BIOMOLECULAR EXPRESSION ON MELATONIN AND VITAMIN-E SUPPLEMENTATION DURING SUMMER AND WINTER IN PIG

A Thesis
Submitted to the
Assam Agricultural University

In partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

VETERINARY PHYSIOLOGY

By

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KHANAPARA, GUWAHATI-781 022

January, 2016

Dedicated
To My
Beloved Parents

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ASSAM AGRICULTURAL UNIVERSITY
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CERTIFICATE I

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All help received by him have been duly acknowledged.

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INSTITUTIONAL ANIMAL ETHICS COMMITTEE

Communication of Decision of the Institutional Animal Ethics Committee (IAEC)

Topic of the PG research:
Biomolecular expression on Melatonin and vitamin E supplementation during summer and winter in pig

Name of the Student:
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[✓] - New review [ ] - Revised review [ ] - Expedited review

Date of review (D/M/Y): 10.2.2014
Date of previous review, if revised application :

Decision of the IAEC

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[ ] - Suggested Revision [ ] - Rejected

Suggestions / Reasons/ Remarks :
Collection of blood to be done humanly following guideline of CPCSEA. An undertaking with this effect may be furnished.

Please note*

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ABSTRACT

The present experiment was conducted to study the changes of various physiological, haematological and hormonal parameters including expression of HSP70 gene in the crossbred pigs (Hampshire × Local) under the agroclimatic condition of Assam. The experiment included a total of 36 numbers of crossbred weaned female pigs. Eighteen (18) animals were subjected to treatment separately during summer and winter. The selected animals were divided into three groups with six pigs in each group consisting of the control group (Treatment 1), one group was fed melatonin @3 mg/animal (Treatment 2) and the other group was fed Vitamin E @100 mg (Treatment 3) for both the seasons. The animals were maintained at AICRP on Pig, College of Veterinary Science, AAU, Khanapara, Guwahati-22.

The physiological parameters such as body temperature, pulse rate and respiration rate were recorded following standard methods. Temperature-Humidity Index was calculated out from the data of ambient temperature and relative humidity by using standard formula. About 5 ml of blood was collected from each experimental animal aseptically at 15 days interval for the whole experimental period. The haematological parameters viz. Haemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC) and total leucocyte count (TLC) were estimated from fresh blood by using MS4 Automated Haematological Cell Counter. The enzyme superoxide dismutase (SOD) and Lactate dehydrogenase (LDH) were estimated by using SOD and LDH assay kit manufactured by Cayman Chemical Company, USA as per manufactures protocol. Growth hormone and Progesterone were estimated by ELISA technique using Elisa kits procured from LDN Immunoassays and services. Melatonin was estimated by ELISA technique using Elisa kits procured from, Genway, Biotech Inc. The level of thermal stress related blood hormones such as triiodothyronine (T_3), thyroxine (T_4) and cortisol hormones were estimated by Radioimmunoassay (RIA) technique. The relative expression of HSP 70 gene was done by Real time PCR.

The Temperature Humidity Index (THI) during the study period was indicative of thermal stress to the experimental animals in the summer as compared to winter season. Physiological parameters *viz*., body temperature, respiration rate and pulse rate were found to be positively correlated with THI. All the physiological parameters showed significant difference ($P<0.01$) between summer and winter seasons irrespective of treatments. Haematological parameters viz. Hb, PCV, TEC was significantly lower during summer while TLC concentration was significantly higher during summer season as compared to winter in all the treatment groups. The mean body weight in the experimental pigs was significantly higher ($P<0.01$) in winter compared to summer.

Serum T_3 concentrations was significantly ($P<0.01$) lower during summer as compared to winter in all the treatment groups. Serum T4 concentration showed significant difference between treatment, between season and also between treatment and season. Serum cortisol concentration showed significant difference between treatment, between season and also between treatment and season. The serum cortisol concentration was found lowest in the melatonin and vitamin E supplemented group in both the seasons as compared to the control group. Significant difference ($P<0.01$) was found in the mean GH values between season with significantly higher values in the winter season. Statistical analysis revealed significant difference ($P<0.01$) in the mean progesterone concentration between treatment and between season. Significant difference ($P<0.01$) was found in the mean age at puberty between treatment with lower age at puberty in the melatonin supplemented group followed by vitamin E supplemented group and control group with highest age at puberty. There was also significant difference ($P<0.01$) in the mean age at puberty between season with lower age at puberty in the winter compared to summer. There was also significant difference ($P<0.01$) between day and season. Serum LDH activity was significantly higher ($P<0.01$) during summer as compared to winter season. The serum SOD activity was found to differ significantly ($P<0.01$) higher between treatment and between season and also between treatment and season. The serum progesterone concentration showed significant difference ($P<0.01$) between treatment and between season. There was also significant difference ($P<0.01$) between day and season. The mean melatonin concentration showed significant difference ($p<0.01$) between groups with significantly higher melatonin concentration in the melatonin supplemented group in both the season. Similarly serum vitamin E concentration was significantly higher ($p<0.01$) in the vitamin E supplemented group than the other two treatment groups in both the seasons. The normalized expression for HSP70 during summer shows that the animals with Melatonin treatment had 1.98 fold lower expression than the animals of control group. Likewise, animals with Vitamin E treatment showed 0.56 fold lower expression than control animals during summer season. During winter, the animals with Melatonin treatment showed 0.70 fold higher expression compared to control animals. Similarly, animals with Vitamin E treatment showed 1.28 fold higher expressions than control animals.

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LIST OF ABBREVIATIONS

Abbreviations		***Full form***
ANOVA	.....	Analysis of variance
DNA	.....	Deoxyribonucleic acid
cDNA	.....	Complementary DNA
d.f.	.....	Degrees of freedom
g/dl	.....	Gram / deciliter
h	.....	Hour
hrs	.....	Hours
HSP	.....	Heat shock protein
U/l	…….	Unit/liter
U/ml	.....	Unit/milliliter
Kg	.....	Kilogram
mg	.....	Milligram
mmol/L	.....	Millimol/Liter
RNA	.....	Ribonucleic acid
mRNA	.....	Messenger RNA
ng	….....	Nanogram
nmol/L	….....	Nanomol/liter
%	…….	percent
PCR	.....	Polymerase chain reaction
PCV	.....	Packed cell volume
pg	.....	Picogram
PR	.....	Pulse rate
RH	.....	Relative humidity
RR	.....	Respiration rate
RT	.....	Rectal temperature
SE	.....	Standard error
SOD	.....	Superoxide dismutase
T_3	.....	Triiodothyronine
T_4	.....	Thyroxine
TEC	.....	Total erythrocyte count
THI	.....	Temperature humidity index
TLC	.....	Total leucocyte count

CHAPTER I

INTRODUCTION

Heat stress is a term used in negative connotation and occurs in animals when there is an imbalance between heat production within the body and its dissipation. It is a universal problem faced by the livestock producers including swine growers. Although there are circumstances beyond the control of the producer, with planning and attention to details there is much the swine producer can do to protect livestock and reduce production losses. Environmental heat stress is a combination of several environmental factors. Most of our perception on heat stress is dependant on temperature, although humidity is readily recognised as an adverse factor at high air temperatures. The temperature humidity index (THI) was defined by Thom (1959) to represent the effects of these two factors.More recently the heat index has been used to empirically represent the combined effects of temperature and humidity, but not in the same proportion as the THI.

Heat is generated in every living cell of an animal as it metabolizes nutrients. As environmental temperature increases, the heat generated within the body of the animal is increasingly more difficult to dissipate to the surroundings. When heat production exceeds heat dissipation, body temperature rises. Controlling that body temperature is critical to survival, and is dependent on the ability of the animal to maintain body temperature within a range defining it as a homeotherm. Survival responses include: increased blood flow to the surface of the skin to increase heat dissipation; increased respiration rate for evaporation of moisture from the lungs and behavioural changes such as the use of a wallow or sprinklers if available to wet the skin surface for increased evaporative heat loss. Each of these steps might be completed without diverting nutrients or energy needed for normal body functions and growth. Reduced feed intake also lessens the amount of heat to be dissipated but reduces the supply of nutrients to be used for.

Swine are particularly susceptible to heat stress because they possess little to no functional sweat glands (Curtis, 1983). In addition, pigs maintain more subcutaneous fat

compared to other species and this prevents effective heat dissipation (Mount *et al*., 1979). Due to inadequate sweat glands, pigs depend on panting as their primary mechanism of heat dissipation (Patience *et al.,* 2005), especially if they don't have access to a wallowing area. The normal body temperature of the pig is 39.2°C (102.5°F) and at ambient temperatures above 22°C heat stress indicators such as increased respiration rates, and rectal temperatures are observed (Huynh *et al*., 2005). Nienaber and Hahn (2007) suggest that fast growing animals near market weight are at increased risk of severe heat stress because of increased metabolic heat due to genetic selection for enhanced lean tissue accretion rates. A 2.1% increase in lean tissue correlates with a metabolic heat production increase of 18.7% (Brown-Brandl *et al.*, 2004). Pigs respond to warm temperatures by increasing respiration rate, maximizing their surface area by laying on the ground, as well as increasing water intake. According to Marple *et al.* (1974), severe physiological changes can be observed in pigs with a rectal temperature reaching 41.5°C (106.7°F). This temperature can be potentially fatal, especially in finishing hogs and lactating sows which have decreased ability to dissipate heat.

Heat stress is one of the wide varieties of factors which cause oxidative stress *in-vivo*. Reactive oxygen species (ROS), the major culprits for causing oxidative stress, are constantly generated *in vivo* as an integral part of metabolism. ROS may cause oxidative stress when their level exceeds the threshold value. They trigger progressive destruction of polyunsaturated fatty acids (PUFA), ultimately leading to membrane destruction. Body employs antioxidants to quench these free radicals. The enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) act by scavenging both intracellular and extracellular superoxide radical and preventing lipid peroxidation of plasma membrane. Non-enzymatic antioxidants include vitamins like vitamins C, A and E, proteins like albumin, transferrin, glutathione (GSH) etc. Antioxidant nutrient supplementation especially vitamins C, A and E, zinc and chromium can be used to attenuate the negative effects of environmental stress. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start

chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.

Vitamin E has a number of different but related functions. One of the most important functions is its role as an intercellular and intracellular antioxidant. This function is closely related to and synergistic with the role of selenium. Selenium has been shown to act in aqueous cell media (cytosol and mitochondrial matrix) by destroying hydrogen peroxide and hydroperoxides via the enzyme glutathione peroxidase (GSHpx) of which it is a co-factor. In this capacity, it prevents oxidation of unsaturated lipid materials within cells, thus protecting fats within the cell membrane from breaking down. If lipid hydroperoxides are allowed to form in the absence of adequate tocopherols, direct cellular tissue damage can result, in which peroxidation of lipids destroys structural integrity of the cell and causes metabolic derangements.

Vitamin E reacts or functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing oxidation of lipids within membranes. At least one important function of vitamin E is to interrupt production of free radicals at the initial stage. Myodystrophic tissue is common in cases of vitamin E-selenium deficiency with leakage of cellular compounds such as creatinine and various transaminases through affected membranes into plasma. Fontaine *et al.* (1977) reported that serum creatine phosphokinase activity increases are associated with the occurrence of subclinical muscular dystrophy and that vitamin E and selenium deficiencies have marked additive effects on the induction of skeletal muscular disease in pigs. The more active the cell (e.g., the cells of skeletal and involuntary muscles), the greater is the inflow of lipids for energy supply and the greater is the risk of tissue damage if vitamin E is limiting. This antioxidant property also ensures erythrocyte stability and maintenance of capillary blood vessel integrity.

Melatonin hormone is secreted by the pineal gland which is direct free radical scavenger acting as indirect antioxidant. Melatonin secretion is regulated by

environmental light or dark cycle via SCN (Suprachiasmatic nucleus) and is considered as body's chronological pacemaker or Zeitgeber. Photoperiod could be the environmental factor mediating seasonality in pig. Administration of melatonin opens up a new methodology to control reproduction. Paterson *et al*. (1992) reported that seasonal inhibition of puberty in domestic gilts was overcome by melatonin administered orally.

Hormonal profiles related to metabolism and stress levels are good marker of animal's adaptability for growth under extreme conditions of summer and winter seasons. Heat shock proteins (HSPs) are a family of proteins which are expressed when cells are exposed to stress stimuli. Among HSPs, HSP-70 is an important part of the cells machinery for protein folding that helps to protect cells from stress. However, very little informations are available regarding the role of HSP-70 during heat stress and winter in pigs. Available literature reveals that very meagre quantum of studies on assessment of environmental stress level and thermo adaptability have been carried out so far in pig under agro climatic condition of the NE region of the country. Such study is essential to find out baseline data on the heat tolerance of pigs by studying some biochemical and haematological parameters especially in weaned pigs. The same parameters can be used to see the effect during cold winter season.

In view of the above facts, the present experiment was conducted in weaned pigs during summer and winter seasons with the following objectives:

1. To study certain physio- biochemical profiles in supplemented pigs.
2. To study the effects of body weight gain on attainment of puberty in supplemented pigs.
3. To study expressions of HSP gene in supplemented pigs.

CHAPTER II

REVIEW OF LITERATURE

In this chapter an attempt has been made to review the available literature related to the present experimental study**.**

2.1 TEMPERATURE-HUMIDITY INDEX (THI)

Davis and Mader (2002) Temperature-Humidity Index (THI) is a suitable climatic marker to correlate climatic stress on physiology and productivity of animals and also a reliable tool for effective management of livestock under different climatic condition

Kadzere *et al.* (2002) reported THI level beyond 72 was indicative of mild heat stress, THI 75 to 78 denoted stressful condition and that beyond 78 could indicate severe stress due to heat and humidity.

Marai *et al.* (2002) observed that Rabbits are very susceptible to heat stress, since they have few functional sweat glands and have difficulty in eliminating excess body heat, when the environmental temperature is high. In female rabbits, conception rate, embryonic development, litter size, litter weight and milk production decreased and age at puberty and pre and post weaning mortality increased by exposure to heat stress. In males, testosterone concentration, spermatogenesis, temporary sterility, sexual desire, ejaculate volume, motility, sperm concentration and total number of spermatozoa in an ejaculate decreased and sperm abnormalities and dead sperm increased by exposure to the same factor. The drastic changes that occurred in rabbits' biological functions were depression in feed intake and feed efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. When exposed to THI 30 or more, rabbits could no longer regulate internal temperature and heat prostration sets in.

Antonio and Andres (2003) observed that Temperature and humidity conditions affected livestock production in Central Argentina. This study evaluated the risk of thermal stress affecting dairy production. The temperature-humidity index (THI) was used to analyze the regional and seasonal effects of temperature and humidity. Statistically, the THI was found to be normally distributed. The probability of occurrence of a daily THI higher than 72 was 40 per cent for Río Cuarto during January. Regional variability of THI indicated a low risk of harmful extreme thermal stress conditions. The probability of THI being 78 or above ranges between 4 and 10 per cent for the main dairy region of Córdoba during January.

2.2 PHYSIOLOGICAL PARAMETERS

Physiological parameters like body temperature, respiration rate and pulse rates give an immediate response to the climatic stress and consequently the level of discomfort/comfort to the animal. These responses have been used as a measure of animal comfort and adaptability to an adverse environment or as a sensitive physiological measure of environmental modification. These responses reflect the degree of stress imposed on animals by climatic parameters. The ability of an animal to withstand the rigors of climatic stress under warm conditions has been assessed physiologically by means of changes in body temperature, pulse rate and respiration rate (Leagates *et al.*, 1991 and Sethi *et al.*, 1994).

Several investigators studied physiological adaptation mechanisms such as rectal temperature, pulse rate and respiration rate in small ruminants (Srikandakumar *et al.*, 2003; Maurya *et al.*, 2004; Marai *et al.*, 2007; Otoikhian *et al.*, 2009; Phulia *et al.*, 2010; Sharma *et al.* 2013;). Body temperature is a good measure of heat tolerance in animals. It represents the resultant of all heat gain and heat loss processes of the body. Rectal temperature is considered as a good index of core body temperature even though there is a considerable variation in different parts of the body core at different times of the day (Srikandakumar *et al.,* 2003). There is activation of heat-regulating mechanism in the heat-stressed animal and an increase in respiratory exchange to maintain the internal body temperature (Marai *et al.*, 2007).

Respiration Rate (RR)

Quiniou and Noblet (1999) reported that the respiration rate in multiparous lactating sows increased from 26 to 124 breaths/min between 18 and 29˚c and this indicates that the evaporative critical temperature was below 22˚c. Higher ambient temperature contributed to higher evaporative heat losses through increased respiratory rates.

