**	0.7446**	1.0000

# = No. of observations (240)

\* = P<0.05

\*\* = P<0.01

**Table 61. Correlation coefficients (r) # of maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of buffaloes during hot-dry season.**

Control group:						
	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0251	1.0000				
RT	0.2421*	0.2387	1.0000			
RR	0.0132	-0.1849*	0.0150	1.0000		
HR	0.0303	-0.0460	0.0310	0.0956	1.0000	
ST	0.0823	0.0390	0.1872*	0.2705**	0.3923**	1.0000
Experimental group:						
	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	0.1309	1.0000				
RT	0.1211	-0.4316**	1.0000			
RR	-0.0243	-0.4658**	0.6260**	1.0000		
HR	-0.0854	-0.4984**	0.6733**	0.7950**	1.0000	
ST	0.0527	-0.4656**	0.7021**	0.8013**	0.8127**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 62. Correlation coefficients (r) # of maximum temperature, plasma hormones, metabolites, milk production and composition in control and experimental group of buffaloes during hot-dry season.**

Variables	Significance	Value of 'r'
<b>Max temp. vs:</b>		
T <sub>4</sub>	P<0.01	0.296
<b>T<sub>4</sub> vs:</b>		
Cortisol	P<0.05	- 0.183
Plasma NEFA	P<0.05	- 0.184
Plasma urea	P<0.01	- 0.236
SCC	P<0.05	- 0.198
<b>Insulin vs:</b>		
Cortisol	P<0.01	- 0.382
Plasma glucose	P<0.01	- 0.257
Plasma NEFA	P<0.01	- 0.497
Plasma urea	P<0.01	- 0.469
<b>Cortisol vs:</b>		
Plasma glucose	P<0.01	0.395
Plasma NEFA	P<0.01	0.516
Plasma urea	P<0.01	0.530
<b>Plasma glucose vs:</b>		
Plasma NEFA	P<0.01	0.413
Plasma urea	P<0.01	0.317
TLC	P<0.05	0.219
Milk NEFA	P<0.01	- 0.417
<b>Plasma NEFA vs:</b>		
Plasma urea	P<0.01	0.629
<b>Plasma urca vs:</b>		
SCC	P<0.05	0.223
<b>MP vs:</b>		
TS	P<0.01	0.998
Fat	P<0.01	0.999
Protein	P<0.01	0.999
Lactose	P<0.01	0.999
Milk NEFA	P<0.01	0.365
Milk urea	P<0.01	0.978
<b>TS vs:</b>		
Fat	P<0.01	0.998
Protein	P<0.01	0.997
Lactose	P<0.01	0.997
Milk NEFA	P<0.01	0.418
Milk urea	P<0.01	0.988
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.728
TLC	P<0.01	0.251
<b>Epithelial cells vs:</b>		
TLC	P<0.01	- 0.480
<b>Fat vs:</b>		
Protein	P<0.01	0.999
Lactose	P<0.01	0.999
Milk NEFA	P<0.01	0.361

Contd...

<b>Variables</b>	<b>Significance</b>	<b>Value of 'r'</b>
Milk urea	P<0.01	0.978
<b>Protein vs:</b>		
Milk NEFA	P<0.01	0.347
Milk urea	P<0.01	0.974
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	0.350
Milk urea	P<0.01	0.975
<b>Milk NEFA vs:</b>		
Milk urea	P<0.01	0.532

**Table 63. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	47.53	46.00	51.18	49.23	47.88	47.68	48.28	47.10	48.70	48.96	48.25	
	$\pm 1.49$	$\pm 1.85$	$\pm 1.39$	$\pm 1.72$	$\pm 3.05$	$\pm 1.62$	$\pm 1.15$	$\pm 1.93$	$\pm 0.94$	$\pm 1.74$		$\pm 0.54$
Experimental	51.73	50.66	52.58	50.11	47.75	50.81	51.01	50.51	49.10	55.48	50.97	
	$\pm 1.26$	$\pm 2.30$	$\pm 1.68$	$\pm 1.44$	$\pm 1.77$	$\pm 1.09$	$\pm 2.16$	$\pm 2.29$	$\pm 2.93$	$\pm 1.36$		$\pm 0.61$
Control	1.83	1.90	1.88	1.94	1.66	1.72	1.80	1.69	2.04	1.82	1.83	
	$\pm 0.12$	$\pm 0.07$	$\pm 0.12$	$\pm 0.13$	$\pm 0.18$	$\pm 0.15$	$\pm 0.14$	$\pm 0.10$	$\pm 0.08$	$\pm 0.16$		$\pm 0.04$
Experimental	1.84	2.06	2.09	2.13	2.03	2.06	1.72	1.92	2.10	1.97	1.99	
	$\pm 0.07$	$\pm 0.08$	$\pm 0.16$	$\pm 0.17$	$\pm 0.07$	$\pm 0.09$	$\pm 0.09$	$\pm 0.10$	$\pm 0.10$	$\pm 0.10$		$\pm 0.03$
Control	7.44	8.21	6.99	9.09	7.40	7.46	8.86	8.36	7.35	7.43	7.86	
	$\pm 0.71$	$\pm 1.37$	$\pm 0.60$	$\pm 1.53$	$\pm 1.50$	$\pm 0.72$	$\pm 0.93$	$\pm 1.27$	$\pm 0.87$	$\pm 1.08$		$\pm 0.33$
Experimental	9.00	8.73	10.86	9.71	9.91	9.04	9.95	9.63	9.66	9.69	9.62	
	$\pm 1.30$	$\pm 0.85$	$\pm 1.44$	$\pm 0.81$	$\pm 1.00$	$\pm 1.12$	$\pm 1.00$	$\pm 0.80$	$\pm 0.88$	$\pm 0.72$		$\pm 0.30$
Control	4.05	4.06	4.28	3.74	5.05	4.46	4.84	4.12	4.07	4.69	4.33 <sup>a</sup>	
	$\pm 0.34$	$\pm 0.39$	$\pm 0.36$	$\pm 0.43$	$\pm 0.71$	$\pm 0.70$	$\pm 0.80$	$\pm 0.23$	$\pm 0.53$	$\pm 0.56$		$\pm 0.16$
Experimental	2.39	2.59	2.64	2.59	2.65	3.03	2.70	2.71	2.47	2.60	2.64 <sup>b</sup>	
	$\pm 0.14$	$\pm 0.20$	$\pm 0.24$	$\pm 0.26$	$\pm 0.29$	$\pm 0.22$	$\pm 0.30$	$\pm 0.23$	$\pm 0.25$	$\pm 0.27$		$\pm 0.32$
											1.17	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 64. Least Square Mean ( $\pm$ S.E.) of plasma glucose, NEFA and urea concentration in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	48.68	49.97	46.02	46.39	50.75	49.99	48.86	49.85	50.50	49.80	49.08 <sup>a</sup>	
	$\pm 0.43$	$\pm 0.54$	$\pm 0.55$	$\pm 0.85$	$\pm 0.38$	$\pm 0.43$	$\pm 0.68$	$\pm 0.92$	$\pm 0.56$	$\pm 0.76$		$\pm 0.28$
Experimental	53.83	53.82	55.23	56.45	59.87	60.75	60.62	60.53	59.59	60.42	58.11 <sup>b</sup>	
	$\pm 1.13$	$\pm 1.59$	$\pm 0.72$	$\pm 0.59$	$\pm 0.68$	$\pm 0.35$	$\pm 0.57$	$\pm 0.87$	$\pm 1.50$	$\pm 0.44$		$\pm 0.45$
Control	205.83	203.33	200.00	200.00	197.50	201.66	204.16	208.33	205.00	206.66	203.25	
	$\pm 4.54$	$\pm 3.33$	$\pm 2.88$	$\pm 3.41$	$\pm 5.28$	$\pm 3.80$	$\pm 3.96$	$\pm 2.10$	$\pm 4.08$	$\pm 2.47$		$\pm 1.15$
Experimental	194.16	194.16	198.33	195.00	207.50	211.66	202.50	199.16	197.50	202.50	200.25	
	$\pm 3.96$	$\pm 4.90$	$\pm 3.33$	$\pm 1.82$	$\pm 4.23$	$\pm 2.78$	$\pm 3.81$	$\pm 4.36$	$\pm 4.78$	$\pm 4.42$		$\pm 1.35$
Control	37.55	38.87	36.75	39.04	37.18	36.17	37.26	36.79	38.25	37.39	37.53	
	$\pm 0.90$	$\pm 1.04$	$\pm 0.77$	$\pm 0.53$	$\pm 0.93$	$\pm 0.87$	$\pm 0.44$	$\pm 1.05$	$\pm 1.05$	$\pm 1.05$		$\pm 0.28$
Experimental	32.95	32.82	31.11	34.16	32.90	32.00	34.06	32.74	35.62	36.24	33.46	
	$\pm 1.01$	$\pm 1.21$	$\pm 0.47$	$\pm 0.54$	$\pm 0.65$	$\pm 0.41$	$\pm 0.68$	$\pm 0.62$	$\pm 0.75$	$\pm 1.15$		$\pm 0.30$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 65. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
Control	101.21	101.46	101.46	101.26	101.66	101.26	100.83	101.20	101.16	101.23	101.27	101.27
	$\pm 0.07$	$\pm 0.18$	$\pm 0.14$	$\pm 0.15$	$\pm 0.13$	$\pm 0.04$	$\pm 0.27$	$\pm 0.05$	$\pm 0.06$	$\pm 0.06$	$\pm 0.05$	
Experimental	101.43	101.10	101.30	101.13	101.26	101.20	101.16	101.30	100.93	101.20	101.20	101.20
	$\pm 0.13$	$\pm 0.04$	$\pm 0.09$	$\pm 0.04$	$\pm 0.04$	$\pm 0.05$	$\pm 0.03$	$\pm 0.04$	$\pm 0.08$	$\pm 0.01$	-	$\pm 0.02$
Control	21.00	21.66	22.66	21.00	20.33	21.00	20.66	20.00	21.00	20.00	20.93 <sup>a</sup>	20.93 <sup>a</sup>
	$\pm 0.85$	$\pm 0.61$	$\pm 1.22$	$\pm 0.85$	$\pm 0.33$	$\pm 0.44$	$\pm 0.42$	$\pm 0.01$	$\pm 0.44$	$\pm 0.51$	$\pm 0.22$	$\pm 0.22$
Experimental	19.00	19.66	20.00	19.66	19.00	19.33	18.33	19.00	19.00	19.00	19.20 <sup>b</sup>	19.20 <sup>b</sup>
	$\pm 0.68$	$\pm 0.33$	$\pm 0.52$	$\pm 0.61$	$\pm 0.45$	$\pm 0.42$	$\pm 0.33$	$\pm 0.45$	$\pm 0.45$	$\pm 0.45$	1.64	$\pm 0.15$
Control	52.66	51.66	51.33	53.33	50.33	51.00	49.66	49.66	49.66	51.00	51.03	51.03
	$\pm 1.42$	$\pm 0.80$	$\pm 1.11$	$\pm 0.66$	$\pm 0.95$	$\pm 1.00$	$\pm 1.08$	$\pm 0.80$	$\pm 0.80$	$\pm 1.00$	$\pm 0.33$	$\pm 0.33$
Experimental	51.00	48.66	51.66	53.00	50.00	48.66	50.66	48.33	50.00	49.66	50.16	50.16
	$\pm 1.00$	$\pm 0.84$	$\pm 1.20$	$\pm 0.45$	$\pm 0.89$	$\pm 0.67$	$\pm 0.84$	$\pm 0.33$	$\pm 0.89$	$\pm 0.80$	$\pm 0.30$	$\pm 0.30$
Control	32.66	35.58	31.16	31.58	32.91	30.16	31.91	32.16	32.75	32.83	32.37	32.37
	$\pm 0.17$	$\pm 1.49$	$\pm 0.11$	$\pm 0.15$	$\pm 0.08$	$\pm 0.11$	$\pm 0.08$	$\pm 0.27$	$\pm 0.11$	$\pm 0.10$	$\pm 0.22$	$\pm 0.22$
Experimental	32.08	34.00	31.25	31.58	32.75	30.08	31.83	31.91	32.75	32.75	32.10	32.10
	$\pm 0.35$	$\pm 0.18$	$\pm 0.11$	$\pm 0.15$	$\pm 0.11$	$\pm 0.08$	$\pm 0.10$	$\pm 0.08$	$\pm 0.17$	$\pm 0.11$	$\pm 0.13$	$\pm 0.13$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 66. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	101.00	100.96	101.20	100.96	101.33	100.96	100.73	100.98	101.03	102.10	102.27 <sup>a</sup>
	$\pm 0.05$	$\pm 0.06$	$\pm 0.14$	$\pm 0.13$	$\pm 0.11$	$\pm 0.06$	$\pm 0.16$	$\pm 0.08$	$\pm 0.09$	$\pm 0.06$	
Experimental	100.80	100.80	100.76	100.70	100.76	100.66	100.70	100.70	100.76	100.63	100.73 <sup>b</sup>
	$\pm 0.08$	$\pm 0.22$	$\pm 0.14$	$\pm 0.20$	$\pm 0.32$	$\pm 0.17$	$\pm 0.14$	$\pm 0.13$	$\pm 0.05$	$\pm 0.12$	
<b>Respiration Rate (breaths per min.)</b>											
Control	19.33	18.66	18.33	17.67	17.33	16.33	17.00	17.67	18.33	17.33	17.80 <sup>a</sup>
	$\pm 0.42$	$\pm 0.67$	$\pm 0.80$	$\pm 0.80$	$\pm 0.42$	$\pm 0.61$	$\pm 0.68$	$\pm 0.61$	$\pm 0.33$	$\pm 0.42$	
Experimental	16.00	15.00	15.67	15.33	14.33	16.00	14.33	15.00	15.00	15.33	15.20 <sup>b</sup>
	$\pm 0.51$	$\pm 0.45$	$\pm 0.61$	$\pm 0.42$	$\pm 0.33$	$\pm 0.51$	$\pm 0.33$	$\pm 0.44$	$\pm 0.44$	$\pm 0.42$	
<b>Heart rate (per min.)</b>											
Control	49.66	48.00	47.33	49.33	46.33	46.33	47.00	47.67	46.00	48.33	47.60 <sup>a</sup>
	$\pm 1.30$	$\pm 0.52$	$\pm 0.67$	$\pm 0.84$	$\pm 0.80$	$\pm 0.80$	$\pm 1.24$	$\pm 0.33$	$\pm 0.89$	$\pm 0.33$	
Experimental	44.67	44.00	44.33	45.67	45.33	44.67	45.00	44.33	44.33	44.67	44.70 <sup>b</sup>
	$\pm 0.42$	$\pm 0.89$	$\pm 0.80$	$\pm 0.61$	$\pm 0.84$	$\pm 0.42$	$\pm 0.45$	$\pm 0.33$	$\pm 0.33$	$\pm 0.42$	
<b>Skin temperature (<math>^{\circ}</math>C)</b>											
Control	30.75	32.50	29.67	30.25	31.33	28.67	30.33	30.25	31.50	31.16	30.64 <sup>a</sup>
	$\pm 0.21$	$\pm 0.26$	$\pm 0.21$	$\pm 0.25$	$\pm 0.17$	$\pm 0.28$	$\pm 0.11$	$\pm 0.17$	$\pm 0.12$	$\pm 0.21$	
Experimental	29.33	31.41	28.33	28.91	29.08	27.42	28.25	29.50	29.91	29.91	29.21 <sup>b</sup>
	$\pm 0.21$	$\pm 0.40$	$\pm 0.11$	$\pm 0.24$	$\pm 0.15$	$\pm 0.15$	$\pm 0.17$	$\pm 0.13$	$\pm 0.24$	$\pm 0.24$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 67. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	7.25	7.25	6.33	6.91	6.83	6.16	6.66	7.33	7.25	7.33	6.93
	$\pm 0.88$	$\pm 0.73$	$\pm 0.99$	$\pm 0.70$	$\pm 0.88$	$\pm 0.65$	$\pm 0.64$	$\pm 0.71$	$\pm 0.76$	$\pm 0.86$	
Experimental	7.00	7.66	7.66	7.91	7.75	8.16	8.08	8.00	8.00	8.25	7.85
	$\pm 0.50$	$\pm 0.61$	$\pm 0.54$	$\pm 0.63$	$\pm 0.60$	$\pm 0.60$	$\pm 0.47$	$\pm 0.54$	$\pm 0.53$	$\pm 0.52$	
	NEFA ( $\mu$ mol/l)										
Control	197.50	200.00	191.66	192.50	195.00	190.00	196.66	203.33	196.66	195.00	195.83
	$\pm 5.28$	$\pm 3.65$	$\pm 3.07$	$\pm 2.81$	$\pm 5.00$	$\pm 3.87$	$\pm 4.77$	$\pm 2.79$	$\pm 4.01$	$\pm 5.32$	
Experimental	188.33	184.16	185.83	186.66	187.50	188.33	188.33	187.50	191.66	188.33	187.66
	$\pm 4.01$	$\pm 4.72$	$\pm 2.39$	$\pm 2.47$	$\pm 2.14$	$\pm 2.47$	$\pm 2.11$	$\pm 2.14$	$\pm 3.80$	$\pm 5.42$	
	Urea (mg%)										
Control	35.22	37.04	34.75	37.54	35.17	34.84	35.96	35.19	36.57	36.05	35.83 <sup>a</sup>
	$\pm 0.95$	$\pm 1.00$	$\pm 0.77$	$\pm 0.53$	$\pm 1.28$	$\pm 0.95$	$\pm 0.33$	$\pm 0.94$	$\pm 1.02$	$\pm 1.25$	
Experimental	31.08	30.32	29.11	32.33	31.23	30.65	32.06	30.42	33.37	34.42	31.50 <sup>b</sup>
	$\pm 1.03$	$\pm 0.88$	$\pm 0.65$	$\pm 0.51$	$\pm 0.56$	$\pm 0.76$	$\pm 0.68$	$\pm 0.58$	$\pm 1.03$	$\pm 1.61$	
											2.51