Huynh *et al.* (2005) reported that for determining the effect of temperature and relative humidity on physiological data (respiration rate, rectal temperature and skin temperature) the mean of individual data per pig per day (three pigs in each chamber) was used. He found that, with increasing temperature respiration rate remained constant at an average 32 breaths per minute until the inflection point (on average 22˚c) which increased by an average 13 breaths/min/˚c in pigs.

Pulse Rate (PR)

Cooperative Extension Service, West Virginia University (2010) reported that the pulse rate in pigs range between 60 to 80 per minute.

Jackson and Cockroft (2014) reported that the pulse rate of 60 to 90 beats/min is normal in adult pigs but increases rapidly if they are stressed.

Rectal Temperature (RT)

Quiniou and Noblet (1999) reported that the rectal temperature in multiparous lactating sows was found constant between 18 and 22^{o}C (38.6^{o}C) but it increased at higher temperature (39-39.4^{o}C between 25 and 29^{o}C). The ambient temperature also affected the gradient observed between the rectal temperature and other estimates of the body temperature.

Huynh *et al.* (2005) reported that for determining the effect of temperature and relative humidity on physiological data (respiration rate, rectal temperature and skin temperature) the mean of individual data per pig per day (three pigs in each chamber) was used. He found that, respiration rate was affected by increasing temperature until the

temperature reached 26.1^{o}C the respiration rate of pig was constant at an average of 39.3^{o}C. Above the inflection point it increased 0.13 ^{o}C / ^{o}C.

2.3 BODY WEIGHT

Chu and Song (2013) reported that the final body weight and average daily gain in fattening pigs were significantly lower ($P<0.05$) in summer than in spring, autumn and winter and it was not different between spring, autumn and winter. The finished body weight in summer was 100.67 kg while that of spring, autumn and winter were 107.83, 107.17 and 107.83 kg respectively. The average daily gain was 0.815, 0.530, 0.810 and 0.850 kg per day spring, summer, autumn and winter respectively.

Korzeniowska *et al.* (2012) reported that the daily body weight gains of pigs finishing in winter season were lower as against those in the summer period by an average of 291.63 g in the first stage and 26.58 g in the second stage.

Quiniou and Noblet (1999) reported an increased body weight loss in the multiparous lactating sows but its estimate chemical composition remained constant. Over the total lactation the body weight loss was significantly affected by temperature; it amounted to 23 kg at 18, 22 and 25^{o}C on average but increased up to 36 kg at 29^{o}C,the value at 27^{o}C being intermediate. A significant interaction between temperature and dietary treatment was observed in connection with a lower body weight loss at 25^{o}C.

Tian *et al.* (2001) reported that in the growing-finishing pigs during the overall experimental period (0-9 weeks, 54-106 kg body weight) growth performance was not significantly affected by dietary vitamin and trace mineral levels. During the first two weeks (21 to 30 kg body weight), average daily gain in the 200% mineral supplemented group was highest and lowest in 200% water soluble supplemented group ($P<0.05$)

Prassana *et al.* (2010) reported that the piglets born in the rainy season recorded significantly higher body weights followed by those born in winter and summer seasons. However the effect of season on birth was found to be non-significant on birth weight of the pigs. Significant effect of season of furrowing on body weights at different pre-

weaning ages indicated a favorable effect of rainy season in general. Genetic group affected all the preweaning body weights highly significant except the body weight at birth.

Mavrogenis and Robinson (1976) reported that gilts born in the fall furrowing season have lower body weight at puberty ($P<0.01$) than those born in the spring. Exteroceptive study associated with the presence of boar caused an 8 kg reduction in the body weight at puberty.

Young *et al.* (1975) reported that all the pigs survived and gained approximately 0.32 kg per day during the trail. The rate of gain was not influenced by supplemental selenium or vitamin E. however, the weekly body weight gain of pigs which did not receive supplemental vitamin E or selenium appeared lower during the last week of the experiment as compared with pigs supplemented with vitamin E or selenium (5.52, 6.98, 6.62 kg)

Jong *et al.* (2000) reported that when the Restricted feeding (RF) pigs were given the opportunity to eat *ad libitum* after 84 days age they had a significantly higher growth rate than the Ad libitum fed (AL) pigs. At weaning and at 35 days of age AL pigs weighed slightly but significantly more than RF pigs.

2.4 HAEMATOLOGICAL (HEMOGLOBIN,PCV,TEC AND TLC)

Normal body temperature is essential for physiological adjustment (Lowe *et al.*, 2001). Thermal stress may cause hyperthermia and potentially have several physiological side effects. It is known to alter the homeostatic mechanisms of animals resulting in impaired erythropoiesis. High environmental temperature increases oxygen consumption of animals through increased respiration rate. The higher oxygen intake increases the partial pressure of oxygen in blood, decreases erythropoiesis, which in turn reduces the number of circulating RBCs and thus PCV and Hb values (Maurya *et al.*, 2007; Temizel *et al.*, 2009).

Gyo-Moon Chu and Young-Min Song (2013) reported that the hemoglobin concentration was not affected much by seasonal variation. In summer it was 13.28 gdL^{-1} , in spring it was 13.33 gdL^{-1} , in autumn it was 13.19 gdL^{-1} and in winter it was found to be 12.78 gdL^{-1} in the fattening pigs.

Mayengbam *et al.* (2014) reported that the average hemoglobin concentration in Zovawk pigs were stable and were found to be 13.41±0.30 in the pre-weaning group, 14.48±0.54 in the grower group and 15.15±0.70 in the adult group. Since, there is no literature available on the hematological profile of Zovawk pigs, the findings were compared in relation to the data available for other exotic breeds of pig.

Korzeniowska *et al.* (2012) reported a higher concentration of hemoglobin in the 1^{st} stage of summer season than the winter season in the fattening pigs.

Adenkola *et al.* (2011) reported that the hemoglobin concentration (g%) in pigs in hot-dry and harmattan season differed significantly($p<0.05$) with values of 13.2±0.6 and 10.64±0.3 respectively. The values indicated that the animals are being severely affected by adverse meteorological conditions prevailing in the tropical Africa, predisposing them to heat or cold stress.

Eze *et al.* (2010) reported that the hemoglobin concentration in pigs raised under intensive system of management were found to be 9.60±1.78 in piglets and 11.29±2.76 in adults.

Adenkola *et al.* (2009) reported the hemoglobin concentration in pigs in harmattan season before short journey, immediately after short journey and day 7 after short journey in experimental and control pigs as 11.07±0.32 & 10.64±0.28, 11.05±0.43 & 11.18±0.33 and 12.43±0.46 & 12.56±1.23 respectively.

Dey *et al.* (2013) reported the hemoglobin values of different pig breeds of Andaman and Nicobar Islands. The Hb (g/dl) values were 17.27±0.35 in Andaman wild pigs, 8.60±2.01 in Nicobari pigs, 8.57±1.37 in Andaman desi pigs and 11.48±2.94 in Large White Yorkshire pigs.

Mayengbam *et al.* (2014) reported that the PCV percentage did not change in the Zovawk pigs from the pre-weaning stage till the growing stage but increased significantly ($p<0.05$) in the adults. Since, there is no literature available on the hematological profile of Zovawk pigs, the findings were compared in relation to the data available for other exotic breeds of pig.

Adenkola *et al.* (2011) reported that the PCV values in the hot-dry and harmattan season in pigs were 39.7±1.9 and 32.00±0.9 and they differed significantly ($p<0.05$).

Dey *et al.* (2013) reported the PCV values of different pig breeds of Andaman and Nicobar Islands. The PCV (%) values were 61.3±0.56 in Andaman wild pigs, 28.89±6.08 in Nicobari pigs, 28.64±4.72 in Andaman desi pigs and 41.35±4.46 in Large White Yorkshire pigs.

Eze *et al.* (2010) reported that the PCV value in pigs raised under intensive managemental system was found to be 31.36±7.1 in piglets and 35.89±10.10 in adults.

Adenkola *et al.* (2009) reported the PCV values recorded on day 7 after the journey rose ($p<0.05$) to 37.38±1.39% in the experimental pigs and the value was significantly ($p<0.05$) higher than 33.54±1.01% recorded in the control pigs post transportation in the harmattan season.

Mayengbam *et al.* (2014) reported that the TEC values in the Zovawk pigs increased significantly ($p<0.05$) from the pre-weaning stage (14.11±0.89) to the grower stage(17.71±0.59) and remained stable in the adult stage (17.68±0.84). Since, there is no literature available on the hematological profile of Zovawk pigs, the findings were compared in relation to the data available for other exotic breeds of pig.

Adenkola *et al.* (2011) reported that the TLC values in pigs during hot-dry and harmattan season in the Northern Guinea Savannah Zone of Algeria were found to be 15920.00±1119.1 and 18836.5±1727.1 which differed significantly ($p<0.05$).

Gyo-Moon Chu and Young-Min Song (2013) reported the TLC values in fattening pigs in summer and winter as 19.40 and 14.05 which varied significantly.

Adenkola *et al.* (2009) reported that pigs administered ascorbic acid and transported by road for four hours during harmattan season the TLC dropped (15830.25±1063.08) ($p<0.05$) in the experimental animals after transportation and the value was significantly ($p<0.05$) lower than the value of 22010.69±1722.00 obtained in the control pigs after the journey.

Mayengbam *et al.* (2014) reported that the TEC values in the Zovawk pigs increased with age with significantly ($p<0.05$) higher values in the adults as compared to the pre-weaning and the growers. Since, there is no literature available on the hematological profile of Zovawk pigs, the findings were compared in relation to the data available for other exotic breeds of pig.

De *et al.* (2013) reported the TEC values of different pig breeds of Andaman and Nicobar Islands. The TEC (10^6 /µl) values were 9.72±0.17 in Andaman wild pigs, 4.52±0.93 in Nicobari pigs, 5.44±0.95 in Andaman desi pigs and 7.43±0.78 in Large White Yorkshire pigs.

Gyo-Moon Chu and Young-Min Song (2013) reported the TEC values in the fattening pigs during summer were found to be 7.31 and in the winter it was 7.05.

2.5 REPRODUCTIVE PARAMETERS

Attainment of Puberty

Paterson *et al.* (1992) reported that the daily feeding of 1mg melatonin increased ($p<0.05$) the proportion of gilts which reached puberty. Among the 27 gilts which were fed melatonin 15(55.6%) reached puberty compared with six of the 25(24%) control gilts.

Mavrogenis and Robinson (1976) reported that the gilts in the fall reached puberty at a younger age and a lower weight ($p<0.01$) than those born in the spring. The presence of boars substantially reduce ($p<0.01$) age and weight at puberty.

Canope and Raynaud (1981) recorded the age and body weight of gilts at puberty as 275 ± 7.0 days and 107 ± 2.7 kg for large white and 171.4 ± 3.5 days and 52.3 ± 2.0 kg for Creoles breed respectively

Lo *et al.* (1985) made an experiment to see the effect of reproductive performance of gilts by different feeding methods during growing period. Female piglets are randomly divided into 3 groups. Group 1 was offered feed *ad lib.* Whereas group 2 and 3 offered 90 and 70 percent of *ad lib.* Feed intake respectively from 20 to 90 kg body weight. Age at puberty averaged 166.2, 167.3 and 174.3 days respectively ($p<0.05$), and body weight at puberty 88.8, 89.2 and 80.3 kg. group 3 vs group 1 and 2 ($p<0.01$)

The age at puberty was recorded by Banerjee (1986) as 5 months, by Frandson (1986) as 3 to 7 months and length of estrus cycle as 18 to 24 days, by Morrow (1986) as 5-8 months and length of oestrus cycle as 21 days, by Soumi *et al.* (1997) as 225 days and by Anderson(1993) as 25 weeks of age. He also reported that there productive cyclicity is of 19 – 23 days.

Krikwood and Thacker (1988) recorded the age at puberty as 150 days in Yorkshire X Landrace gilts when fed 3 kg of diet per day from 120 days of age to onset of puberty (control). On the other hand, when gilts were fed only 2.0 kg per day they exhibited pubertal oestrus at 165 days of age (restricted). In 3rd group they fed 2.00 kg per day from 120 to 150 days of age, but increased thereafter to 3.5 kg per day until mated (flushed) and recorded age at puberty as 165 days.

Litter Size

Quiniou and Noblet (1999) reported that higher average litter size were obtained at 27˚C that at other temperatures (i.e., 18, 22, 25 and 29˚C) in multiparous lactating sows.

2.6 SERUM CONCENTRATION OF T_3 and T_4 ,CORTISOL

Exposure of animals to heat stress activates the hypothalamo-pituitary –adrenal axis (Abilay *et al.,* 1975) and hence estimation of concentrations of hormones such as

cortisol and thyroxine could be one of the important indicators for assessment of stress in animals.

Triiodothyronine (T_3) and Thyroxine (T_4)

Kallfelz and Erali (1973) studied the thyroid status in suckling, young adult and mature pigs and reported that the serum T_4 concentration decreased significantly with age. They also found that the T_3 values were highest in young adult animals. The respective values for T_3 (%) and T_4(μg / 100 ml of blood) were 30.1 ± 2.52 and 8.40 ± 0.54, 3.17 ±1.18 and 4.70 ± 0.45 and 32.6 ±2.20 and 2.10 ± 6.42 in suckling, young adult and mature pigs.

Reap *et al.* (1978) reported the normal serum T_4 and T_3 values in pigs as 3.32±0.80 and 1.70± 4.68 μg/dl respectively whereas Anderson *et al.* (1988) reported that the concentration of total T_4, free T_4,total T_3 and free T_3 in pig serum as 53 ng/ml,21.7pg/ml,760pg/ml and 2.74 pg/ml respectively

Djurdjevic *et al.* (1992) studied the effect of different dietary levels on serum level of T_3 and T_4 in Swedish X Big Yorkshire X German Landrace gilts and found that normal level of T_3 and T_4 as 1.48 ± 0.28 and 46.57 ± 11.37 respectively at 4 months of age which were remained unchanged until first oestrus (about 6.5 months of age) in all gilts with a significant lower concentration of T_3 and T_4 only at 1-3 days before parturition.

It was demonstrated by earlier workers that the optimization of the reproductive status was dependant on the thyroxin level. A higher T_3 value was found in non-pregnant than in pregnant Landrace pigs. The plasma level of total T_4 in large white X Landrace pigs was 3.5± 0.5 (range- 3.0 to 4.5) μg/100 ml serum. (Sutherland and Irvine, 1973)

Baltaci *et al.* (2004) reported that suppressing effect of melatonin on thyroid functions, as evidenced from TSH, T_3, and T_4 level could be avoided by dietary zinc supplementation.

Herpin *et al.* (2014) reported the thyroxine concentration in Meishan pigs-80.5, Large white pigs-85.6 and in composite line pigs-71.6.

Cortisol

Gyo-Moon Chu and Young-Min Song (2013) reported that the plasma cortisol concentration in the fattening pigs in summer (5.67 μgdL^{-1}) significantly increased ($p<0.05$) compared to winter (2.57 μgdL^{-1}).

Fagundes *et al.* (2008) reported that the pigs at high temperature showed significantly higher average cortisol level ($p<0.01$) than the comfort temperature ones (7.06 and 4.82 mg/dL). Increase in serum cortisol was continuous and linear ($p<0.05$) during the experimental period suggesting the cortisol as a possible indicator of the heat stress in growing-finishing pigs.

Averos *et al.* (2007) reported that the plasma cortisol concentration in the pigs transported to slaughter under commercial conditions were found to be 3.00±0.21 in female, 3.68±0.22 in male, 2.83±0.24 in winter and 3.85±0.19 in summer.

Bonnette *et al.* (1990) reported that in pigs following supplementation of two supplemental vitamin E levels the cortisol levels decreased during the first week following weaning and then increased linearly ($p<0.01$) over time.

Herpin *et al.* (2014) reported the cortisol level in Meishan pigs-150.6, Large white pigs-114.4 and in composite line pigs-127.

Increase in cortisol secretion is a classical response to stress (Kannan *et al.*, 2000). Plasma cortisol level increases during acute heat stress and decreases during the chronic phase. The increase in plasma cortisol level during acute heat stress is attributed to the fact that glucocorticoid hormones have hyperglycaemic action through the gluconeogenesis pathway, thus enhancing glucose formation in heat stressed animals.