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 68. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										CD	Overall average
	1	2	3	4	5	6	7	8	9	10		
	<b>Total solids (%)</b>											
Control	17.17 $\pm 0.02$	17.19 $\pm 0.01$	17.18 $\pm 0.01$	17.50 $\pm 0.03$	17.16 $\pm 0.01$	17.18 $\pm 0.01$	17.18 $\pm 0.01$	17.18 $\pm 0.01$	17.19 $\pm 0.01$	17.20 $\pm 0.01$		17.21 <sup>a</sup> $\pm 0.03$
Experimental	17.50 $\pm 0.02$	17.49 $\pm 0.02$	17.63 $\pm 0.03$	17.76 $\pm 0.02$	17.72 $\pm 0.01$	17.71 $\pm 0.01$	17.79 $\pm 0.02$	17.70 $\pm 0.02$	17.75 $\pm 0.02$	17.73 $\pm 0.02$	<b>0.20</b>	17.68 <sup>b</sup> $\pm 0.02$
	<b>Fat (%)</b>											
Control	6.96 $\pm 0.13$	6.97 $\pm 0.14$	6.97 $\pm 0.14$	6.99 $\pm 0.14$	7.01 $\pm 0.14$	6.98 $\pm 0.14$	6.99 $\pm 0.14$	7.03 $\pm 0.09$	7.02 $\pm 0.10$	7.05 $\pm 0.10$		7.00 $\pm 0.04$
Experimental	7.24 $\pm 0.15$	7.23 $\pm 0.13$	7.25 $\pm 0.13$	7.26 $\pm 0.12$	7.25 $\pm 0.08$	7.34 $\pm 0.10$	7.27 $\pm 0.10$	7.29 $\pm 0.08$	7.29 $\pm 0.12$	7.35 $\pm 0.13$	-	7.28 $\pm 0.04$
	<b>Protein (%)</b>											
Control	4.36 $\pm 0.08$	4.47 $\pm 0.05$	4.39 $\pm 0.05$	4.44 $\pm 0.05$	4.22 $\pm 0.08$	4.22 $\pm 0.08$	4.27 $\pm 0.05$	4.42 $\pm 0.04$	4.19 $\pm 0.07$	4.50 $\pm 0.06$		4.35 <sup>a</sup> $\pm 0.02$
Experimental	4.87 $\pm 0.08$	4.87 $\pm 0.05$	4.90 $\pm 0.05$	5.02 $\pm 0.05$	5.02 $\pm 0.04$	5.07 $\pm 0.08$	4.93 $\pm 0.04$	5.04 $\pm 0.06$	5.13 $\pm 0.05$	5.10 $\pm 0.06$	<b>0.16</b>	4.99 <sup>b</sup> $\pm 0.02$
	<b>Lactose (%)</b>											
Control	4.90 $\pm 0.02$	4.90 $\pm 0.01$	4.94 $\pm 0.04$	4.98 $\pm 0.02$	4.99 $\pm 0.03$	4.98 $\pm 0.03$	4.98 $\pm 0.03$	4.99 $\pm 0.03$	4.97 $\pm 0.02$	5.00 $\pm 0.03$		4.96 $\pm 0.01$
Experimental	4.97 $\pm 0.03$	5.15 $\pm 0.04$	5.17 $\pm 0.06$	5.17 $\pm 0.02$	5.16 $\pm 0.03$	5.12 $\pm 0.03$	5.17 $\pm 0.05$	5.17 $\pm 0.04$	5.14 $\pm 0.02$	5.14 $\pm 0.02$	-	5.13 $\pm 0.01$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 69. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental buffaloes during hot-humid season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
	<b>Somatic cell count (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	1.09 $\pm 0.02$	0.98 $\pm 0.03$	1.03 $\pm 0.02$	0.98 $\pm 0.03$	1.01 $\pm 0.03$	1.02 $\pm 0.03$	0.96 $\pm 0.04$	0.96 $\pm 0.03$	0.96 $\pm 0.04$	0.94 $\pm 0.04$	1.02 $\pm 0.05$	1.00 $\pm 0.01$
Experimental	1.07 $\pm 0.02$	0.99 $\pm 0.03$	0.92 $\pm 0.05$	0.96 $\pm 0.05$	0.98 $\pm 0.03$	0.96 $\pm 0.03$	0.99 $\pm 0.03$	0.96 $\pm 0.03$	0.96 $\pm 0.05$	0.96 $\pm 0.05$	0.91 $\pm 0.03$	0.97 $\pm 0.01$
	<b>Epithelial cells (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	0.71 $\pm 0.04$	0.66 $\pm 0.04$	0.65 $\pm 0.03$	0.72 $\pm 0.04$	0.68 $\pm 0.05$	0.71 $\pm 0.03$	0.71 $\pm 0.03$	0.56 $\pm 0.04$	0.56 $\pm 0.06$	0.65 $\pm 0.06$	0.65 $\pm 0.07$	0.67 $\pm 0.01$
Experimental	0.68 $\pm 0.03$	0.68 $\pm 0.04$	0.63 $\pm 0.04$	0.63 $\pm 0.04$	0.69 $\pm 0.04$	0.63 $\pm 0.04$	0.66 $\pm 0.05$	0.65 $\pm 0.04$	0.65 $\pm 0.05$	0.69 $\pm 0.05$	0.56 $\pm 0.03$	0.65 $\pm 0.01$
	<b>TLC (<math>\times 10^5</math> cells/ml of milk)</b>											
Control	0.38 $\pm 0.02$	0.31 $\pm 0.03$	0.38 $\pm 0.01$	0.26 $\pm 0.03$	0.33 $\pm 0.03$	0.30 $\pm 0.03$	0.24 $\pm 0.03$	0.39 $\pm 0.03$	0.30 $\pm 0.02$	0.30 $\pm 0.02$	0.40 $\pm 0.03$	0.33 $\pm 0.01$
Experimental	0.39 $\pm 0.03$	0.30 $\pm 0.03$	0.29 $\pm 0.02$	0.32 $\pm 0.02$	0.28 $\pm 0.03$	0.32 $\pm 0.02$	0.33 $\pm 0.03$	0.31 $\pm 0.04$	0.26 $\pm 0.02$	0.26 $\pm 0.02$	0.35 $\pm 0.03$	0.31 $\pm 0.01$

**Table 70. Analysis of Variance (ANOVA) of complete data on plasma thyroxine, triiodothyronine, insulin, cortisol, glucose, NEFA and urea of buffaloes during hot-humid season.**

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	36.56	0.12	4.24	1.27	5.66	48.00	10.33*
Between Groups	1	222.25**	0.80**	92.82**	86.34**	2446.5**	270.00	496.78**
Between Periods	9	24.17	0.12	2.21	0.74	48.35**	101.75	13.92**
All effect interaction	9	13.22	0.058	3.04	0.45	19.58**	172.77	6.24
Error	95	19.59	0.087	6.97	1.05	3.79	90.01	3.98

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

**Table 71. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of buffaloes during hot-humid season.**

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	0.12*	1.74	2.31	0.80
Between Groups	3	3.60**	352.06**	487.66**	128.25**
Between Periods	9	0.28**	6.34**	20.08**	31.17**
All effect interaction	27	0.092	1.85	4.51	0.60
Error	195	0.051	1.84	4.10	0.53

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

\* P<0.05, \*\* P<0.01

**Table 72. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration, heart rate and skin temperature of buffaloes during hot-humid season.**

Source effect	d.f.	Mean Sum of Squares											
		RT		RR		HR		ST					
		M	E	M	E	M	E	M	E				
Between Animals	5	0.070	0.0052	0.77	2.80	1.03	2.46	0.82	0.40				
Between Groups	1	0.17	2.55**	90.13**	202.80**	22.53*	252.30**	2.26	61.63**				
Between Periods	9	0.24**	0.10	4.23*	4.07*	17.71**	6.18	18.11**	13.52**				
Periods x Groups	9	0.17**	0.066	0.94	2.65	4.90	4.81	0.73	0.59				
Animals x Groups	5	0.0000	0.0000	0.0073	0.0012	0.013	0.013	0.0053	0.0017				
Error	90	0.045	0.68	2.05	1.68	5.23	3.16	0.76	0.24				

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P < 0.05, \*\* P < 0.01

Table 73. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of buffaloes during hot-humid season.