The decline which occurs in chronic stress is attributed to the fact that cortisol was found to be thermogenic in nature and consequently the reduction of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing a rise in metabolic heat production in hot environment. Hence these combined stressed animals have the capacity to adjust the cortisol level to the minimum possible increase to elicit thermal stress relieving effects. This indicates the role of the adrenal cortex gland in adaptation to stress (Marai and Habeeb, 2010). On the other hand, glucocorticoids function as vasodilators and facilitates heat loss; have stimulatory effect on proteolysis and lypolysis. Hence, providing energy to the animal to offsets the effect of reduction of feed intake (Cunningham and Klein, 2007). Furthermore, cortisol have been found to suppress immune functions of the animals (Weiss, 2009).

The association between stress and increased cortisol is well documented in goats (Ali and Hayder, 2008; Sejian and Srivastava, 2010a). Sejian *et al.* (2010b) found significant increase in the serum cortisol level in Marwari goats when they were exposed to thermal stress in a psychrometric chamber (40^{O}C and 60% RH) for 4 hrs a day for days in comparison to control goats. Sejian *et al.* (2010a) also reported significant increase in serum cortisol concentration in Malpura ewes exposed to thermal and nutritional stress. But they found reduction of cortisol level in nutritionally stressed ewes. This showed the differential adaptive capacity of the animals. As such, cortisol plays an important role in all types of stress. The stressors induce release of cortisol by activation of the hypothalamic-pituitary- adrenal axis (Minton, 1994). Bhan *et al.* (2012) reported significant increase in cortisol level in both growing as well as adult Sahiwal cattle in summer in comparison to winter season. They also recorded significant higher level of cortisol level at afternoon than the morning level in both the groups of cattle.

Kaushish *et al.* (1997) reported significant breed difference for serum cortisol concentration in goats. They recorded lower cortisol concentration (17.6 ± 2.68 ng/ml) in Beetal kids compared to Black Bengal kids (48.1± 3.68 ng/ml). They also reported significant increase in serum cortisol level (38.5 ± 5.13 to 54.3 ± 2.68 ng/ml) in Beetel kids, but the increase was non significant (43.7 ± 5.32 to 55.5 ± 5.72 ng/ml) in Black Bengal kids after a short term thermal stress alone or thermal stress coupled with water

withholding. They concluded that Black Bengal goats were more adapted to hot humid conditions than Beetal goats.

2.7 GROWTH HORMONE CONCENTRATION

Growth hormone (GH), a 191-amino acid polypeptide, is synthesized by somatotroph cells in the anterior pituitary. GH induces protein synthesis and nitrogen retention, and impairs glucose tolerance by antagonizing insulin's action. GH stimulates lipolysis, leading to increased circulating fatty-acid levels, reduced omental fat mass, and enhanced lean body mass. GH promotes sodium, potassium, and water retention, and elevates serum levels of inorganic phosphate.

Siers and Swiger (1971) reported that the decrease in circulating GH level seen as animals become larger and older is primarily due to their increased size.

Trenkle (1971) reported that diet had no marked effect on the concentration of plasma growth hormone in sheep

Growth hormone is a peptide synthesized and secreted by the pituitary that reaches target tissues through the peripheral blood stream. Its regulation is mediated by somatostatin (an inhibitor) and by GH-RH (a stimulator). In turn, Somatomedin-C inhibits GH release, stimulating somatostatin synthesis and inhibiting GH-RH synthesis in the hypothalamus (Berelowitz *et al.*, 1981).

Etherton *et al.* (1986, 1987, 1992 and 1998) reported that following daily injection with effective doses of GH @ 30, 70,100 µg/kg BW/days, to growing pigs for 8-11 weeks showed an average daily gain by 10%, 19% and 20%. With GH @ 100 µg/kg BW/days, muscle growth is increased by as much as 62 %.

Campbell *et al.* (1990) reported the growth hormone administration improved growth rate by 13%, 22% and 16% and feed conversion efficiency by 19%, 34% and 32% in boar, gilts and barrows respectively.

Heinrichs *et al.* (1997) reported that serum GH levels were significantly greater in fasted than fed animals (5 ± 2 vs. 27 ± 12 vs. 29 ± 7 ng / ml in the fed, 15-h fast, 30- h fast groups, respectively; $P < 0.02$). They also reported that in growth plate, fasting significantly increased growth plate GH receptor mRNA levels compared with controls (100 ± 10% vs. 200 ± 12 vs. 197 ± 23 in the fed, 15-h fast, 30-h fast groups, respectively; $P < 0.001$).

Baudet *et al.* (2009) reported that Growth hormone is essential for postnatal growth in mammals. Along with Growth, GH affects the metabolism of carbohydrate, protein and fats (Moller and Jorgensen, 2009).

2.8 PROGESTERONE

Saikia (2007) reported the mean serum progesterone concentration on day 14 days before treatment of probiotics, 7 day before treatment of probiotics, day of probiotic treatment and the day of estrus as 0.47 ±0.04, 0.51±0.05, 0.49±0.04 and 0.42±0.05 ng/ml; 0.46±0.05, 0.50±0.06, 0.48±0.05 and 0.42±0.05 ng/ml; and 0.47±0.06, 0.51±0.04, 0.48±0.06 and 0.40±0.06 ng/ml, in gilts of group A, B and C respectively.

Callaghan (1978) indicated that plasma progesterone level in prepubertal gilts averaged 0.5 ng/ml when gilts were induced to oestrus by treating 400 IU PMSG and 200 IU HCG.

Esbenshade *et al.* (1982) reported that the mean concentration of plasma progesterone concentration ranged from 0.1 to 0.3 ng/ml between 6 day before to the day on which gilts exhibited first oestrus. The low progesterone concentration in all the treatment groups during summer and winter may be attributed to the absence of functional corpus luteum.

2.9 ENZYME ACTIVITY

LDH

Gyo-Moon Chu and Young-Min Song (2013) reported that the plasma LDH concentration in the fattening pigs was significantly higher ($p<0.05$) in summer (937.70 UL^{-1}) than in winter.

Korzeniowska *et al.* (2012) reported that LDH concentration in the fattening pigs in the winter season were : Mean-2106.25,SD-292.29 in the 1st stage and Mean-1706.60 and SD-293.64 in the 2nd stage whereas in the summer season it was ,Mean-2178.64 and SD-427.65 in the 1st stage and Mean-1831.08 and SD-376.98 in the 2nd stage.

Averos *et al.* (2007) reported that the LDH concentration in pigs transported for slaughter under commercial conditions were found to be, 1.203±38.34 in female, 1.114±40.20 in male, 1.201±45.98 in winter and 1.109±33.75 in summer.

SOD

High environmental temperature challenges the animal's ability to maintain energy, thermal, hormonal and mineral balance. Thermal stress stimulates excessive production of reactive oxygen species (ROS), such as superoxide anion (O^{-}_{2}), hydroxyl ion (OH) and hydrogen peroxide (H_2O_2), which are continuously produced in the course of normal aerobic metabolism. Reactive Oxygen species modulate multiple cellular processes, including proliferation, differentiation and signaling. These free radicals can damage healthy cells if they are not eliminated. This may be reflected as disturbed physiology and altered biochemical profile of the animal (Bernabucchi *et al.*, 2002).

The major defense in detoxification of superoxide anion and hydrogen peroxide, are superoxide dismutase (SOD), catalase and glutathione peroxidase (McCord and Fridovich., 1969; Chance *et al.*, 1979). The enzymatic antioxidant i.e. superoxide dismutase provides protection against ROS generated due to thermal stress. Superoxide dismutase along with catalase and glutathione peroxidase (GPx) scavenges both

intracellular and extracellular superoxide radicals and prevents lipid peroxidation (Agarwal and Prabhakaran, 2005).

Mitochondrial SOD readily converts the bulk of mitochondrial superoxide ions to H_2O_2. Thus, SOD protects the cell from the damage due to the secondary generation of highly reactive hydroxyl group from superoxide ion to H_2O_2 (Miyazaki *et al.,* 1991). Antioxidant enzyme activities are sensitive markers of oxidative stress as their levels may increase or decrease in response to reactive oxygen species. Superoxide dismutase that catalizes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress (Halliwell and Chirico, 1993). Many types of diseases or stresses have been associated with low antioxidant levels and high free radical compound production

2.10 SERUM CONCENTRATION OF MELATONIN AND VITAMIN E

Melatonin

Harlow (1987) opined that action of the pineal gland hormone requires the activity of specific receptors located primarily in the brain and in the peripheral tissues thereby affecting the metabolism directly or indirectly. Pineal gland melatonin is a known antioxidant hormone which functions via a number of pathways to reduce oxidative stress. It acts as a direct free radical scavenger, as an indirect antioxidant and has ability to augment the activities of other antioxidants.

Seijan and Srivastava (2010a) reported that melatonin plays an important role in relieving heat stress by influencing cardiovascular system and evaporative heat loss It also interacts with other hormones to alleviate heat stress possibly with thyroxine and successfully modify adrenal function to relieve thermal stress

Singh *et al.* (2014) reported that the melatonin concentration was significantly higher in winter in comparison to summer, which may be due to natural melatonin level. The higher level of melatonin in winter compared to summer may be due to the diurnal variations for melatonin secretion in winter that may give a natural advantage to the animals for ameliorating cold stress.

Singh *et al.* (2014) reported that the clinically immune parameters such as lymphocyte count and stimulation ratio of T lymphocytes presented a day/night rhythm prominently in winter. They also observed that the oxidative load in terms of malonedialdehyde was always low during night while antioxidants such as superoxide dismutase catalase and total antioxidant status were high during nighttime

Paterson *et al.* (1992) compared to implants melatonin administered orally is effective in overcoming the seasonal inhibition of puberty in domestic gilts

Vitamin E

Mitssioulis and Judson (2000) reported that on the day of collection (0 day), the plasma vitamin E concentration (mg/l) in the animals sampled from each species ranged from 1.5-2.8 in pigs, 6.7 to 10.2 in cattle and 0.2 to 1.7 in sheep.

Niculita *et al.* (2007) reported that the serum from Vitamin E supplemented pigs had the highest concentrate ion of alpha-tocopherol throughout the 1st 4 weeks of the experiment. They also reported that the blood levels of vitamin E were 2.7 to 3.5 times higher in the vitamin E supplemented pigs than those in the basal diet.

2.11 HEAT SHOCK PROTEINS (HSPs) GENES EXPRESSION

Zhang *et al*. (2012) reported that the HSP-70 mediates distinct stress related functions in different tissues during transportation of pigs**.**

The HSPs are originally identified as proteins whose expression was markedly increased by heat shock (Lindquist, 1986). Several HSPs are expressed even in unstressed cells and play important function in normal cell physiology. The intensity and duration of the heat stimulus needed for HSP expression vary considerably from tissue to tissue. Induction of HSP expression typically starts within minutes after the initiation of heat stress, with peak expression occurring upto several hours later. Importantly, several experiments have found that, during the period of hyperthermia and shortly thereafter, the HSPs become the predominant proteins synthesized by cells (Lindquist, 1986). Interestingly, most HSP genes lack introns (Lindquist, 1986), which may facilitate their

rapid expression and which may also help explain how they can be expressed in the presence of stressors (such as heat) that can interfere with RNA splicing.

In mammalian cells, non-lethal heat shock produces changes in gene expression and in the activity of expressed proteins, resulting in what is referred to as a cell stress response (Jaattela, 1999; Lindquist, 1986). This response characteristically includes an increase in thermo tolerance that is temporally associated with increased expression of HSPs. Heat induced changes in gene expression occur both during hyperthermia as well as after return to normothermia.

Regulation of heat shock protein gene transcription is mediated by the interaction of the heat shock factor (HSF). The HSFs, present in the cytosol, are bound by heat shock proteins (HSPs) and maintained in an inactive state (Morimoto, 1998). A broad array of physiological stimuli ("stressors") are thought to activate HSFs, causing them to separate from HSPs. HSFs are phosphorylated by protein kinases (Kim *et al.*, 1997) and translocate into nucleus (Morimoto, 1998; Pockley, 2001). In the nucleus trimerization occurs. These HSF trimer complexes bind to heat shock elements (HSE) in the promoter region of the HSP gene. HSP mRNA is then transcribed and leaves the nucleus for the cytosol, where new HSP is synthesized (Sarge *et al.*, 1993).

Upon stress the most prominent HSPs present in the nucleolus are the inducible HSP70 and HSP110 (Welch and Suhan, 1985). Some of the HSPs translocate to the nucleolus, which suggests a specific and unique role in the repair and protection of these cellular structures (Collier and Schlesinger, 1986). Upon recovery after heat shock, HSP70 leave the nucleolus to accumulate back in the cytoplasm (Welch and Feramisco, 1984; Welch and Suhan, 1985).

Proteins that lose their normal three-dimensional conformation provoke HSP synthesis through the activation of HSF. During and after heat-shock, cytosolic proteins normally aggregate and their solubility is reduced and HSPs play a major role in preventing aggregation of proteins (Vidair *et al.*, 1996). Along with the release during heat stress they themselves are subject to strict autoregulation by multiple molecular mechanisms (Lindquist, 1993). Heat shock protein chaperoning is a permanent cellular

event during both stressed and non-stressed conditions. However, heat shock or other stresses upregulate the synthesis and translocation of various HSPs to other cellular compartments, which suggests that during evolution tissues developed intrinsic defense mechanisms for reusing unfolding proteins in various cellular compartments.

Enhanced synthesis of HSPs was detected in highly purified T cells during heat stress (febrile temperatures less than or equal to 41^{o}C), three major HSPs with approximate molecular weights of 110, 90, and 75 were detected in these T cell populations. Enhanced HSP synthesis reflected augmented transcription of *hsp* genes which was dependant on the continued presence of hyperthermic stress (Ciavarra and Simeone, 1990). Heat shock induces HSP70 in the bovine lymphocytes (Guerriero and Raynes, 1990), bovine peripheral blood mononuclear cells (PBMCs) (Kamwanja *et al.*, 1994; Lacetera *et al.*, 2006) and buffalo PBMCs (Patir and Upadhyay, 2007).

The HSP70 level was found to increase by 200 and 2.5 times in serum and lymphocytes, respectively, compared to control animals under natural conditions when buffaloes were exposed at 42^{0}C and 70 per cent RH (Mishra *et al.*, 2011). The mRNA level of HSP70 in lymphocytes was increased with increase in THI; the mRNA level of HSP70 at high temperature was higher than others ($P<0.01$) in dairy cows (Liu *et al.*, 2010). The HSP70 concentration in Angus cattle increased from 0.07 to 0.25 μg/million cells when the temperature was enhanced from 38.5^{o}C to 42.4^{o}C whereas in Brahman cattle, it increased from 0.07 to 0.26 μg/million cells when the temperature was enhanced from 38.5^{o}C to 42.0^{o}C (Kamwanja *et al.*, 1994). *In vitro* studies showed that HSP70 was produced in heat-stressed lung cells (Fargnoli *et al.*, 1990), hepatocytes and liver (Heydari *et al.*, 1995; Hall *et al.*, 2000) and myocardium (Gray *et al.*, 2000) suggesting that HSPs provides protection from toxic effects of thermal stress. The HSPs are released at specific temperatures and have critical temperatures for different livestock species viz; 42°C (bovine and horse), 43°C (sheep) or 44°C (chicken). Proteins with molecular weights of 70 and 90 kDa were synthesized in all species. Additional proteins were found in bovine, ovine and chicken lymphocytes (Guerriero and Raynes, 1990).

In buffaloes, higher intensity and duration of temperature exposure cause higher HSP70 induction in lymphocytes to maintain cellular homeostasis (Patir and Upadhyay, 2010). Irrespective of stocking density, transportation under hot and humid tropical conditions significantly increased HSP70 densities ($P<0.05$) in the kidneys of goats (Zulkifli *et al.,* 2010). Dangi *et al.* (2012) found significant increase ($P<0.05$) in mRNA expression of HSP60, HSP90 and ubiquitin in different age groups during peak summer season as compared with peak winter season in both tropical (Barbari) and temperate region goats (Pashmina). Similarly, significant increase ($P<0.05$) in mRNA expression of HSP70 was observed in tropical goats during summer season in comparison to winter season. However, no significant difference was observed in HSP70 expression between summer and winter season in the temperate region goats. In another experiment recently, Sharma *et al.* (2013) also documented significant increase ($P<0.05$) in mRNA expression of HSP60, HSP70, HSP90 and ubiquitin in Barbari goats when they were exposed to 35-40^{o}C in a psychrometric chamber @ 6 h/day for 11 days. They further observed that expression of HSP60 increased manifold in melatonin treated groups at 40^{o}C exposure temperature.

Heat shock proteins not only enhance heat tolerance but also give capacity to resist hypoxia, ischemia and inflammation (DiDomenico *et al.,* 1982). Further, the response of individual to heat stress depends on the variables such as species, and breed, duration of exposure, severity of stress and the type of immune response (Kelley *et al.,* 1982). Schimidt and Abdulla (1988) found that when incubation temperature of lymphocyte was increased, it resulted in reduced biosynthesis of P35, an IL-1 precursor protein, in human blood adherent monocytes, whereas heat shock proteins (HSP70 and HSP90) synthesis was increased.

CHAPTER III

MATERIALS AND METHODS

Detailed accounts of animals needed for the study, plan of study, procedures and methods adopted to carry out the present investigation are described below.

The experimental design was approved by the Institutional Animal Ethics Committee, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022.

3.1 PLACE OF WORK

The present study was carried out at the Department of Veterinary Physiology and AICRP on Pig, College of Veterinary Science, Khanapara, Guwahati and National Research Centre on Pig, Rani.

3.2 PERIOD OF WORK

The experimental study was carried out during two different seasons: Summer (June, July & August, 2014) and winter (December, 2013 & January and February 2014)

3.3 EXPERIMENTAL DESIGN

The present experiment included 36 nos. of weaned, healthy and uniform sized crossbred (Hampshire X Assam local) female pigs (Fig. 3.1). Eighteen (18) animals were subjected to treatment separately during summer and winter. The selected animals were divided into three groups with six pigs in each group consisting of the control group (Treatment 1), animals of one group was fed melatonin (Meloset) @3 mg/animal (Treatment 2) and the other group was fed Vitamin E (Evion) @100 mg (Treatment 3) for both the seasons. The animals were fed as per standard feeding practices of the farm. For identification of the animals, numbers were imprinted by trimming the body hairs.

After the end of three months of treatment period for both summer and winter season the animals were observed till furrowing to determine the litter size.

3.4 PHYSIOLOGICAL PARAMETERS

Rectal temperature was recorded with a digital thermometer by keeping the thermometer in contact with the rectal mucosa for about 2 minutes and expressed in

degree celsius. Pulse rate was recorded by feeling the coccygeal artery and expressed in min^{-1}. Respiration rate were recorded by visual observation of the inward and outward abdominal movement and expressed in min^{-1}. The datas were recorded twice daily at weekly interval at 7 AM in the morning and at 4.00 PM in the evening and average was calculated as the final reading. Care was taken to induce minimum disturbance to the animals.

To determine the age at puberty boar was introduced to the individual animals and the behavioral and physical signs were observed.

3.5 TEMPERATURE-HUMIDITY INDEX (THI)

Temperature-Humidity Index was calculated out from the data of ambient temperature and relative humidity by using the formula of Mader *et al.* (2006). The dry bulb temperature and relative humidity were recorded daily from June to August, 2014 and December 2013 to February 2015 from the Automatic Weather Station (AWS) installed in the College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, where the experimental animals were reared. Temperature-Humidity Index was calculated for the entire period using the following formula:

$$THI = (0.8 \times Tdb) + [(RH/100) \times (Tdb - 14.4)] + 46.4$$

3.6 BLOOD COLLECTION

About 5 ml of blood was collected (Fig. 3.2) from each experimental animal aseptically at 15 days interval for the whole experimental period. One part of the blood sample was kept in the anticoagulant vial for estimation of Heat Shock Proteins (HSPs), haematological parameters, LDH and SOD enzyme. The other part of the blood was allowed to coagulate and serum was separated and stored at $-20^{o}C$ for estimation of hormones.

3.7 BODY WEIGHT

Body weight of the animals were measure in digital balance (Fig. 3.3) till four months after weaning.

Fig. 3.1. ANIMALS MAINTAINED IN THE FARM

Fig. 3.2. BLOOD COLLECTION

Fig. 3.3. BODY WEIGHT MEASUREMENT IN THE DIGITAL BALANCE

3.8 ESTIMATION OF HAEMATOLOGICAL PROFILE

The haematological parameters *viz*., Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) were estimated from fresh blood by using MS4 Automated Haematological Cell Counter.

3.9 ESTIMATION OF ENZYME ACTIVITY

The enzyme superoxide dismutase (SOD) and Lactate Dehydrogenase (LDH) was estimated by using SOD and LDH assay kit manufactured by Cayman Chemical Company, USA as per manufactures protocol. The estimation used 96 wells ELISA plates namely Dynamica, Halo MPR 96 visible Microplate Readers (Australia). The results obtained were expressed in U/ml for SOD and U/L for LDH.

3.10 ESTIMATION OF VITAMIN-E

Vitamin E was estimated by Ferric Chloride Dipyridyl Method (Emmerie-Engel Reaction). The absorbancy was determined at 520mμ. The difference between the absorbancies of the control sample and the test sample was converted to mg of tocopherol.

3.11 HORMONE ASSAY

The level of thermal stress related blood hormones such as triiodothyronine (T_3), thyroxine (T_4) and cortisol were estimated by Radioimmunoassay (RIA) technique using RIA kits supplied by Immunotech, France. The tracer I-125 was used in the estimation technique which involved competition between free and isotope tagged hormones for binding to the limited antibody sites and subsequently quantification was made through calibration curve. The estimation used 6 well Automatic gamma counter procured from Stratec W. Germany. The intra and inter assay co-efficient of variation were found to be 6.3 per cent and 7.7 per cent for Triiodothyronine, 6.2 per cent and 8.6 per cent for thyroxine and 5.8 per cent and 9.2 per cent for cortisol.

Growth hormone and Progesterone were estimated by ELISA technique using Elisa kits procured from LDN Immunoassays and services. Melatonin was estimated by ELISA technique using Elisa kits procured from, Genway, Biotech Inc.

3.12 ESTIMATION OF HSP70 BY REAL TIME PCR

Procedure

a) RNA protect Animal Blood Tubes (Qiagen make) were used for collection and RNA stabilization of blood.

i) The tubes were stored at room temperature (15 – 25 °C) and labelled.

ii) After collection of blood from the animals, 500 µl blood was added immediately to RNA protect Animal Blood Tube.

iii) The tubes were immediately closed and gently inverted 8-10 times. To ensure efficient cell lysis the tubes were kept at 15-25 °C for at least 2 hours and stored upright at -20 °C until estimation was done.

b) mRNA preparation

i) The RNAprotect Animal blood Tubes was centrifuged for 3 min at 5000xg and the blood was transferred from the tube (100 µl) to a new 1.5 ml collection tube.

ii) The supernatant was removed and 1 ml RNase free water was added to the pellet.

iii) The pellet was dissolved by vortexing and centrifuged for 3 min at 5000 x g.

iv) The entire supernatant was removed by decanting or pipetting and discarded.

v) 240 µl of buffer RSB was added and the pellet was dissolved by vortexing.

vi) Pipetting of the sample was done into a supplied 1.5 ml tube. 200 µl of buffer RBT and 20 µl proteinase K was added and mixed properly. Incubation was done for 10 min at 55 °C in a shaker incubator at 400-1400 rpm. After incubation, the temperature was set to 65 °C.

vii) Pipetting of the sample was done into a QIAshredder spin column placed in a 2 ml collection tube and centrifuged for 3 min at full speed.

viii) The flow-through supernatant was carefully transferred to a new 1.5 ml collection tube without disturbing the pellet.

ix) 240µl ethanol (96-100%) was added and mixed by vortexing.

x) Pipetting of the sample was done into an RNeasy MinElute spin column placed in a 2 ml collection tube and centrifuged for 1 min.

xi) 350µl of buffer RW1 was added to the spin column and centrifuged for 15 sec.

xii) 10 µl of DNase I stock solution was added to 70 µl buffer RDD in a 1.5 ml microcentrifuge tube and mixed properly.

xiii) Pipetting of the DNase I incubation mix (80 µl) was done directly onto the spin column membrane and incubated at 20-30 °C for 15 min.

xiv) 350 µl buffer RW1 was added to the spin column and centrifuged for 15 sec.

xv) 500 µl buffer RPE was added to the spin column and centrifuged for 15 sec.

xvi) 500 µl of 80% ethanol was added to the spin column and centrifuged for 2 min.

xvii) The spin column was placed in a new 2 ml collection tube and centrifuged at full speed for 5 min.

xviii) The spin column was placed in a new 1.5 ml collection tube. 14-30 µl of buffer REB was pipetted directly onto the membrane and centrifuged for 1 min.

xix) Incubation of RNA elute was done for 5 min at 65 °C in a shaker incubator without shaking. After incubation the RNA elute was chilled immediately on ice.

c) cDNA preparation

cDNA was prepared by using Verso cDNA synthesis Kit (Thermo Scientific) following manufacturer's protocol.

The volume of each component was for a 20 µl final reaction.

	Volume	Final concentration
5 x cDNA synthesis buffer	4 µl	1x
dNTP mix	2 µl	500 µM each
RNA primer	1 µl	
RT enhancer	1 µl	
Verso Enzyme Mix	1 µl	
Template (RNA)	1-5 µl	1ng
Water, nuclease free	To 20 µl	
Total volume	20 µl	

Reverse transcription cycling program

	Temperature	Time	Number of cycles
cDNA synthesis	42°C	30 min	1 cycle
Inactivation	95 °C	2 min	1 cycle

PCR

PCR (Fig. 3.4) for HSP 70 (Fig. 3.5) and GAPDH (Fig. 3.6) genes was carried out to check the specificity of primers used and confirm the size of the amplicons.

PCR Composition

10X Taq DNA polymerase buffer (with 15 mM Mg^{+2})	2.5 µl
Forward Primer (10 pmol)	1.0 µl
Reverse Primer (10 pmol)	1.0 µl
dNTPs mix (10 mM each)	2.5 µl
Cdna	1.0 µl
Taq Polymerase	0.2 µl
Nuclease free water	16.8 µl
Total	25 µl

PCR Cycling Condition

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	95	10 min.	1
Denaturation	95	15 sec.	35
Annealing	60	30 sec.	
Extension	72	30 sec.	
Final extension	72	5 min.	1

Primer sequences

The following primers were used for qPCR.

Sl. No	**Primer Sequence (5' – 3')**	**Accession No.**	**Amplicon size**
1.	HSP 70 Forward Primer GCCCTGAATCCGCAGAATA	HM025989.2	152 bp
2.	HSP 70 Reverse Primer TCCCCACGGTAGGAAACG		
7.	Primer GAPDH Forward GAAGGTCGGAGTGAACGGAT	Lallawmkimi *et al*., 2013	149 bp
8.	Primer GAPDH Reverse CATGGGTAGAATCATACTGGAACA		

Poly Acrylamide Gel Electrophoresis (PAGE)

Confirmation of specific products for HSP 70 and GAPDH genes were done using Poly Acrylamide Gel Electrophoresis (PAGE)**.** The gel was run until the marker dyes migrated the desired distance followed by silver staining procedure. The bands were visualized under UV light using white light converter and recorded in a gel documentation system (Gel Doc XR + BioRad, USA).

qPCR

qPCR of HSP70 was carried out using SYBR Green Chemistry in Applied Biosystem Step One Plus platform. GAPDH was used as internal control. All samples were prepared in triplicate. Reactions were performed to a total volume of 20µl. Reaction mixture and Non-Template Control (NTC) comprised of the following:

Components	Volume (µl)	Non-Template Control (NTC) (µl)
SYBR Green Master Mix (2x)	10	10
Forward Primer	1	1
Reverse Primer	1	1
cDNA (0.002-.2 µg/ µl)	2	-
Nuclease Free Water	6	8
Total	20	20

The thermal cycling conditions were set using the PCR thermal-cycling conditions specified in the following table:

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	95	10 min.	HOLD
Denaturation	95	15 sec.	40
Annealing	60	30 sec.	
Extension	72	30 sec.	

For melt curve analysis: 1 minute at 95°C, followed by ramping down to 60°C, collect fluorescence data continuously over the temperature range (60-95°C)

3.13 STATISTICAL ANALYSIS

The data were analysed as per the method of Snedecor and Cochran (1994) by using SAS Enterprise guide (as per version 9.3).

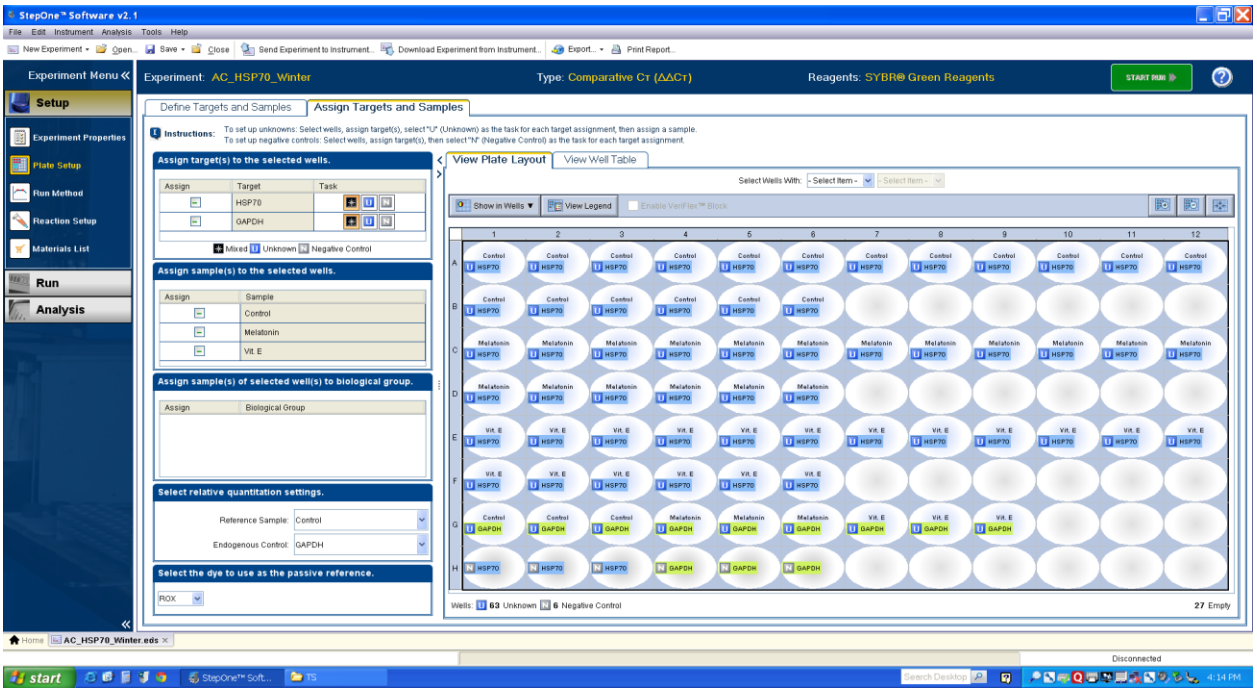

Fig. 3.4. PCR plate set up

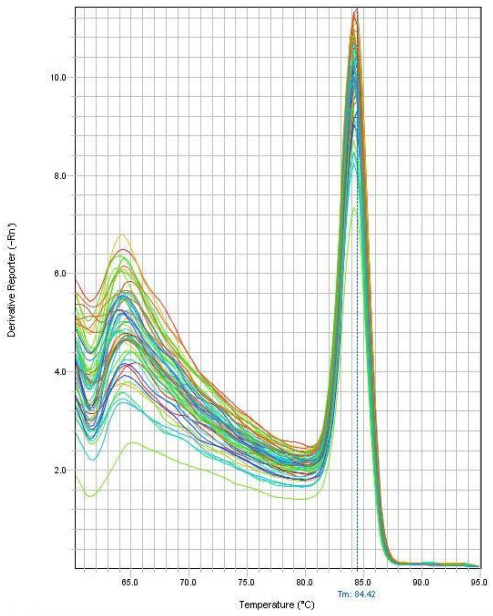

Fig. 3.5. Melt curve of HSP 70 gene

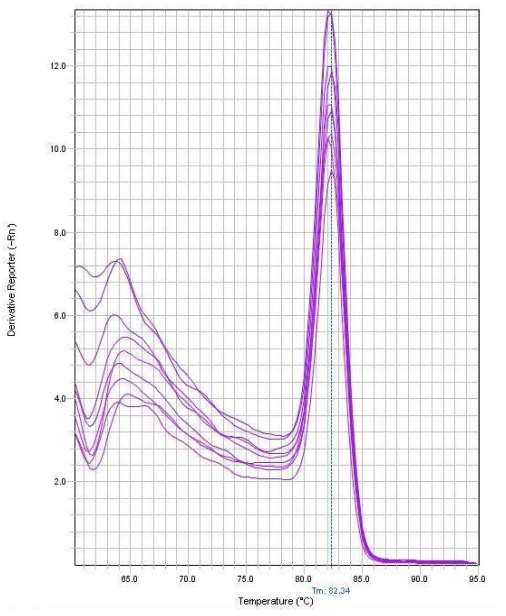

Fig. 3.6. Melt curve of GAPDH gene

CHAPTER IV

RESULTS AND DISCUSSION

4.1 RELATIVE HUMIDITY, TEMPERATURE AND TEMPERATURE HUMIDITY INDEX (THI)

The mean relative humidity, temperature and temperature humidity indexes in different months of two different seasons are presented in table 4.1 and 4.2 and Fig. 4.1.