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	fat	protein	lactose	NEFA	urea
Between Animals	5	79.17**	0.037	0.0043	0.0037	0.0032	0.69**	0.052*	0.015*	20.5	9.68
Between Groups	1	30.83**	6.52**	0.027	0.010	0.0046	2.29**	12.41**	0.87**	2000.8**	563.46**
Between Periods	9	0.44	0.084*	0.015	0.013	0.016**	0.012	0.047*	0.023**	55.92	14.63**
All effect interaction	9	0.73	0.046	0.0076	0.010	0.010*	0.035	0.085**	0.0076	61.48	5.78
Error	95	0.93	0.033	0.0089	0.012	0.0048	0.65	0.021	0.059	89.71	4.81

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

**Table 74. Correlation coefficients (r) # of pooled data on maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in buffaloes during hot-humid season.**

	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0134	1.0000				
RT	-0.0042	-0.1450*	1.0000			
RR	0.0611	-0.1462*	0.5550**	1.0000		
HR	0.0866	-0.2110**	0.4729**	0.6572**	1.0000	
ST	0.3394**	-0.1737*	0.4706**	0.6124**	0.5144**	1.0000

# = No. of observations (240)

\* =  $P < 0.05$

\*\* =  $P < 0.01$

Table 75. Correlation coefficients (r) # of maximum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of buffaloes during hot-humid season.

Control group:						
	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0113	1.0000				
RT	0.0382	0.1030	1.0000			
RR	0.0829	0.1947*	0.1831*	1.0000		
HR	0.1161	0.0565	0.0383	0.1931*	1.0000	
ST	0.4805**	-0.0425	0.0312	0.1206	0.0302	1.0000
Experimental group:						
	Max. temp.	MP	RT	RR	HR	ST
Max temp.	1.0000					
MP	-0.0163	1.0000				
RT	-0.0394	-0.1903*	1.0000			
RR	0.0895	-0.2115*	0.3344**	1.0000		
HR	0.1200	-0.2982**	0.2711**	0.4820**	1.0000	
ST	0.3982**	-0.0937	0.3258**	0.4689**	0.2727**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 76. Correlation coefficient (r) # of maximum temperature, plasma hormones, metabolites, milk production and composition in control and experimental group of buffaloes during hot-humid season.**

Variables	Significance	Value of 'r'
<b>Max temp. vs:</b>		
Plasma glucose	P<0.05	- 0.190
<b>T<sub>4</sub> vs:</b>		
Plasma glucose	P<0.05	0.208
Plasma urea	P<0.05	- 0.201
MP	P<0.01	0.314
Protein	P<0.05	0.205
Lactose	P<0.01	0.252
Milk urea	P<0.05	- 0.204
<b>T<sub>3</sub> vs:</b>		
Cortisol	P<0.01	- 0.279
MP	P<0.01	0.264
TS	P<0.01	0.271
Fat	P<0.01	0.265
Protein	P<0.01	0.268
Lactose	P<0.05	0.192
<b>Insulin vs:</b>		
Cortisol	P<0.01	- 0.233
Plasma glucose	P<0.01	0.227
Plasma urea	P<0.01	- 0.289
MP	P<0.01	0.352
TS	P<0.01	0.348
Fat	P<0.01	0.224
Protein	P<0.01	0.356
Lactose	P<0.01	0.312
Milk urea	P<0.01	- 0.303
<b>Cortisol vs:</b>		
Plasma glucose	P<0.01	- 0.497
Plasma urea	P<0.01	0.341
MP	P<0.01	- 0.593
TS	P<0.01	- 0.539
Fat	P<0.01	- 0.258
Protein	P<0.01	- 0.605
Lactose	P<0.01	- 0.412
Milk NEFA	P<0.05	0.222
Milk urea	P<0.01	0.359
<b>Plasma glucose vs:</b>		
Plasma urea	P<0.01	- 0.528
MP	P<0.01	0.776
TS	P<0.01	0.681
SCC	P<0.05	- 0.179
Fat	P<0.01	0.347
Protein	P<0.01	0.774
Lactose	P<0.01	0.639
Milk urea	P<0.01	- 0.532

Contd...

Variables	Significance	Value of 'r'
<b>Plasma NEFA vs:</b>		
Milk NEFA	P<0.01	0.559
<b>Plasma urea vs:</b>		
MP	P<0.01	- 0.616
TS	P<0.01	- 0.442
Fat	P<0.01	- 0.250
Protein	P<0.01	- 0.557
Lactose	P<0.01	- 0.518
Milk NEFA	P<0.01	0.384
Milk urea	P<0.01	0.957
<b>MP vs:</b>		
TS	P<0.01	0.683
Fat	P<0.01	0.484
Protein	P<0.01	0.804
Lactose	P<0.01	0.652
Milk NEFA	P<0.01	- 0.349
Milk urea	P<0.01	- 0.632
<b>TS vs:</b>		
TLC	P<0.05	- 0.179
Fat	P<0.01	0.258
Protein	P<0.01	0.719
Lactose	P<0.01	0.593
Milk NEFA	P<0.01	- 0.328
Milk urea	P<0.01	- 0.456
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.735
TLC	P<0.01	0.235
Lactose	P<0.01	- 0.333
<b>Epithelial cells vs:</b>		
TLC	P<0.01	- 0.454
Lactose	P<0.01	- 0.239
<b>TLC vs:</b>		
Milk NEFA	P<0.01	0.227
<b>Fat vs:</b>		
Protein	P<0.01	0.424
Lactose	P<0.01	0.290
Milk urea	P<0.01	- 0.247
<b>Protein vs:</b>		
Lactose	P<0.01	0.657
Milk NEFA	P<0.01	- 0.358
Milk urea	P<0.01	- 0.542
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	- 0.298
Milk urea	P<0.01	- 0.490
<b>Milk NEFA vs:</b>		
Milk urea	P<0.01	0.354

**Table 77. Least Square Mean ( $\pm$ S.E.) of plasma thyroxine, triiodothyronine, insulin and cortisol concentration in control and experimental buffaloes during winter season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	61.81	60.45	61.80	62.65	58.33	60.41	60.81	64.63	64.85	66.21		62.19
	$\pm 1.87$	$\pm 2.36$	$\pm 2.12$	$\pm 1.57$	$\pm 1.64$	$\pm 2.46$	$\pm 0.91$	$\pm 1.36$	$\pm 3.20$	$\pm 2.76$		$\pm 0.68$
Experimental	63.80	65.76	65.76	64.78	64.51	66.30	67.90	60.63	58.86	62.25	-	64.05
	$\pm 1.64$	$\pm 1.19$	$\pm 1.44$	$\pm 1.91$	$\pm 2.12$	$\pm 2.82$	$\pm 2.05$	$\pm 1.28$	$\pm 0.91$	$\pm 2.89$		$\pm 0.65$
Control	1.73	1.82	1.77	2.02	1.75	1.74	1.72	1.64	1.55	1.67		1.74
	$\pm 0.11$	$\pm 0.12$	$\pm 0.14$	$\pm 0.12$	$\pm 0.13$	$\pm 0.12$	$\pm 0.07$	$\pm 0.16$	$\pm 0.07$	$\pm 0.06$		$\pm 0.04$
Experimental	1.67	1.74	1.76	1.91	1.72	1.69	1.68	1.69	1.69	1.47	-	1.70
	$\pm 0.07$	$\pm 0.13$	$\pm 0.06$	$\pm 0.06$	$\pm 0.08$	$\pm 0.15$	$\pm 0.06$	$\pm 0.07$	$\pm 0.07$	$\pm 0.14$		$\pm 0.03$
Control	8.48	9.37	8.68	9.64	7.43	8.73	9.83	8.35	8.60	8.38		8.75
	$\pm 1.48$	$\pm 1.71$	$\pm 0.69$	$\pm 1.50$	$\pm 0.50$	$\pm 0.75$	$\pm 1.55$	$\pm 1.07$	$\pm 0.87$	$\pm 0.95$		$\pm 0.35$
Experimental	7.79	8.94	11.49	9.43	9.89	10.81	9.56	9.98	10.59	11.03	-	9.95
	$\pm 0.65$	$\pm 0.39$	$\pm 1.19$	$\pm 0.73$	$\pm 0.91$	$\pm 0.96$	$\pm 0.64$	$\pm 0.91$	$\pm 0.72$	$\pm 0.68$		$\pm 0.27$
Control	1.97	2.05	1.88	2.03	2.13	1.86	1.84	2.03	2.15	1.87		1.98
	$\pm 0.09$	$\pm 0.10$	$\pm 0.05$	$\pm 0.04$	$\pm 0.06$	$\pm 0.06$	$\pm 0.06$	$\pm 0.06$	$\pm 0.14$	$\pm 0.15$		$\pm 0.03$
Experimental	2.35	2.00	1.99	1.98	1.95	1.99	2.12	1.84	2.14	1.81	-	2.02
	$\pm 0.06$	$\pm 0.07$	$\pm 0.09$	$\pm 0.11$	$\pm 0.10$	$\pm 0.11$	$\pm 0.04$	$\pm 0.14$	$\pm 0.19$	$\pm 0.09$		$\pm 0.04$