All the three parameters except relative humidity were found significantly different (P<0.01) between seasons i.e., temperature and the temperature humidity index was more in summer than in winter, whereas within seasons they were not significant.

TABLE 4.1. RELATIVE HUMIDITY, TEMPERATURE AND TEMPERATURE HUMIDITY INDEX (THI) (MEAN± SE) DURING SUMMER AND WINTER SEASON

Season	RH (%)	T (^{o}C)	THI
	Mean± SE	Mean± SE	Mean± SE
Summer	81.97±1.12	29.28±0.30	82.01±0.50
Winter	68.75±5.98	17.91±0.14	63.16±0.30

TABLE 4.2. ANOVA FOR RELATIVE HUMIDITY, TEMPERATURE AND TEMPERATURE HUMIDITY INDEX (THI) DURING SUMMER AND WINTER SEASON

Parameters	Source of Variation	Sum of Squares	df	Mean Square	F	P Value
Humidity	Between Season	261.888	1	261.888	4.714	0.096^{NS}
	Within Season	222.233	4	55.558		
	Total	484.121	5			
Temperature	Between Season	193.688	1	193.688	1163.757	<0.001**
	Within Season	0.666	4	0.166		
	Total	194.354	5			
THI	Between Season	532.984	1	532.984	1058.593	<0.001**
	Within Season	2.014	4	0.503		
	Total	534.998	5			

**P(<0.001)

The present findings are in close relation to those reported by Kadzere *et al.* (2002). They reported that THI level beyond 72 was indicative of mild heat stress, THI 75 to 78 denoted stressful condition and that beyond 78 could indicate severe stress due to heat and humidity. Antonio and Andres (2003) observed that Temperature and humidity conditions affected livestock production in Central Argentina. This study evaluated the risk of thermal stress affecting dairy production. The temperature-humidity index (THI) was used to analyze the regional and seasonal effects of temperature and humidity. Statistically, the THI was found to be normally distributed. The probability of occurrence of a daily THI higher than 72 was 40 per cent for Río Cuarto during January. Regional variability of THI indicated a low risk of harmful extreme thermal stress conditions. The probability of THI being 78 or above ranges between 4 and 10 per cent for the main dairy region of Córdoba during January. Davis and Mader, 2002 reported that the Temperature-Humidity Index (THI) is a suitable climatic marker to correlate climatic stress on physiology and productivity of animals and also a reliable tool for effective management of livestock under different climatic condition. Stress is a reaction of body to stimuli that disturb homeostasis often with detrimental effects. Domestic animals undergo various kinds of stress such as physical, nutritional, chemical, psychological and thermal stress. Thermal stress is the perceived discomfort and physiological strains associated with an exposure to an extreme hot or cold environment. Thermal stress includes both heat stress during extreme summer season as well as cold stress during extreme winter season. In tropical and subtropical regions high ambient temperature is the major constraint on animal production (Marai *et al.*, 2007), whereas extreme low temperature in temperate regions is also detrimental to the livestock. The effect of high temperature is further aggravated when heat stress is accompanied by high ambient humidity. In hot climates, high ambient temperature, humidity, wind speed and high direct and indirect solar radiation are the main environmental stressing factors that impose strains on animals (Silanikove, 2000). Excessive heat stress may cause hyperthermia and potentially have several physiological side effects. These include electrolyte imbalances (West *et al.*, 1991), oxidative stress and enzymatic dysfunction (David *et al.*, 2001), aberration of reproductive functions (Roth *et al.*, 2002), reduced meat quality (Kadim *et al.*, 2004) and eventually severe economic losses resulting from increased mortalities and decreased overall animal performance.

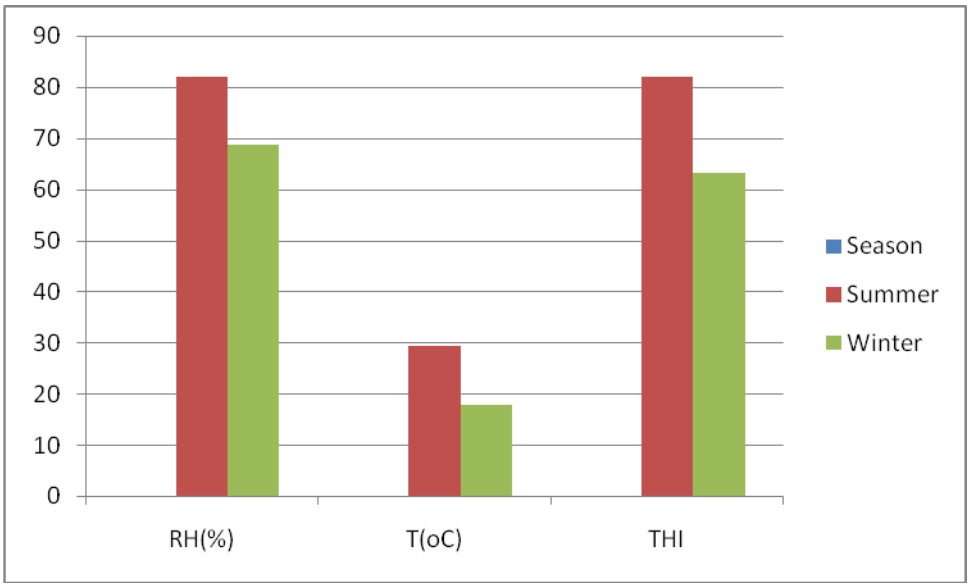

Fig. 4.1. RELATIVE HUMIDITY, TEMPERATURE AND TEMPERATURE HUMIDITY INDEX (THI) DURING SUMMER AND WINTER SEASON

4.2 PHYSIOLOGICAL PARAMETERS

4.2.1 Rectal Temperature (°C)

The mean rectal temperature in the three treatment groups during different seasons (summer and winter) are presented in Table 4.3 and 4.4 and Fig 4.2 .The mean rectal temperature during summer in the three treatment groups was found to be 39.163±0.0255 in treatment group 1, 39.103±0.0198 in treatment group 2 and 39.118±0.0204 in treatment group 3. On the other hand the mean rectal temperature was lower during winter in the three treatment groups and it was found to be 38.776±0.0092 in treatment group 1, 38.807±0.0063 in treatment group 2 and 38.813±0.0064 in treatment group 3. Statistical analysis revealed significant difference ($P<0.01$) in the rectal temperature between seasons. There was also significant difference ($P<0.01$) between the treatment and season.

TABLE 4.3. RECTAL TEMPERATURE (ºC, MEAN± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	39.163±0.0255[a]	38.776±0.0092[b]	38.970±0.0158[a]
2	39.103±0.0198[a]	38.807±0.0063[b]	38.955±0.0121[a]
3	39.118±0.0204[a]	38.813±0.0064[b]	38.965±0.0124[a]
AGGREGATE	39.128±0.0127[**a**]	38.798±0.0043[**b**]	38.963±0.0078

Values having same superscript do not differ significantly

TABLE 4.4. ANOVA FOR RECTAL TEMPERATURE IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	0.659	5	0.132	1.692	0.133
Treatment	0.066	2	0.033	0.423	0.655
Error(Treatment)	0.526	10	0.053		
Season	46.939	1	46.939	602.224	0.000
Treatment × Season	0.720	2	0.360	4.620	0.010
Error (Season)	133.049	1707	.078		
Total	181.959	1727			

In the present study the rectal temperature during summer was found to be 39.163±0.0255 in treatment group 1, 39.103±0.0198 in treatment group 2 and 39.118±0.0204 in treatment group 3 .On the other hand the mean rectal temperature was lower during winter in the three treatment groups and it was found to be 38.776±0.0092 in treatment group 1, 38.807±0.0063 in treatment group 2 and 38.813±0.0064 in treatment group 3.

The present findings are in close accordance with the findings reported by Huynh *et al*. (2005) who reported that the rectal temperature was affected by increasing

temperature. Until the temperature reached 26.1˚C, the rectal temperature of pigs was constant at an average of 39.3˚C. Above that inflection point, it increased 0.13˚C/˚C. Also the present findings are in close proximity with the findings reported by Quiniou and Noblet (1999) who reported that the rectal temperature in the multiparous lactating sows was found constant between 18 and 22˚C (38.6˚C) but it increased at higher temperature (39-39.4˚C between 25 and 29˚C). Rectal temperature increases when the physiological mechanism of an animal fails to negate the excessive heat load. It might be due to temperature associated with the season which could exceed the comfort zone of the animals, resulting in imbalance in the heat energy produced and dissipated.

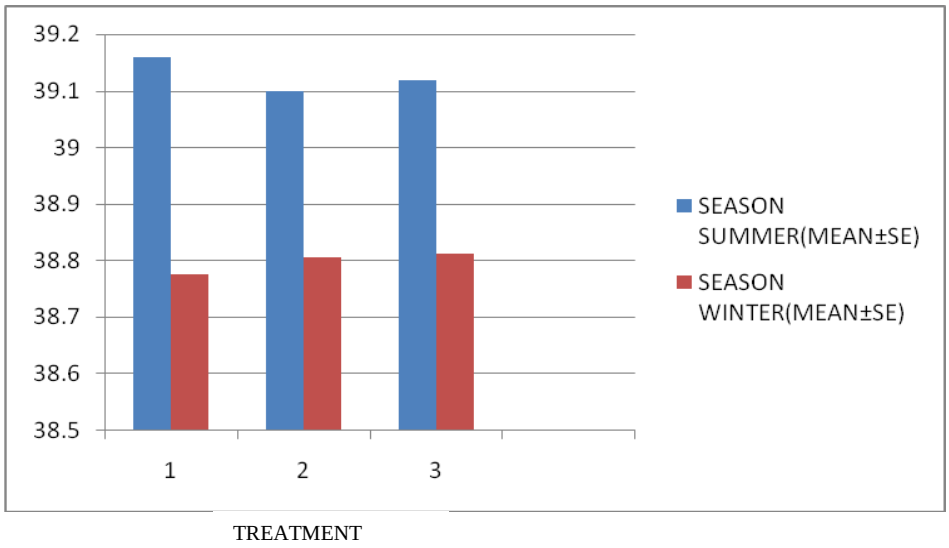

Fig. 4.2. RECTAL TEMPERATURE (ºC) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.2.2 Respiration Rate (breaths/min)

The mean respiration rate in the three treatment groups during summer and winter are presented in Table 4.5 and 4.6 and Fig 4.3 The mean respiration rates during summer was found to be 61.99±0.380 in treatment group 1, 62.47±0.375 in treatment group 2 and 61.82±0.359 in treatment group 3. On the other hand the mean respiration rate was lower during winter in the three treatment groups and it was found to be 43.52±0.234 in treatment group 1, 43.31±0.221 in treatment group 2 and 43.24±0.208 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean respiration rate between seasons.

TABLE 4.5. RESPIRATION RATE (BREATHS/MIN, MEAN± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	61.99±0.380[a]	43.52±0.234[b]	52.76±0.445[a]
2	62.47±0.375[a]	43.31±0.221[b]	52.89±0.455[a]
3	61.82±0.359[a]	43.24±0.208[b]	52.53±0.439[a]
AGGREGATE	62.09±0.214[**a**]	43.36±0.128[**b**]	52.73±0.258

Values having same superscript do not differ significantly

TABLE 4.6. ANOVA FOR RESPIRATION RATE IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	284.940	5	56.988	2.122	0.060
Treatment	37.293	2	18.646	0.694	0.500
Error(Treatment)	207.409	10	20.741		
SEASON	151631.348	1	151631.348	5646.405	0.000
Treatment * SEASON	39.918	2	19.959	0.743	0.476
Error (Season)	45840.620	1707	26.854		
Total	198041.527	1727			

In the present study the mean respiration rates during summer was found to be 61.99 ± 0.380 in treatment group 1, 62.47 ± 0.375 in treatment group 2 and 61.82 ± 0.359 in treatment group 3 whereas it was lower during winter in the three treatment groups and it was 43.52 ± 0.234 in treatment group 1, 43.31±0.221 in treatment group 2 and 43.24 ± 0.208 in treatment group 3.

The present findings are in close proximity with the findings of Huynh *et al.* (2005) who reported that with increasing temperature respiration rate remained constant at an average of 32 breaths / min until the inflection point (on average 22˚C) which increased by an average of 13 breaths /min/˚C in pigs. Similar findings have been reported by Quiniou and Noblet (1999) who found that respiration rate in multiparous lactating sows increased from 26 to 124 breaths/min between 18 and 29˚C and this indicates that the evaporative critical temperature was below 22˚C. The increase or decrease in respiration rates is an adaptive mechanism of an animal to maintain homoeothermy. In domestic animals respiration rate increases due to the activation of thermoreceptors in the skin when they are exposed to higher ambient temperature (Hafez, 1968). Such activation of the receptors in turn sends neural signals to the hypothalamus that increases respiratory activity to accelerate heat loss from the body by respiratory evaporation (Al-Haidary, 2004). An evaporative heat loss from the respiratory tract is regarded as one of the primary mechanism for maintenance of heat balance to maintain the internal body temperatures (Marai *et al.*, 2007)

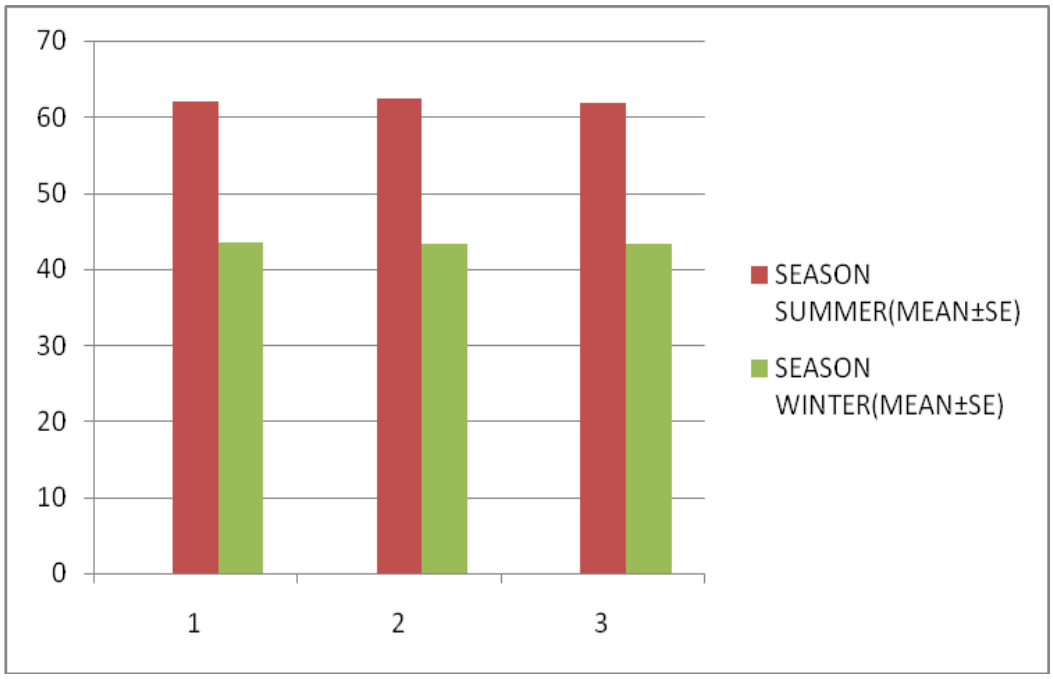

Fig. 4.3. RESPIRATION RATE (BREATHS/MIN) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.2.3 Pulse Rate (Beats/Min)

The mean pulse rate in the three treatment groups during summer and winter are presented in Table 4.7 and 4.8 Fig. 4.4. The mean pulse rate during summer was found to be 75.09 ± 0.129 in treatment group 1, 75.27 ± 0.123 in treatment group 2 and 75.77 ± 0.122 in treatment group 3. On the other hand the mean pulse rate was comparatively lower during winter in the three treatment groups and it was found to be 71.98 ± 0.084 in treatment group 1, 72.65 ± 0.103 in treatment group 2 and 72.50 ± 0.115 in treatment group 3. Statistical analysis revealed significant difference ($P<0.01$) in the mean pulse rate between treatment and between seasons. There was also significant difference ($P<0.05$) in the mean pulse rate between treatment and season.