**Table 78. Least Square Mean ( $\pm$ S.E.) of plasma glucose, NEFA and urea concentration in control and experimental buffaloes during winter season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	51.96	51.17	53.32	52.91	52.51	52.55	52.20	52.86	53.38	52.59	52.59 <sup>a</sup>
	$\pm 0.87$	$\pm 1.04$	$\pm 1.05$	$\pm 1.30$	$\pm 0.89$	$\pm 0.88$	$\pm 0.70$	$\pm 0.78$	$\pm 1.11$	$\pm 0.79$	
Experimental	53.14	53.74	55.81	55.22	58.83	58.14	57.60	56.73	56.18	57.52	56.29 <sup>b</sup>
	$\pm 1.50$	$\pm 1.05$	$\pm 1.43$	$\pm 1.19$	$\pm 1.15$	$\pm 1.62$	$\pm 0.92$	$\pm 0.56$	$\pm 1.26$	$\pm 0.57$	
											2.78
Control	193.33	201.66	198.33	193.33	196.66	201.66	197.50	195.83	198.33	198.33	197.50 <sup>a</sup>
	$\pm 1.66$	$\pm 3.07$	$\pm 4.21$	$\pm 4.01$	$\pm 2.47$	$\pm 3.80$	$\pm 2.14$	$\pm 5.06$	$\pm 4.59$	$\pm 3.57$	
Experimental	180.83	181.66	184.16	180.00	175.83	178.33	183.33	179.16	182.50	180.00	180.58 <sup>b</sup>
	$\pm 2.38$	$\pm 1.66$	$\pm 3.00$	$\pm 4.28$	$\pm 2.38$	$\pm 4.21$	$\pm 2.47$	$\pm 4.36$	$\pm 3.59$	$\pm 3.16$	
											9.10
Control	29.81	28.93	29.37	29.81	28.42	28.17	28.69	28.38	28.02	30.62	29.02
	$\pm 1.03$	$\pm 0.81$	$\pm 0.87$	$\pm 0.84$	$\pm 0.63$	$\pm 0.87$	$\pm 0.91$	$\pm 0.67$	$\pm 0.52$	$\pm 0.56$	
Experimental	26.80	26.28	26.93	26.63	28.97	24.67	25.06	27.08	24.11	26.08	26.26
	$\pm 1.00$	$\pm 1.06$	$\pm 1.02$	$\pm 1.18$	$\pm 1.12$	$\pm 1.22$	$\pm 1.21$	$\pm 1.22$	$\pm 1.19$	$\pm 1.61$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 79. Least Square Mean ( $\pm$ S.E.) of morning rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during winter season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	100.90	100.63	100.10	99.63	100.13	99.16	99.73	99.36	99.80	100.13		99.96
	$\pm 0.04$	$\pm 0.06$	$\pm 0.10$	$\pm 0.26$	$\pm 0.06$	$\pm 0.26$	$\pm 0.22$	$\pm 0.38$	$\pm 0.28$	$\pm 0.04$		$\pm 0.09$
Experimental	100.96	100.73	100.16	100.26	100.53	100.13	99.70	100.10	100.16	100.26	-	100.30
	$\pm 0.03$	$\pm 0.08$	$\pm 0.10$	$\pm 0.08$	$\pm 0.22$	$\pm 0.19$	$\pm 0.46$	$\pm 0.37$	$\pm 0.06$	$\pm 0.06$		$\pm 0.08$
	<b>Rectal Temperature (<math>^{\circ}</math>F)</b>											
Control	11.33	11.33	13.33	13.66	11.33	12.33	12.00	12.66	12.66	11.33		12.20
	$\pm 0.42$	$\pm 0.42$	$\pm 0.66$	$\pm 0.61$	$\pm 0.42$	$\pm 0.33$	$\pm 0.73$	$\pm 0.66$	$\pm 0.42$	$\pm 0.42$		$\pm 0.19$
Experimental	12.66	11.66	11.33	11.00	12.00	10.66	11.00	10.66	11.66	12.00	-	11.46
	$\pm 0.51$	$\pm 0.73$	$\pm 0.51$	$\pm 0.42$	$\pm 0.44$	$\pm 0.50$	$\pm 0.42$	$\pm 0.33$	$\pm 0.73$	$\pm 0.66$		$\pm 0.17$
	<b>Respiration Rate (breaths per min.)</b>											
Control	46.33	46.00	46.00	44.66	45.33	44.33	44.66	44.66	44.83	45.33		45.21
	$\pm 0.61$	$\pm 0.73$	$\pm 0.51$	$\pm 0.42$	$\pm 0.42$	$\pm 0.33$	$\pm 0.42$	$\pm 0.42$	$\pm 0.40$	$\pm 0.66$		$\pm 0.17$
Experimental	45.33	45.33	45.33	44.66	45.00	45.16	44.33	44.33	45.00	45.33	-	44.98
	$\pm 0.42$	$\pm 0.66$	$\pm 0.42$	$\pm 0.42$	$\pm 0.44$	$\pm 0.40$	$\pm 0.33$	$\pm 0.33$	$\pm 0.44$	$\pm 0.42$		$\pm 0.14$
	<b>Heart rate (per min.)</b>											
Control	30.91	30.00	30.00	29.66	30.00	29.00	29.08	29.08	29.41	29.91		29.70
	$\pm 0.08$	$\pm 0.12$	$\pm 0.01$	$\pm 0.10$	$\pm 0.01$	$\pm 0.22$	$\pm 0.08$	$\pm 0.35$	$\pm 0.15$	$\pm 0.20$		$\pm 0.09$
Experimental	31.00	30.16	29.91	29.91	29.66	29.58	29.16	29.25	29.33	30.16	-	29.81
	$\pm 0.01$	$\pm 0.10$	$\pm 0.08$	$\pm 0.08$	$\pm 0.10$	$\pm 0.15$	$\pm 0.10$	$\pm 0.11$	$\pm 0.10$	$\pm 0.10$		$\pm 0.07$
	<b>Skin temperature (<math>^{\circ}</math>C)</b>											

Table 80a. Least Square Mean ( $\pm$ S.E.) of evening rectal temperature, respiration rate, heart rate and skin temperature in control and experimental buffaloes during winter season.

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	101.26	101.00	100.83	100.80	100.76	100.53	100.56	100.40	100.70	100.74	100.74 <sup>a</sup>
	$\pm 0.06$	$\pm 0.05$	$\pm 0.06$	$\pm 0.05$	$\pm 0.06$	$\pm 0.13$	$\pm 0.13$	$\pm 0.12$	$\pm 0.11$	$\pm 0.04$	
Experimental	101.36	101.20	100.93	101.03	101.16	101.03	100.70	100.90	101.00	100.86	101.02 <sup>b</sup>
	$\pm 0.09$	$\pm 0.04$	$\pm 0.06$	$\pm 0.05$	$\pm 0.14$	$\pm 0.09$	$\pm 0.24$	$\pm 0.24$	$\pm 0.05$	$\pm 0.04$	
	Respiration Rate (breaths per min.)										
Control	14.00	13.66	14.66	15.33	14.66	15.66	15.00	15.66	15.66	14.00	14.83 <sup>a</sup>
	$\pm 0.51$	$\pm 0.33$	$\pm 0.42$	$\pm 0.42$	$\pm 0.42$	$\pm 0.33$	$\pm 0.44$	$\pm 0.61$	$\pm 0.80$	$\pm 0.51$	
Experimental	16.00	16.00	16.00	16.66	17.00	16.50	17.33	16.33	16.00	16.66	16.45 <sup>b</sup>
	$\pm 0.51$	$\pm 0.73$	$\pm 0.51$	$\pm 0.42$	$\pm 0.44$	$\pm 0.50$	$\pm 0.42$	$\pm 0.33$	$\pm 0.73$	$\pm 0.66$	
	Heart rate (per min.)										
Control	48.66	47.66	49.00	48.33	47.33	48.66	47.00	46.66	47.00	47.66	47.80
	$\pm 0.42$	$\pm 0.61$	$\pm 0.44$	$\pm 0.61$	$\pm 0.66$	$\pm 0.42$	$\pm 0.44$	$\pm 0.42$	$\pm 0.44$	$\pm 0.61$	
Experimental	49.66	49.66	49.66	49.00	48.00	47.33	48.33	48.00	47.66	48.66	48.60
	$\pm 1.61$	$\pm 0.61$	$\pm 0.61$	$\pm 0.68$	$\pm 0.01$	$\pm 0.66$	$\pm 0.61$	$\pm 1.03$	$\pm 0.61$	$\pm 0.66$	
	Skin temperature ( $^{\circ}$ C)										
Control	31.50	30.66	30.75	30.33	30.58	29.83	30.00	29.83	30.16	30.50	30.41
	$\pm 0.12$	$\pm 0.16$	$\pm 0.11$	$\pm 0.16$	$\pm 0.15$	$\pm 0.27$	$\pm 0.12$	$\pm 0.30$	$\pm 0.16$	$\pm 0.18$	
Experimental	31.83	30.75	30.50	30.83	30.25	30.25	30.00	30.08	30.25	30.66	30.54
	$\pm 0.21$	$\pm 0.11$	$\pm 0.12$	$\pm 0.10$	$\pm 0.11$	$\pm 0.11$	$\pm 0.12$	$\pm 0.08$	$\pm 0.11$	$\pm 0.24$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 80b. Body weight, feed and water intake of buffaloes during hot-dry, hot-humid and winter season.**

	Weeks of experiment				Overall average
	1	2	3	4	
<b>Body weight (kg)</b>					
<b>Hot-dry</b>					
Control	503.81	504.16	504.08	504.50	504.13±18.05
Experimental	520.00	522.16	523.83	526.58	523.14±11.91
<b>Hot-humid</b>					
Control	522.00	522.75	523.41	523.50	522.91±24.62
Experimental	516.33	518.25	520.83	523.00	519.60±32.68
<b>Winter</b>					
Control	515.08	516.33	517.41	519.16	516.99±24.23
Experimental	545.41	547.25	550.08	550.91	548.41±15.11
<b>Feed intake (kg/day)</b>					
<b>Hot-dry</b>					
Control	42.50	38.90	37.70	38.25	39.34±0.54 <sup>a</sup>
Experimental	39.90	42.10	46.38	47.25	43.91±0.87 <sup>b</sup>
<b>Hot-humid</b>					
Control	44.50	42.70	37.28	39.00	40.87±0.83 <sup>a</sup>
Experimental	43.40	44.60	43.90	48.16	45.02±0.54 <sup>b</sup>
<b>Winter</b>					
Control	46.22	43.97	41.18	42.25	43.41±0.87
Experimental	45.50	44.29	47.25	48.75	46.45±0.49
<b>Water intake (litres/day)</b>					
<b>Hot-dry</b>					
Control	70.49	62.47	60.98	64.28	64.56±1.05 <sup>a</sup>
Experimental	69.28	60.97	55.28	51.50	59.26±1.93 <sup>b</sup>
<b>Hot-humid</b>					
Control	54.78	48.47	52.99	49.50	51.44±0.74
Experimental	55.48	47.88	44.92	41.75	47.51±1.47
<b>Winter</b>					
Control	44.33	39.50	42.60	41.50	41.99±0.51
Experimental	41.50	45.65	43.90	43.79	43.71±0.43

Values with different superscripts <sup>a, b</sup> in a column differ (P<0.05)

Table 81. Least Square Mean ( $\pm$ S.E.) of milk production, milk NEFA and urea concentration in control and experimental buffaloes during winter season.

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	7.50	8.33	7.75	7.83	7.83	8.41	8.33	8.55	8.41	7.58		8.05
	$\pm 0.85$	$\pm 0.89$	$\pm 0.91$	$\pm 1.08$	$\pm 0.91$	$\pm 1.12$	$\pm 1.19$	$\pm 1.06$	$\pm 0.85$	$\pm 0.66$		$\pm 0.29$
Experimental	9.00	8.83	8.91	8.91	8.58	8.58	8.91	8.75	8.75	8.75	-	8.81
	$\pm 0.80$	$\pm 0.82$	$\pm 0.70$	$\pm 0.82$	$\pm 0.78$	$\pm 0.91$	$\pm 0.95$	$\pm 1.06$	$\pm 0.71$	$\pm 0.81$		$\pm 0.22$
	NEFA ( $\mu$ mol/l)											
Control	185.83	194.16	190.00	184.16	190.00	189.16	190.00	189.16	189.16	188.33		189.00 <sup>a</sup>
	$\pm 2.38$	$\pm 3.00$	$\pm 4.47$	$\pm 2.38$	$\pm 1.82$	$\pm 2.00$	$\pm 4.47$	$\pm 4.36$	$\pm 3.27$	$\pm 3.33$		$\pm 1.01$
Experimental	174.16	175.83	180.00	180.83	181.66	180.83	181.66	183.33	184.16	184.16	7.88	180.66 <sup>b</sup>
	$\pm 3.57$	$\pm 4.40$	$\pm 2.88$	$\pm 4.28$	$\pm 4.83$	$\pm 3.09$	$\pm 2.88$	$\pm 2.81$	$\pm 4.83$	$\pm 3.96$		$\pm 0.97$
	Urea (mg%)											
Control	26.94	27.30	27.37	28.14	25.99	27.73	26.94	25.42	27.89	28.79		27.25
	$\pm 0.95$	$\pm 0.79$	$\pm 0.97$	$\pm 1.19$	$\pm 0.94$	$\pm 0.68$	$\pm 0.90$	$\pm 0.87$	$\pm 1.21$	$\pm 0.63$		$\pm 0.30$
Experimental	24.52	23.96	24.43	24.46	26.64	22.84	23.23	27.43	23.85	24.20	-	24.56
	$\pm 0.94$	$\pm 1.71$	$\pm 1.08$	$\pm 1.55$	$\pm 1.47$	$\pm 1.32$	$\pm 1.40$	$\pm 1.11$	$\pm 1.58$	$\pm 1.46$		$\pm 0.44$

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 82. Least Square Mean ( $\pm$ S.E.) of total solids, milk fat, protein and lactose in control and experimental buffaloes during winter season.**

Group	Periods of experiment										Overall average
	1	2	3	4	5	6	7	8	9	10	
Control	17.98	17.98	17.96	18.04	18.03	17.96	17.99	17.98	18.00	17.99	17.99 <sup>a</sup>
	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	
Experimental	17.96	18.07	18.05	18.06	18.03	18.05	18.07	18.01	18.00	18.06	18.04 <sup>b</sup>
	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	
<b>Total solids (%)</b>											
Control	7.24	7.22	7.21	7.25	7.27	7.29	7.26	7.19	7.26	7.30	7.25
	$\pm 0.29$	$\pm 0.30$	$\pm 0.29$	$\pm 0.28$	$\pm 0.28$	$\pm 0.30$	$\pm 0.31$	$\pm 0.25$	$\pm 0.30$	$\pm 0.29$	
Experimental	7.91	7.90	7.91	7.91	7.92	7.90	7.89	7.91	7.91	7.92	7.91
	$\pm 0.30$	$\pm 0.32$	$\pm 0.30$	$\pm 0.28$	$\pm 0.29$	$\pm 0.28$	$\pm 0.30$	$\pm 0.30$	$\pm 0.30$	$\pm 0.29$	
<b>Fat (%)</b>											
Control	4.59	4.63	4.57	4.61	4.64	4.65	4.63	4.65	4.65	4.64	4.62 <sup>a</sup>
	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	
Experimental	4.74	4.80	4.89	4.92	4.95	5.06	5.06	5.10	5.09	5.06	4.97 <sup>b</sup>
	$\pm 0.03$	$\pm 0.04$	$\pm 0.05$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.06$	$\pm 0.01$	$\pm 0.04$	$\pm 0.04$	
<b>Protein (%)</b>											
Control	5.03	5.02	4.98	5.01	4.98	5.00	5.02	4.99	5.07	5.04	5.01 <sup>a</sup>
	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.03$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	
Experimental	5.13	5.15	5.17	5.22	5.21	5.24	5.25	5.22	5.22	5.29	5.21 <sup>b</sup>
	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	
<b>Lactose (%)</b>											
Control	5.03	5.02	4.98	5.01	4.98	5.00	5.02	4.99	5.07	5.04	5.01 <sup>a</sup>
	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.03$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.01$	
Experimental	5.13	5.15	5.17	5.22	5.21	5.24	5.25	5.22	5.22	5.29	5.21 <sup>b</sup>
	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	