TABLE 4.7. PULSE RATE (BEATS/MIN, MEAN ± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	75.09 ± 0.129^{b}	71.98 ± 0.084^{d}	73.53 ± 0.101^{b}
2	75.27 ± 0.123^{b}	72.65 ± 0.103^{c}	73.96 ± 0.097^{a}
3	75.77 ± 0.122^{a}	72.50 ± 0.115^{c}	74.13 ± 0.108^{a}
AGGREGATE	$75.37\pm0.073^{\mathbf{a}}$	$72.38\pm0.059^{\mathbf{b}}$	73.88±0.059

Values having same superscript do not differ significantly

TABLE 4.8. ANOVA FOR PULSE RATE IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	192.331	5	38.466	10.803	.000
Treatment	109.404	2	54.702	15.363	.000
Error (Treatment)	145.179	10	14.518		
Season	3882.002	1	3882.002	1090.238	.000
Treatment × Season	32.480	2	16.240	4.561	.011
Error (Season)	6078.101	1707	3.561		
Total	**10439.498**	**1727**			

In the present study the mean pulse rate during summer in the three treatment groups was 75.37 ± 0.073 whereas in winter it was 72.38 ± 0.059.

The present findings are in close proximity with the findings reported by 4-H youth Development, Extension Service West Virginia University (2010) who reported the pulse rate in pigs in a range of 60 to 80 per minute. Similar pulse rate was reported by Jackson and Cockroft (2014). They reported a pulse rate of 60-90 beats/min adult pigs but also suggested that the rates increase rapidly if the animals are stressed. The concomitant increase in the pulse rate along with the RR and the RT could be considered as an intrinsic physiologic mechanism. It reflects the homeostasis of circulation along with the general metabolic status.

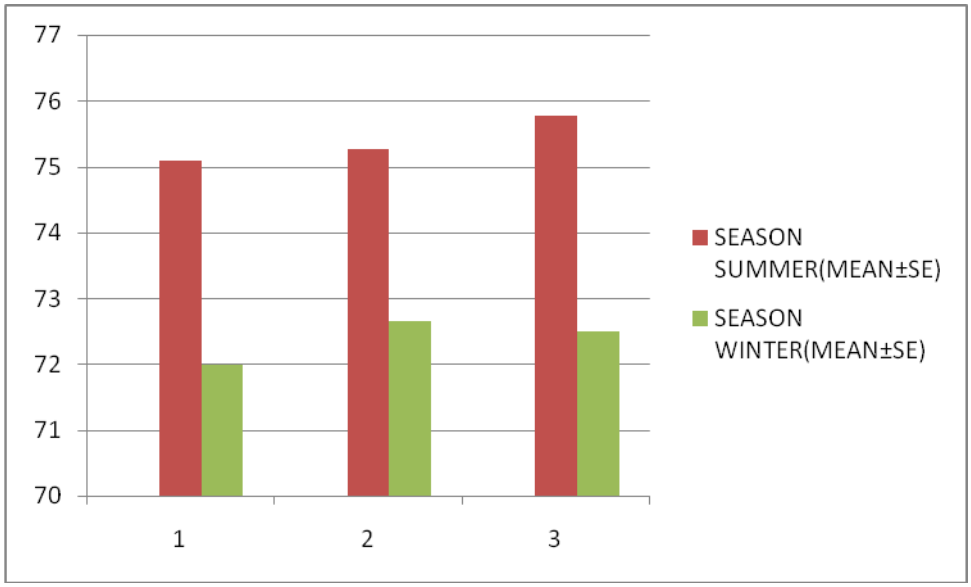

FIG. 4.4. PULSE RATE (BEATS/MIN) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.3 HEMATOLOGICAL PARAMETERS

4.3.1 HEMOGLOBIN

The mean hemoglobin concentration in the three treatment groups during summer and winter are presented in Table 4.9 and 4.10 and Fig 4.5. The mean haemoglobin concentration during summer was found to be 10.91±0.14 in treatment group 1, 11.13 ±

0.14 in treatment group 2 and 11.00 ± 0.13 in treatment group 3. On the other hand the mean hemoglobin concentration was comparatively high during winter in the three treatment groups and it was found to be 14.45 ± 0.10 in treatment group 1, 14.11 ± 0.07 in treatment group 2 and 14.33 ± 0.08 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean haemoglobin concentration between treatment. There was also significant difference ($P<0.05$) in the mean haemoglobin concentration between treatment and season.

TABLE 4.9. SERUM HEMOGLOBIN (g/dl, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	10.91 ± 0.14^{b}	14.45 ± 0.10^{a}	12.68 ± 0.23^{a}
2	11.13 ± 0.14^{b}	14.11 ± 0.07^{a}	12.62 ± 0.19^{a}
3	11.00 ± 0.13^{b}	14.33 ± 0.08^{a}	12.66 ± 0.21^{a}
AGGREGATE	$11.01\pm0.08^{\mathbf{b}}$	$14.30\pm0.05^{\mathbf{a}}$	12.65±0.12

Values having same superscript do not differ significantly

TABLE 4.10. ANOVA FOR HEMOGLOBIN CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	5.239	5	1.048	2.372	0.041
Treatment	0.142	2	0.071	0.161	0.852
Error (Treatment)	6.037	10	0.604		
Season	581.938	1	581.938	1317.484	<0.001
Treatment × Season	2.728	2	1.364	3.088	0.048*
Error (Season)	86.132	195	0.442		
Total	682.216	215			

In the present study the mean haemoglobin concentration during summer was 10.91±0.14 in treatment group 1, 11.13±0.14 in treatment group 2 and 11.00±0.13 in treatment group 3 whereas it was comparatively higher during winter and it was 14.45±0.10 in treatment group 1, 14.11±0.07 in treatment group 2 and 14.33±0.08 in treatment group 3.

The present findings show a slight variability with the findings of Korzeniowska *et al*. (2012). He reported a higher concentration of hemoglobin in the 1st stage of summer season than the winter season in the fattening pigs. Although Gyo-Moon Chu and Young-Min Song (2013) reported that the hemoglobin concentration was not affected much by seasonal variation. In summer it was 13.28 gdL^{-1} , in spring it was 13.33 gdL^{-1} , in autumn it was 13.19 gdL^{-1} and in winter it was found to be 12.78 gdL^{-1} in the fattening pigs, which are in close association with the present findings. On the other hand Mayengbam *et al.* (2012) reported a variation in the haemoglobin concentration in different age groups of Zovawk pigs. They found that the average hemoglobin concentration in Zovawk pigs were 13.41±0.30 in the pre-weaning group, 14.48±0.54 in the grower group and 15.15±0.70 in the adult group. Adenkola *et al.* (2011) illustrated the effect of adverse meteorological condition on the haemoglobin concentration of pigs. He reported that the hemoglobin concentration (g%) in pigs in hot-dry and harmattan season differed significantly ($p<0.05$) with values of 13.2±0.6 and 10.64±0.3 respectively

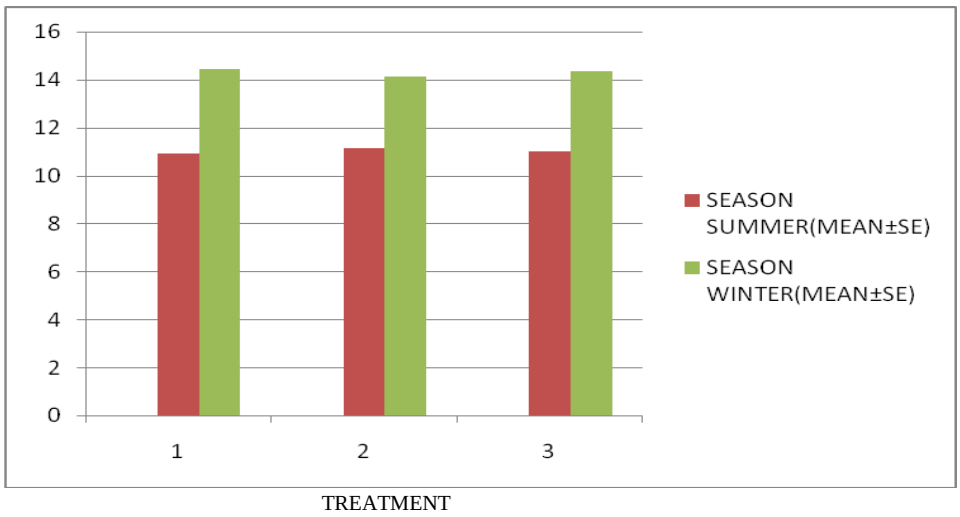

Fig. 4.5. HEMOGLOBIN (g/dl) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.3.2 Packed Cell Volume (PCV) (%)

The mean PCV % in the three treatment groups during summer and winter are presented in Table 4.11 and 4.12 and Fig 4.6. The mean PCV % during summer was found to be 30.81 ± 0.15 in treatment group 1, 32.00 ± 0.17 in treatment group 2 and 32.38 ± 0.16 in treatment group 3. On the other hand the mean PCV% was comparatively high during winter in the three treatment groups and it was found to be 43.42±0.25 in treatment group 1, 43.12 ± 0.26 in treatment group 2 and 43.01±0.23 in treatment group 3.Statistical analysis revealed significant difference (P<0.01) in the mean PCV% between treatment and between season. There was also significant difference (P<0.01) in the mean PCV % between treatment and season.

TABLE 4.11. PCV (%, MEAN±SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	30.81±0.15^{c}	43.42±0.25^{a}	37.11±0.76^{b}
2	32.00±0.17^{b}	43.12±0.26^{a}	37.56±0.68^{ab}
3	32.38±0.16^{b}	43.01±0.23^{a}	37.69±0.65^{a}
AGGREGATE	31.73±0.11$^{\mathbf{b}}$	43.18±0.14$^{\mathbf{a}}$	37.46±0.40

Values having same superscript do not differ significantly

TABLE 4.12. ANOVA FOR PCV IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	10.325	5	2.065	1.486	0.196
Treatment	13.310	2	6.655	4.790	0.009**
Error (Treatment)	42.105	10	4.211		
Season	7080.909	1	7080.909	5097.036	<0.001**
Treatment × Season	38.008	2	19.004	13.680	<0.001**
Error (Season)	270.898	195	1.389		
Total	7455.556	215			

In the present study the mean PCV % during summer was found to be 30.81±0.15 in treatment group 1, 32.00±0.17 in treatment group 2 and 32.38±0.16 in treatment group 3 whereas it was comparatively high during winter with values of 43.42±0.25 in treatment group 1, 43.12±0.26 in treatment group 2 and 43.01±0.23 in treatment group 3.

Similar findings were reported by Mayengbam *et al*. (2014) who reported that the PCV percentage in the Zovawk pigs did not change significantly from the preweaning stage (45.00±1.00) till the growing stage (47.00±1.58) but increased significantly in adults (57.89±1.52). Also the findings reported by Adenkola *et al.* (2011) were similar to the findings in the present study. He reported that the PCV% in the hot-dry and harmattan season in pigs were 39.7±1.9 and 32.00±0.9 and they differed significantly. The present findings are also in close proximity with the findings reported by Onunkwo *et al.* (2010) who reported that the PCV values in pigs raised under intensive management system was found to be 31.36±7.1 in piglets and 35.81±10.10 in adults. Adenkola *et al.* (2009) recorded the PCV values on day 7 after the journey rose to 37.38±1.39 % in the experimental pigs and the value was significantly ($P<0.05$) higher than 33.54±1.01% recorded in the control pigs post transportation in the harmattan season. De *et al.* (2013) reported a comparative PCV % in different pig breeds of Andaman and Nicobar Islands. He reported that the PCV % in Andaman wild pig as 61.3±0.56, Nicobari pig as 28.89±6.08, Andaman desi pig as 28.64±4.72 and in Large white Yorkshire as 41.35±4.46 respectively.

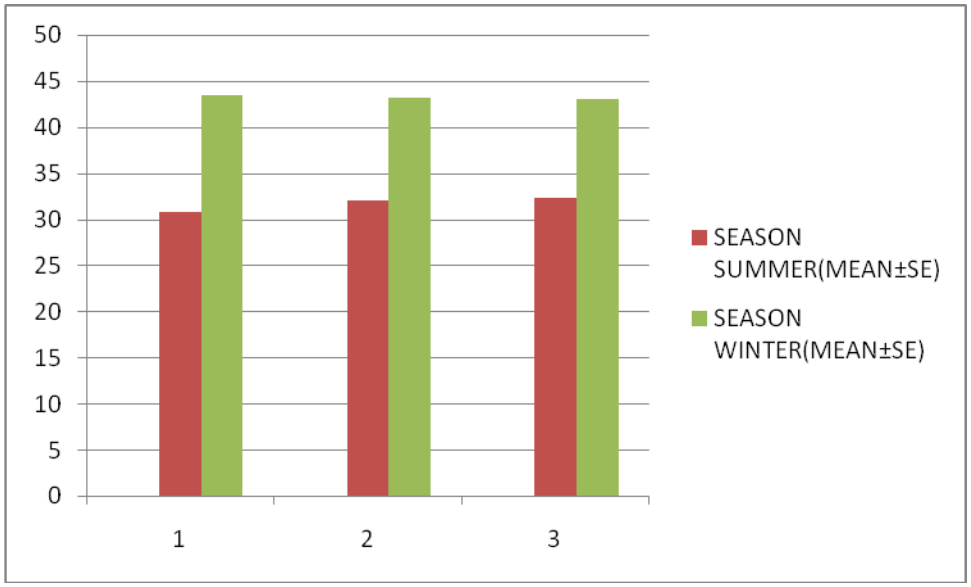

Fig. 4.6. PCV (%) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.3.3 Total Erythrocytic Count (million/cmm)

The mean TEC values in the three treatment groups during summer and winter are presented in table 4.13 and 4.14 and fig 4.7. The mean TEC values during summer was found to be 6.18±0.03 in treatment group 1, 6.02±0.04 in treatment group 2 and 5.98±0.06 in treatment group 3. On the other hand the mean TEC was comparatively high during winter in the three treatment groups and it was found to be 7.08±0.07 in treatment group 1, 7.20±0.07 in treatment group 2 and 7.13±0.07 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean TEC values between season. There was also significant difference ($P<0.01$) in the mean TEC values between treatment and season.

TABLE 4.13. TEC (million/cmm, MEAN± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	6.18±0.03^{b}	7.08±0.07^{a}	6.63±0.07^{a}
2	6.02±0.04^{bc}	7.20±0.07^{a}	6.61±0.08^{a}
3	5.98±0.06^{c}	7.13±0.07^{a}	6.56±0.08^{a}
AGGREGATE	6.06±0.03$^{\mathbf{b}}$	7.14±0.04$^{\mathbf{a}}$	6.60±0.04

Values having same superscript do not differ significantly

TABLE 4.14. ANOVA FOR TEC IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	8.591	5	1.718	26.311	<0.001
Treatment	0.198	2	0.099	1.513	0.223
Error (Treatment)	7.109	10	0.711		
SEASON	62.576	1	62.576	958.222	<0.001**
Treatment * SEASON	0.826	2	0.413	6.322	0.002**
Error (Season)	12.734	195	0.065		
Total	92.033	215			

In the present study the mean TEC value during summer was 6.18±0.03 in treatment group 1, 6.02±0.04 in treatment group 2 and 5.98±0.06 in treatment group 3 as compared to winter where the values were higher in the three treatment groups and it was found to be 7.08±0.07 in treatment group 1, 7.20±0.07 in treatment group 2 and 7.13±0.07 in treatment group 3.

The present findings are in close proximity with the findings of De *et al*. (2013) who reported a comparative TEC value in different pig breeds of Andaman and Nicobar Islands. He reported that the TEC in Andaman wild pig as 9.72±0.17, Nicobari pig as

4.52±0.93, Andaman desi pig as 5.44±0.95 and in Large white Yorkshire as 7.43±0.78 respectively. Mayengbam *et al.* (2014) reported that the TEC values in Zovawk pigs increased with age with higher significant values in the adults 11.25±0.69 in comparison to pre-weaning group 8.11±0.17 and the growers 10.25±0.29 ($P<0.05$).On the other hand Gyo-Moon Chu and Young-Min Song (2013) reported that the TEC values in the fattening pigs during summer was found to be 7.13 and it was 7.05 during the winter season.

It is a matter of general understanding that blood is the most important specialised tissue of the body that creates an open channel system to provide the equal amount of nutrients, hormones and other important factors to different organs. It is also of crucial importance for the maintenance of physiologic equilibrium in the body (Geneser, 1986).However this equilibrium may be disturbed due to certain physiological and pathological conditions. The knowledge of the haematological values is useful in diagnosing various pathological and metabolic disorders which can adversely affect the productive performance of the animals (Pyne and Maira, 1981; Dutta *et al*., 1988). Therefore, the importance of haematological indices in the animal husbandry is well acknowledged. These indices may vary depending on factors such as age, sex, weather, stress, season and physical exercise (Kaneko *et al*., 1999).

In the present investigation the mean Hb (g/dl), PCV (%) and TEC (million/cmm) was found to be significantly lower($P<0.01$) in summer compared to winter season. These variations may be attributed to the fact that high environmental temperature during summer season increases the oxygen consumption of animals through increased respiration rate. The higher oxygen intake increases the partial pressure of oxygen in the blood, decreases erythropoiesis, which in turn reduces the number of circulating RBCs and thus PCV and Hb values (Maurya *et al*., 2007). Another explanation for the decreased Hb and PCV values during thermal stress could be due to the increased attack of reactive oxygen molecules on the erythrocyte membrane, which is rich in lipid content, and ultimately lysis of RBC or inadequate nutrient availability for haemoglobin biosynthesis due to decrease voluntary intake as the animal consumes less feed during thermal stress (Srikandakumar *et al*., 2003).

During summer the decrease in the Hb values may be attributed to haemodilution where more water is infused into the circulatory system for evaporative cooling (El-Nouty *et al*., 1990). At high temperature, peripheral vasodilation and redistribution of cardiac output are associated with expansion of blood volume and resulting in haemodilution (Hales, 1973; Ganong, 2003).The lowering in the values of Hb and PCV during summer in the present study might be in part attributed to haemodilution. Vasodilation which occurs in thermal stressed animals causes a decline in the hydrostatic blood pressure below the blood colloidal pressure so that more interstitial fluid passes into the intravascular compartment (Kamal *et al*., 1972).

On the other hand the increase in the values of Hb, PCV and TEC during winter as compared to summer may be attributed in part to haemoconcentration. At low temperature peripheral vasoconstriction and redistribution of cardiac output are associated with reduction of blood volume and thereby resulting in haemoconcentration (Cunningham and Klein, 2007)

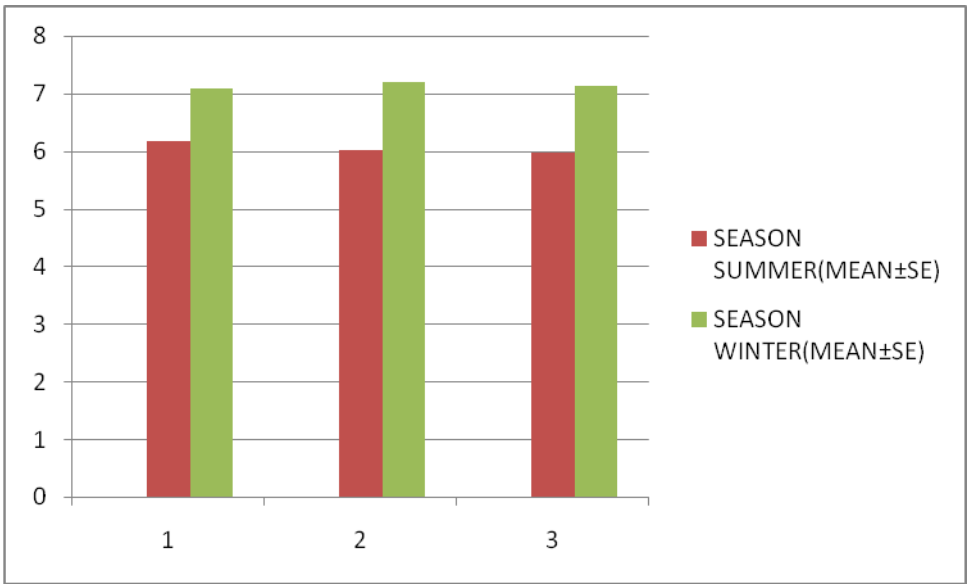

Fig. 4.7. TEC (million/cmm) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.3.4 Total Leukocytic Count (thousand/cmm)

The mean TLC values in the three treatment groups during summer and winter are presented in Table 4.15 and 4.16 and Fig 4.8. The mean TLC values during summer was found to be 22.66±0.07 in treatment group 1, 22.35±0.06 in treatment group 2 and 22.63±0.05 in treatment group 3. On the other hand the mean TLC value was comparatively low during winter in the three treatment groups and it was found to be 18.36±0.07 in treatment group 1, 18.59±0.09 in treatment group 2 and 18.40±0.07 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean TLC values between season. There was also significant difference ($P<0.01$) in the mean TLC values between treatment and season.

TABLE 4.15. TLC(thousand/cmm, MEAN±SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	22.66±0.07^{a}	18.36±0.07^{c}	20.51±0.26^{a}
2	22.35±0.06^{b}	18.59±0.09^{c}	20.47±0.23^{a}
3	22.63±0.05^{a}	18.40±0.07^{c}	20.51±0.25^{a}
AGGREGATE	22.55±0.04$^{\mathbf{a}}$	18.45±0.05$^{\mathbf{b}}$	20.50±0.14

Values having same superscript do not differ significantly

TABLE 4.16. ANOVA FOR TLC IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	df	Mean Square	F	P Value
Replication	3.954	5	0.791	6.556	<0.001
Treatment	0.094	2	0.047	0.389	0.678
Error (Treatment)	10.762	10	1.076		
SEASON	905.118	1	905.118	7503.591	<0.001**
Treatment * SEASON	3.150	2	1.575	13.056	<0.001**
Error (Season)	23.522	195	0.121		
Total	946.600	215			

In the present study the mean TLC values during summer was 22.66 ± 0.07 in treatment group 1, 22.35 ± 0.06 in treatment group 2 and 22.63±0.05 in treatment group 3. A slightly lower value trend was observed during winter with values of 18.36±0.07 in treatment group 1, 18.59±0.09 in treatment group 2 and 18.40±0.07 in treatment group 3.

The present findings are in close proximity with the findings of Gyo-Moon Chu and Young-Min Song (2013). They reported a higher TLC value in the fattening pigs during summer and it was found to be 19.40 as compared to winter where it was found to be 14.05, which varied significantly. Mayengbam *et al*. (2014) reported the TLC values in different age groups of Zovawk pigs. Theye reported that the TLC values showed significant increase from the pre-weaning stage 14.11±0.89 to the grower stage 17.71±0.59 and remained stable to the adult stage 17.68±0.84. Also Adenkola *et al*. (2011) reported the TLC values in pigs during Hot-Dry and Harmattan season in the Northern Guinea Savanna zone of Nigeria were found to be 15920.00±1119.1 and 18836.5±1727.1 which differed significantly ($P<0.05$). The impact of stress factors on the pigs was illustrated by Adenkola *et al*. (2009) who reported the TLC values in pigs transported by road for four hours during the Harmattan season. The TLC values dropped (15830±1063.08) in the experimental pigs after transportation and the values were significantly lower than the value of 22010.69±1722.00 obtained in the control pigs after the journey.

The present investigation reveals higher TLC values during summer compared to winter. Leukocytes are cells of the immune system found in the blood and lymphatic system and are involved in defending the body against both infectious diseases and foreign materials (Maton *et al*., 2008). McGlone and Pond (2002) reported that the normal TLC values in pigs increases two folds when the pigs have an active infection or stress. Therefore, the result indicates that summer months are more stressful than winter. Also the increase in the TLC values during summer may be due to the state of stress that stimulates the anterior pituitary gland to secrete ACTH. The circulating ACTH in turn induces the adrenal cortex to produce glucocorticoids, involved in the mobilisation of neutrophils from the pool into the peripheral circulation (Adenkola *et al*., 2009)

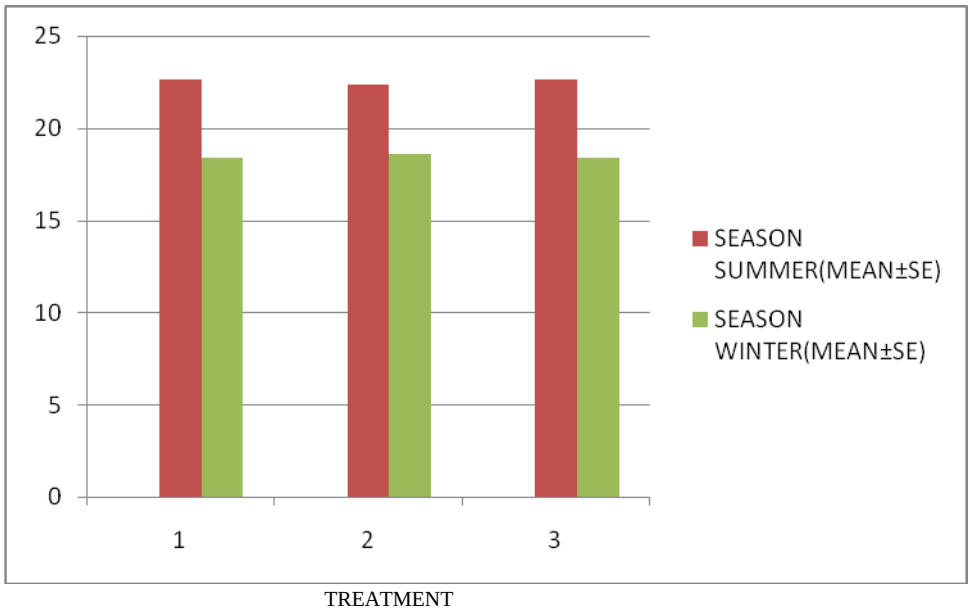

Fig. 4.8. TLC (thousand/cmm) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.4 BODY WEIGHT (KGs)

The mean body weight values in the three treatment groups during summer and winter are presented in table 4.17 and 4.18 and fig 4.9 The mean body weight value in the three treatment groups during summer was found to be 22.95 ±0.77 ^{A} whereas it was 25.60± 0.93^{B} during winter. The mean body weight values during summer and winter (aggregate) was found to be 24.09±1.05^{B} for treatment group 1, 24.38±1.06^{A} for treatment group 2 and 24.35±1.05^{A} for treatment group 3 .Statistical analysis revealed significant difference (P<0.01) in the mean body weight values between treatment and between season. There was also significant difference (P<0.01) between day and season.

TABLE 4.17. BODY WEIGHT (KG, MEAN± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Season	Treatment	Day																Aggregate	
		0		15		30		45		60		75		90		105			
		Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Summer	**1**	10.25	0.02	12.40	0.14	16.15	0.10	20.36	0.25	24.65	0.16	29.10	0.07	33.06	0.15	37.63	0.40	22.95	1.35
	2	10.32	0.07	12.99	0.13	16.34	0.15	20.86	0.32	24.55	0.16	29.49	0.15	33.62	0.24	38.94	0.11	23.39	1.39
	3	10.32	0.05	13.01	0.08	16.25	0.16	20.51	0.20	25.12	0.11	29.59	0.20	33.37	0.24	38.39	0.18	23.32	1.37
	Aggregate	**10.30**	**0.03**	**12.80**	**0.09**	**16.25**	**0.08**	**20.58**	**0.15**	**24.77**	**0.10**	**29.39**	**0.10**	**33.35**	**0.13**	**38.32**	**0.19**	**23.22**	**0.78**
Winter	**1**	10.34	0.06	12.96	0.08	17.23	0.22	21.83	0.23	26.96	0.26	32.66	0.26	37.70	0.27	42.75	0.16	25.30	1.61
	2	10.25	0.09	12.92	0.08	17.74	0.21	22.38	0.23	27.99	0.16	32.90	0.20	38.28	0.20	44.07	0.22	25.81	1.66
	3	10.15	0.17	13.02	0.10	17.92	0.12	22.67	0.15	28.11	0.23	32.71	0.28	37.98	0.32	43.18	0.21	25.72	1.62
	Aggregate	**10.25**	**0.07**	**12.97**	**0.05**	**17.63**	**0.13**	**22.29**	**0.14**	**27.69**	**0.17**	**32.76**	**0.14**	**37.99**	**0.16**	**43.33**	**0.17**	**25.61**	**0.93**
	Overall	**10.30**	**0.03**	**12.80**	**0.09**	**16.25**	**0.08**	**20.58**	**0.15**	**24.77**	**0.10**	**29.39**	**0.10**	**33.35**	**0.13**	**38.32**	**0.19**	23.22	0.78

Values having same superscript do not differ significantly

TABLE 4.18. ANOVA FOR BODY WEIGHT IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	df	Mean Square	F	P Value
Replication	1.387	5	.277	.245	0.942
SEASON	412.060	1	412.060	363.958	<.001**
Replication × Season	1.165	5	0.233	0.206	0.960
Treatment	12.442	2	6.221	5.495	0.005**
Season × Treatment	0.068	2	0.034	0.030	0.971
Day	30290.774	7	4327.253	3822.107	<0.001
Treatment × Day	8.648	14	0.618	0.546	0.904
Error	284.173	251	1.132		
Total	31010.717	287			

In the present study the mean body weight in the three treatment groups during summer was found to be 22.95 ±0.77 whereas it was 25.60± 0.93 during winter.

The present findings are in close association with the findings reported by Gyo-Moon Chu and Young-Min Song (2013). They reported that the final body weight and average daily gain in fattening pigs were significantly lower (P<0.05) in summer than in spring, autumn and winter and it was not different between spring, autumn and winter. The finished body weight in summer was 100.67 kg while that of spring, autumn and winter were 107.83, 107.17 and 107.83 kg respectively. On the other hand Korzeniowska *et al*. (2012) reported that the daily body weight gains of pigs finishing in winter season were lower as against those in the summer period by an average of 291.63 g in the first stage and 26.58 g in the second stage.

Quiniou and Noblet (1999) reported the effect of temperature on the variation in body weight. They reported an increased body weight loss in the multiparous lactating sows but its estimate chemical composition remained constant. Over the total lactation the body weight loss was significantly affected by temperature; it amounted to 23 kg at 18, 22 and 25^{o}C on average but increased up to 36 kg at 29˚C,the value at 27^{o}C being intermediate. A significant interaction between temperature and dietary treatment was

observed in connection with a lower body weight loss at 25˚C. The effect of vitamin and trace mineral supplementation on the body weight gain in growing-finishing pigs was demonstrated by Tian *et al*. (2001). He reported that in the growing-finishing pigs during the overall experimental period (0-9 weeks, 54-106 kg body weight) growth performance was not significantly affected by dietary vitamin and trace mineral levels. During the first two weeks (21 to 30 kg body weight), average daily gain in the 200% mineral supplemented group was highest and lowest in 200% water soluble supplemented group ($p<0.05$).

The body weight gain in the three treatment groups during summer was lower than in winter which may be attributed to the effect of heat stress that hampered the body weight gain in summer. It may also be due to decreased feed intake which is commonly seen when animal suffers from thermal stress to maintain homoethermy and to counteract or lower the metabolic heat production ultimately leading to decreased weight gain.