Values with different superscripts <sup>a, b</sup> in a column differ ( $P < 0.05$ )

**Table 83. Least Square Mean ( $\pm$ S.E.) of somatic cell count (SCC), epithelial cells and TLC in control and experimental buffaloes during winter season.**

Group	Periods of experiment										Overall average	
	1	2	3	4	5	6	7	8	9	10		CD
Control	1.06	0.95	0.91	1.13	0.99	0.99	1.05	1.06	0.99	0.97		1.01
	$\pm 0.02$	$\pm 0.04$	$\pm 0.04$	$\pm 0.17$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$	$\pm 0.01$	$\pm 0.03$	$\pm 0.03$		$\pm 0.02$
Experimental	0.99	1.02	0.97	0.94	0.93	0.98	0.94	0.98	1.01	1.02	-	0.98
	$\pm 0.05$	$\pm 0.03$	$\pm 0.03$	$\pm 0.03$	$\pm 0.04$	$\pm 0.04$	$\pm 0.05$	$\pm 0.04$	$\pm 0.04$	$\pm 0.02$		$\pm 0.01$
Control	0.59	0.52	0.48	0.61	0.58	0.58	0.60	0.62	0.56	0.58		0.57
	$\pm 0.01$	$\pm 0.03$	$\pm 0.03$	$\pm 0.10$	$\pm 0.05$	$\pm 0.03$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.04$		$\pm 0.01$
Experimental	0.58	0.56	0.59	0.52	0.55	0.57	0.54	0.60	0.60	0.60	-	0.57
	$\pm 0.04$	$\pm 0.02$	$\pm 0.04$	$\pm 0.03$	$\pm 0.02$	$\pm 0.04$	$\pm 0.05$	$\pm 0.03$	$\pm 0.03$	$\pm 0.02$		$\pm 0.01$
Control	0.46	0.43	0.43	0.51	0.41	0.41	0.44	0.44	0.43	0.38		0.43
	$\pm 0.02$	$\pm 0.01$	$\pm 0.04$	$\pm 0.07$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.02$	$\pm 0.03$		$\pm 0.01$
Experimental	0.41	0.45	0.37	0.42	0.39	0.41	0.40	0.38	0.40	0.42	-	0.40
	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$		$\pm 0.01$

**Table 84. Analysis of Variance (ANOVA) of complete data on plasma thyroxine, triiodothyronine, insulin, cortisol, glucose, NEFA and urea of buffaloes during winter season.**

Source effect	d.f.	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	89.09**	0.13	5.56	0.023	26.26**	228.20**	6.52
Between Groups	1	103.77**	0.04	43.29**	0.038	411.93**	8585.20**	228.60**
Between Periods	9	11.23	0.13	4.11	0.12*	14.85**	48.17	9.63
All effect interaction	9	69.48**	0.023	6.08	0.10	8.62	38.91	6.43
Error	95	21.53	0.065	6.19	0.058	5.93	63.20	6.16

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid

\* P < 0.05, \*\* P < 0.01

Table 85. Analysis of Variance (ANOVA) of pooled data on rectal temperature, respiration rate, heart rate and skin temperature of buffaloes during winter.

Source effect	d.f.	Mean Sum of Squares			
		RT	RR	HR	ST
Between Animals	5	0.79**	4.11*	3.87*	0.10
Between Groups	3	13.19**	321.58**	199.14**	10.54**
Between Periods	9	2.27**	1.92	7.96 **	6.67**
All effect interaction	27	0.27	3.34**	1.49	0.15
Error	195	0.16	1.57	1.68	0.14

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

\* P<0.05, \*\* P<0.01

**Table 86. Analysis of Variance (ANOVA) of complete data on rectal temperature, respiration rate, heart rate and skin temperature in buffaloes during winter season.**

Source effect	d.f.	Mean Sum of Squares											
		RT			RR			HR			ST		
		M	E	M	E	M	E	M	E	M	E		
Between Animals	5	0.77**	0.26**	3.74	2.72	0.98	3.84	0.13	0.062				
Between Groups	1	3.53**	2.29**	9.63*	78.40**	1.63	19.20**	0.35	0.46				
Between Periods	9	2.14**	0.50**	2.98	2.74	3.07*	6.35**	3.71**	3.03**				
Periods x Groups	9	0.34	0.091	5.55*	2.03	0.80	2.23	0.18	0.21				
Animals x Groups	5	0.0004	0.0002	0.011	0.0050	0.0040	0.011	0.0003	0.0004				
Error	90	0.24	0.062	2.41	1.60	1.31	1.97	0.11	0.17				

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\*  $P < 0.05$ , \*\*  $P < 0.01$

**Table 87. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea of buffaloes during winter.**

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	55.18**	0.0031	0.0043	0.0033	0.0023	2.21**	0.015*	0.0029	296.83**	9.58
Between Groups	1	21.64**	0.059**	0.028	0.0001	0.024**	13.08**	3.50**	1.14**	2083.3**	217.65**
Between Periods	9	0.63	0.0067**	0.010	0.0051	0.0051	0.0037	0.066**	0.0097	51.01	2.81
All effect interaction	9	0.83	0.0055**	0.021	0.010	0.0052	0.0035	0.036**	0.0081	57.87	15.39
Error	95	4.48	0.0018	0.018	0.0010	0.0046	0.44	0.0067	0.0056	47.35	8.33

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count, NEFA = Non esterified fatty acid

\* P<0.05, \*\* P<0.01

**Table 88. Correlation coefficients (r) # of pooled data on minimum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in buffaloes during winter season.**

	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	-0.0471	1.0000				
RT	0.2560**	-0.0200	1.0000			
RR	-0.0561	0.0493	0.4179**	1.0000		
HR	0.1983**	-0.0374	0.5524**	0.6623**	1.0000	
ST	0.4879**	0.0288	0.6201**	0.3988**	0.5794**	1.0000

# = No. of observations (240)

\* =  $P < 0.05$

\*\* =  $P < 0.01$

**Table 89. Correlation coefficients (r) # of minimum temperature, milk production, rectal temperature, respiration rate, heart rate and skin temperature in control and experimental group of buffaloes during winter season**

Control group:						
	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	-0.0364	1.0000				
RT	0.4081	-0.0506	1.0000			
RR	-0.1494	0.0122	0.2442**	1.0000		
HR	0.3149**	-0.0739	0.6066**	0.4744**	1.0000	
ST	0.5484**	0.0012	0.6832**	0.2731**	0.5521**	1.0000
Experimental group:						
	Min. temp.	MP	RT	RR	HR	ST
Min temp.	1.0000					
MP	-0.0486	1.0000				
RT	0.2274*	0.1105	1.0000			
RR	0.0253	0.1201	0.5691**	1.0000		
HR	0.1601	0.0171	0.5301**	0.7787**	1.0000	
ST	0.5468**	0.0952	0.5534**	0.5053**	0.6100**	1.0000

# = No. of observations (120), \* = P<0.05, \*\* = P<0.01

**Table 90. Correlation coefficient (r) # of minimum temperature, plasma hormones, metabolites, milk production and composition in control and experimental group of buffaloes during winter season.**

Variables	Significance	Value of 'r'
<b>Min. temp. vs:</b>		
Plasma glucose	P<0.05	- 0.193
Protein	P<0.01	- 0.289
<b>T<sub>4</sub> vs:</b>		
Plasma glucose	P<0.01	0.245
Lactose	P<0.05	0.189
Milk NEFA	P<0.05	- 0.174
<b>T<sub>3</sub> vs:</b>		
MP	P<0.05	- 0.195
<b>Insulin vs:</b>		
MP	P<0.05	0.209
Fat	P<0.05	0.220
Protein	P<0.01	0.252
Lactose	P<0.01	0.253
<b>Plasma glucose vs:</b>		
Plasma NEFA	P<0.01	- 0.538
MP	P<0.01	0.490
Protein	P<0.01	0.608
Lactose	P<0.01	0.490
Milk NEFA	P<0.01	- 0.287
<b>Plasma NEFA vs:</b>		
Plasma urea	P<0.01	0.305
MP	P<0.01	- 0.525
Fat	P<0.01	- 0.256
Protein	P<0.01	- 0.639
Lactose	P<0.01	- 0.505
Milk NEFA	P<0.01	0.612
Milk urea	P<0.01	0.227
<b>Plasma urea vs:</b>		
MP	P<0.01	- 0.412
Fat	P<0.05	- 0.184
Protein	P<0.01	- 0.428
Lactose	P<0.01	- 0.399
Milk urea	P<0.01	0.725
<b>MP vs:</b>		
TLC	P<0.01	- 0.261
Fat	P<0.01	0.329
Protein	P<0.01	0.679
Lactose	P<0.01	0.630
Milk NEFA	P<0.01	- 0.298
Milk urea	P<0.01	- 0.411
<b>SCC vs:</b>		
Epithelial cells	P<0.01	0.864
TLC	P<0.01	0.715
<b>Epithelial cells vs:</b>		
TLC	P<0.01	0.272

Contd...

Variables	Significance	Value of 'r'
<b>Fat vs:</b>		
Lactose	P<0.01	0.315
Milk NEFA	P<0.01	- 0.278
<b>Protein vs:</b>		
Lactose	P<0.01	0.743
Milk NEFA	P<0.01	- 0.279
Milk urea	P<0.01	- 0.289
<b>Lactose vs:</b>		
Milk NEFA	P<0.01	- 0.299
Milk urea	P<0.01	- 0.282

**Table 91. Analysis of Variance (ANOVA) of complete data on plasma thyroxine, triiodothyronine, insulin, cortisol, glucose, NEFA and urea in cows and buffaloes during hot-dry, hot-humid and winter season.**

Source effect	d.f	Mean Sum of Squares						
		T <sub>4</sub>	T <sub>3</sub>	Insulin	Cortisol	Glucose	NEFA	Urea
Between Animals	5	127.61	0.60	8.81	3.04**	6.94	218.33	34.63**
Between Species	1	1354.70**	2.33*	459.23**	8.94**	10512.55**	118323.5**	4823.42**
Between Seasons	2	1159.70**	12.38**	335.08**	345.36**	329.16**	238337.8**	8408.30**
Between Groups	1	3329.79**	1.94	14.64	1.40	46.44**	19116.80**	11436.97**
Between Periods	9	160.61	0.87	13.60*	0.66	98.10**	73.91	7.78
Species x Seasons	2	199.91	1.46	16.18	0.49	235.66**	2838.78**	272.96**
Species x Groups	1	103.42	0.072	66.58**	0.86	3.50	245.00	134.83**
Species x Periods	9	120.81	0.81	11.81*	0.46	69.76**	551.09**	22.62**
Seasons x Groups	2	8375.46**	1.50	18.56**	17.68**	2109.17**	304661.1**	29842.68**
Seasons x Periods	18	173.46	0.59	5.78	0.73	8.55**	130.59	12.19
Groups x Periods	9	113.57	0.85	2.49	0.40	6.23	136.09	5.57
Species x Seasons x Groups	2	85.17	0.57	27.02**	2.59*	547.40**	18024.72**	732.62**
Species x Seasons x Periods	18	154.22	0.66	6.09	0.40	18.61**	113.28	10.22
Species x Groups x Periods	9	149.40	0.67	5.85	0.53	13.82**	150.09	2.89
Seasons x Groups x Periods	18	172.54	0.57	2.30	0.60	9.34**	160.34	13.77*
All effect interaction	18	154.62	0.47	5.44	0.30	14.80**	375.72**	23.53**
Error	595	142.36	0.64	5.70	0.68	4.49	112.95	7.95

T<sub>4</sub> = Thyroxine, T<sub>3</sub> = Triiodothyronine, NEFA = Non esterified fatty acid  
 \* P<0.05, \*\* P<0.01

**Table 92. Analysis of Variance (ANOVA) of complete data on morning and evening rectal temperature, respiration rate, heart rate and skin temperature in cows and buffaloes during hot-dry, hot-humid and winter season.**

Source effect	d.f.	Mean Sum of Squares											
		RT		RR		HR		ST					
		M	E	M	E	M	E	M	E				
Between Animals	5	0.23	0.15	58.48	4.43	3.91	6.60	0.59	1.03				
Between Species	1	118.92**	269.13**	13364.45**	44195.67**	4540.08**	35771.69**	195.20**	404.25**				
Between Seasons	2	119.81**	44.36**	7053.94**	6708.26**	7850.55**	8563.13**	3290.21**	2062.64**				
Between Groups	1	0.52	44.80**	22.40	2307.66**	84.04**	3831.83**	0.025	470.61**				
Between Periods	9	1.11**	0.77**	42.18	11.52*	6.11	38.69**	9.00**	6.76**				
Species x Seasons	2	3.07**	47.09**	336.11**	3247.51**	252.08**	4638.33**	40.95**	946.83**				
Species x Groups	1	0.0000	0.0000	0.0002	0.0013	0.0000	0.0008	0.0000	0.0000				
Species x Periods	9	1.38**	0.47**	16.85	8.49	22.32**	30.13**	4.88**	3.12**				
Seasons x Groups	2	0.10	-7.58**	64.89	569.08**	17.11*	786.45**	1.81	33.37**				
Seasons x Periods	18	0.71	1.04**	23.71	10.39**	18.25**	8.78	6.84**	3.17**				
Groups x Periods	9	0.20	0.18	120.69*	1.69	3.15	6.38	0.43	0.72				
Error	660	0.26	0.16	58.49	5.02	5.14	8.62	0.90	0.74				

RT = Rectal temperature, RR = Respiration rate, HR = Heart rate, ST = Skin temperature

M = Morning, E = Evening

\* P < 0.05, \*\* P < 0.01

**Table 93. Analysis of Variance (ANOVA) of complete data on milk production, total solids, SCC, epithelial cells, TLC, fat, protein, lactose, NEFA and urea in buffaloes during hot-dry, hot-humid and winter season.**

Source effect	d.f.	Mean Sum of Squares									
		MP	TS	SCC	Epithelial cells	TLC	Fat	Protein	Lactose	NEFA	Urea
Between Animals	5	79.31**	0.18**	0.0031	0.0061	0.0048	1.93**	0.13*	0.78	148.58	21.59
Between Species	1	110.84**	22.24**	0.15**	0.015	0.067**	28.93**	41.70**	3.45**	90675.55**	4371.56**
Between Seasons	2	3202.33**	1246.40*	12.65**	1.78**	5.22**	607.12**	28.61**	0.52**	243482.0**	8604.56**
Between Groups	1	9013.55**	724.62**	2.64**	1.17**	0.28**	172.81**	2.10**	0.026	13868.89**	10198.21**
Between Periods	9	5.01	0.044	0.014	0.010	0.0049	0.192	0.54**	0.015	73.61	4.87
Species x Seasons	2	48.33**	1.16**	0.00021	0.011	0.010	2.64**	4.30**	0.32**	4398.57**	373.10**
Species x Groups	1	3.40	3.55**	0.000085	0.0001	0.0006	0.96**	0.018	0.0009	866.80**	235.90**
Species x Periods	9	5.91	0.076**	0.0049	0.011	0.0048	0.058	0.62**	0.073**	304.79**	23.18*
Seasons x Groups	2	3124.83**	735.65**	8.53**	2.42**	1.87**	335.20**	7.68**	0.42**	253178.6**	29325.03**
Seasons x Periods	18	1.17	0.018	0.018*	0.019**	0.014**	0.042	0.027	0.0095	62.41	7.82
Groups x Periods	9	1.15	0.029	0.013	0.014*	0.012**	0.082	0.052	0.0081	118.81	6.87
Species x Seasons x Groups	2	42.21**	7.05**	0.0005	0.005	0.0030	0.075	0.76**	0.0035	21365.05**	728.48**
Species x Seasons x Periods	18	1.29	0.072*	0.012	0.0080	0.0045	0.027	0.080*	0.0069	143.90	13.09
Species x Groups x Periods	9	0.86	0.12**	0.010	0.0071	0.014**	0.010	0.037	0.0071	56.69	4.26
Seasons x Groups x Periods	18	1.12	0.038	0.081	0.0092	0.0094*	0.023	0.059	0.0068	91.34	15.60
All effect interaction	18	1.08	0.042	0.012	0.0097	0.0068	0.026	0.15**	0.022	403.19**	24.98**
Error	595	5.39	0.030	0.0099	0.0073	0.0047	0.19	0.048	0.0084	115.82	10.86

MP = Milk production, TS = Total solids, SCC = Somatic cell count, TLC = Total leucocyte count

\* P<0.05, \*\* P<0.01

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