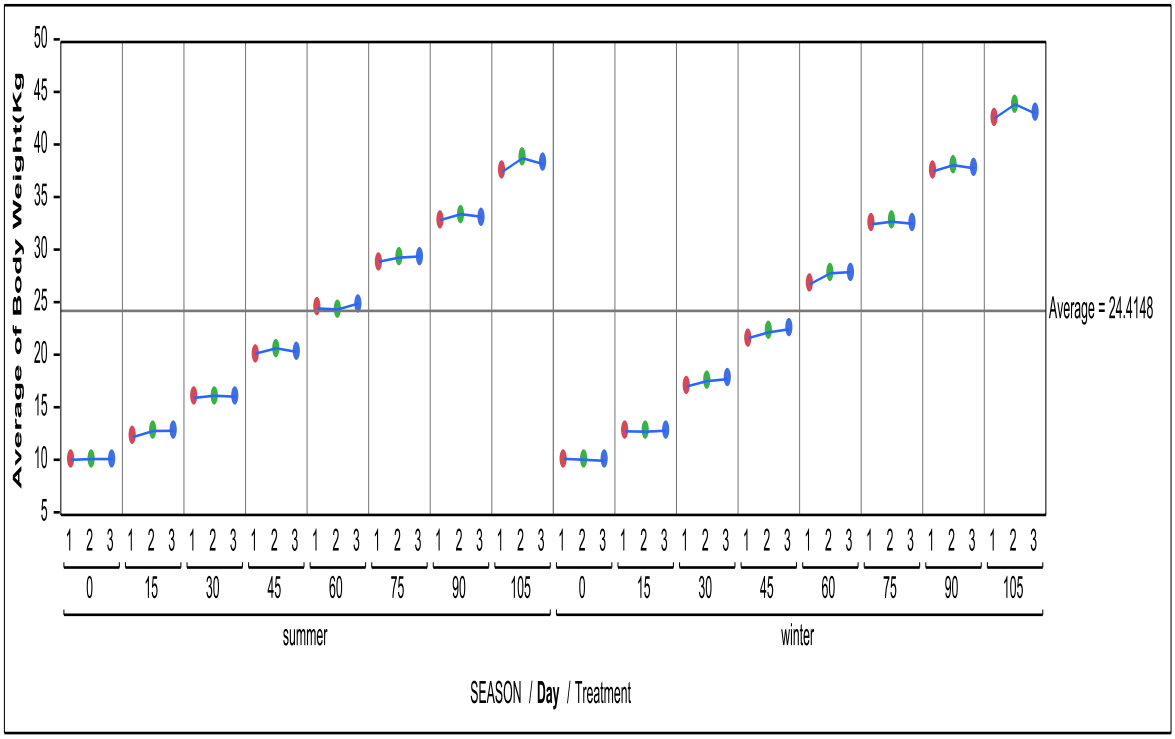

Fig. 4.9. AVERAGE BODY WEIGHT (kg) IN THE THREE TREATMENT GROUPS DURING SUMMER AND WINTER

4.5 REPRODUCTIVE PARAMETERS

4.5.1 Age at Puberty (Days)

The mean age at puberty in the three treatment groups during summer and winter are presented in Table 4.19 and 4.20 and Fig 4.10. The mean age at puberty during summer was found to be 173.17±1.11 in treatment group 1, 153.17±1.17 in treatment group 2 and 166.33 ± 1.20 in treatment group 3. On the other hand the mean age at puberty was comparatively lower during winter in the three treatment groups and it was found to be 159.33±1.23 in treatment group 1, 136.00±1.41 in treatment group 2 and 151.33±1.93 in treatment group 3. Statistical analysis revealed significant difference ($P<0.01$) in the mean age at puberty between treatment. There was also significant difference ($P<0.01$) in the mean age at puberty between season.

TABLE 4.19. AGE AT PUBERTY (DAYS, MEAN± SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	173.17±1.11	159.33±1.23	166.25±2.23[a]
2	153.17±1.17	136.00±1.41	144.58±2.73[c]
3	166.33±1.20	151.33±1.93	158.83±2.51[b]
AGGREGATE	164.22±2.11[a]	148.89±2.49[b]	156.56±2.07

Values having same superscript do not differ significantly

TABLE 4.20. ANOVA FOR AGE AT PUBERTY IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	df	Mean Square	F	P Value
Replication	56.222	5	11.244	1.857	0.162
Treatment	2910.056	2	1455.028	240.280	<0.001**
Error (Treatment)	190.611	10	19.061		
Season	2116.000	1	2116.000	349.431	<0.001**
Season × Treatment	17.167	2	8.583	1.417	0.273
Error (Season)	90.833	15	6.056		
Total	5380.889	35			

In the present study the mean age at puberty during summer was found to be 173.17±1.11 in treatment group 1, 153.17±1.17 in treatment group 2 and 166.33±1.20 in treatment group 3 whereas it was lower during winter in the three treatment groups and it was 159.33±1.23 in treatment group 1, 136.00±1.41 in treatment group 2 and 151.33±1.93 in treatment group 3.

The present findings are in accordance with the findings of Krikwood and Thacker (1988) who recorded the age at puberty as 150 days in Yorkshire × Landrace gilts when fed 3 kg of diet per day from 120 days of age to onset of puberty (control). On the other hand, when gilts were fed only 2.0 kg per day they exhibited pubertal oestrus at 165 days of age (restricted). In 3rd group they fed 2.00 kg per day from 120 to 150 days of age, but increased thereafter to 3.5 kg per day until mated (flushed) and recorded age at puberty as 165 days. Similar finding was reported by Lo *et al.* (1985) who made an experiment to see the effect of reproductive performance of gilts by different feeding methods during growing period. Female piglets were randomly divided into 3 groups. Group 1 was offered feed *ad lib.* whereas group 2 and 3 offered 90 and 70 percent of *ad lib.* feed intake respectively from 20 to 90 kg body weight. Age at puberty averaged 166.2, 167.3 and 174.3 days respectively ($p<0.05$), and body weight at puberty 88.8, 89.2

and 80.3 kg. group 3 vs group 1 and 2 (p<0.01)On the other hand Paterson *et al.* (1992) reported that daily feeding of 1 mg melatonin increased the proportion of gilts which reach puberty. Among the 24 gilts which were fed melatonin 15 (56.6%) reached puberty compared with six of the 25 (24%) control gilts. Also the findings are in close proximity with the findings of Mavogenis and Robison (1976) who reported that gilts born in fall reached puberty at younger age and a lower weight than those born in spring.

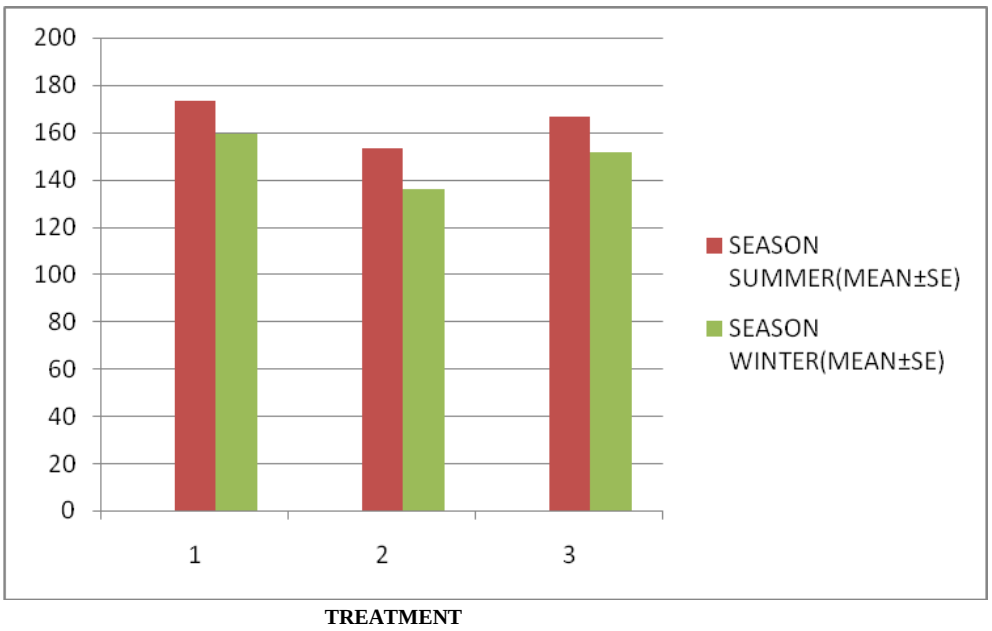

Fig. 4.10. AGE AT PUBERTY (DAYS) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.5.2 Litter Size (Nos.)

The mean litter size in the three treatment groups during summer and winter are presented in table 4.21 and 4.22 and fig 4.11 The mean litter size during summer was found to be 4.50±0.22 in treatment group 1, 5.50±0.43 in treatment group 2 and 5.00±0.26 in treatment group 3. On the other hand the mean pulse rate was comparatively high during winter in the three treatment groups and it was found to be 5.00±0.45 in treatment group 1, 5.83±0.31 in treatment group 2 and 5.17±0.31 in treatment group 3.Statistical analysis revealed significant difference (P<0.05) in the litter size between treatment.

Similar findings were reported by Quiniou and Noblet (1999). They reported that the highest average litter size were obtained at 27ºC than at other temperatures (18, 22, 25 and 29ºC) in the multiparous lactating sows. The present findings support the fact that high THI have a negative effect on litter size. At the same time it also attributes to the fact that antioxidant feeding during thermal stress has a positive effect on the litter size.

TABLE 4.21. LITTER SIZE (Nos., MEAN±SE) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	4.50±0.22	5.00±0.45	4.75±0.25[a]
2	5.50±0.43	5.83±0.31	5.67±0.26[b]
3	5.00±0.26	5.17±0.31	5.08±0.19[ab]
AGGREGATE	5.00±0.20	5.33±0.21	5.17±0.15

Values having same superscript do not differ significantly

TABLE 4.22. ANOVA FOR LITTER SIZE IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	df	Mean Square	F	P Value
Replication	3.333	5	0.667	1.017	0.442
Treatment	5.167	2	2.583	3.941	0.042*
Error (Treatment)	7.500	10	0.750		
Season	1.000	1	1.000	1.525	0.236
Season * Treatment	0.167	2	0.083	0.127	0.882
Error (Season)	9.833	15	0.656		
Total	27.000	35			

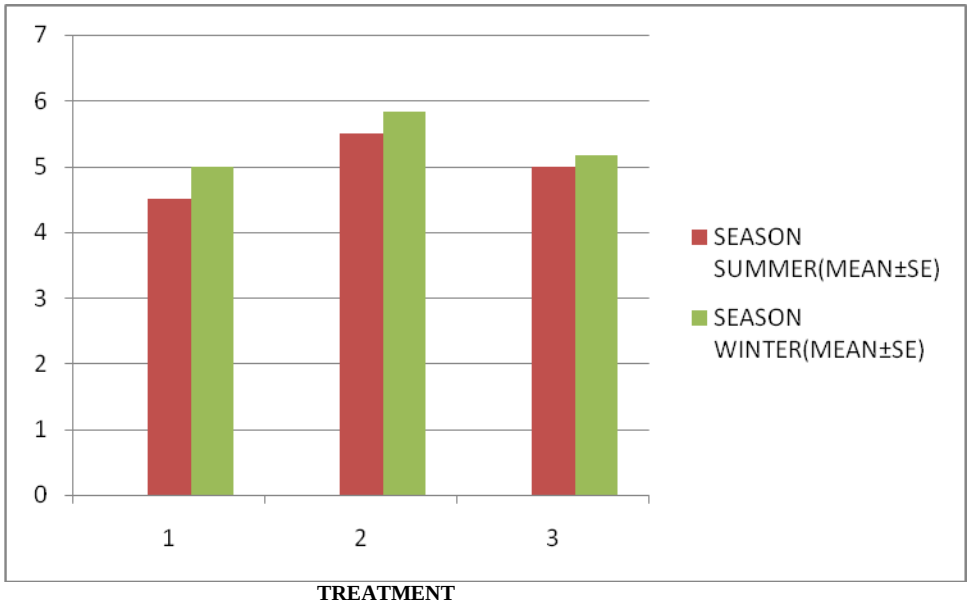

FIG. 4.11. LITTER SIZE (nos.) IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.6 HORMONAL PARAMETERS

4.6.1 Serum T3 (Triiodothyronine) (ng/ml)

The mean T3 values in the three treatment groups during summer and winter are presented in Table 4.23 and 4.24 and Fig 4.12. The mean T3 values during summer was found to be 0.63±0.01 in treatment group 1, 0.62±0.01 in treatment group 2 and 0.65±0.01 in treatment group 3. On the other hand the mean T3 value was comparatively high during winter in the three treatment groups and it was found to be 1.22±0.01 in treatment group 1, 1.23±0.01 in treatment group 2 and 1.23±0.01 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean T3 values between season.

TABLE 4.23. SERUM T_3 (ng/ml, MEAN±SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	0.63±0.01[b]	1.22±0.01[a]	0.92±0.04[a]
2	0.62±0.01[b]	1.23±0.01[a]	0.92±0.04[a]
3	0.65±0.01[b]	1.23±0.01[a]	0.94±0.04[a]
AGGREGATE	0.63±0.01[**b**]	1.22±0.01[**a**]	0.93±0.02

Values having same superscript do not differ significantly

TABLE 4.24. ANOVA FOR SERUM T_3 CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	0.069	5	0.014	3.295	0.007
Treatment	0.011	2	0.005	1.298	0.275
Error (Treatment)	0.167	10	0.017		
Season	18.898	1	18.898	4499.477	<.001**
Treatment × Season	0.011	2	0.005	1.279	0.281
Error (Season)	0.819	195	0.004		
Total	19.975	215			

In the present study the T3 values during summer was 0.63±0.01 in treatment group 1, 0.62±0.01 in treatment group 2 and 0.65±0.01 in treatment group 3. A higher T3 value was obtained during winter in the three treatment groups and it was 1.22±0.01 in treatment group 1, 1.23±0.01 in treatment group 2 and 1.23±0.01 in treatment group 3. The mean T4 values during summer was 21.33±0.11 in treatment group 1, 21.25±0.13 in treatment group 2 and 21.23±0.10 in treatment group 3. On the other hand the mean T4 value was comparatively high during winter in the three treatment groups and it was found to be 31.55±0.16 in treatment group 1, 31.18±0.23 in treatment group 2 and 32.01±0.12 in treatment group 3.

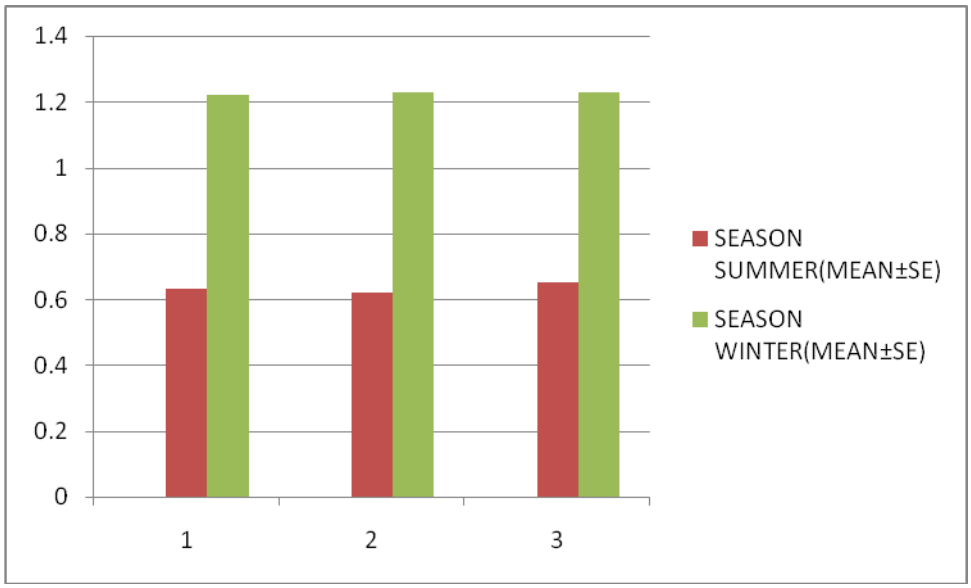

Fig. 4.12. SERUM T_3 (ng/ml) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.6.2 Serum T4 (Thyroxine)(ng/ml)

The mean T4 values in the three treatment groups during summer and winter are presented in Table 4.25 and 4.26 and Fig 4.13. The mean T4 values during summer was found to be 21.33±0.11 in treatment group 1, 21.25±0.13 in treatment group 2 and 21.23±0.10 in treatment group 3. On the other hand the mean T4 value was comparatively high during winter in the three treatment groups and it was found to be

31.55±0.16 in treatment group 1, 31.18±0.23 in treatment group 2 and 32.01±0.12 in treatment group 3. Statistical analysis revealed significant difference (P<0.01) in the mean T4 values between treatment and between season. There was also significant difference (P<0.01) between treatment and season.

TABLE 4.25. SERUM T_4 (ng/ml, MEAN±SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	21.33±0.11^{c}	31.55±0.16^{ab}	26.44±0.61^{ab}
2	21.25±0.13^{c}	31.18±0.23^{b}	26.22±0.60^{b}
3	21.23±0.10^{c}	32.01±0.12^{a}	26.62±0.64^{a}
AGGREGATE	21.27±0.06$^{\mathbf{b}}$	31.58±0.11$^{\mathbf{a}}$	26.43±0.36

Values having same superscript do not differ significantly

TABLE 4.26. SERUM T_4 CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	32.999	5	6.600	12.418	<0.001
Treatment	5.822	2	2.911	5.478	0.005**
Error (Treatment)	27.113	10	2.711		
SEASON	5739.474	1	5739.474	10799.288	<0.001**
Treatment * Season	6.650	2	3.325	6.256	0.002**
Error (Season)	103.636	195	0.531		
Total	5915.694	215			

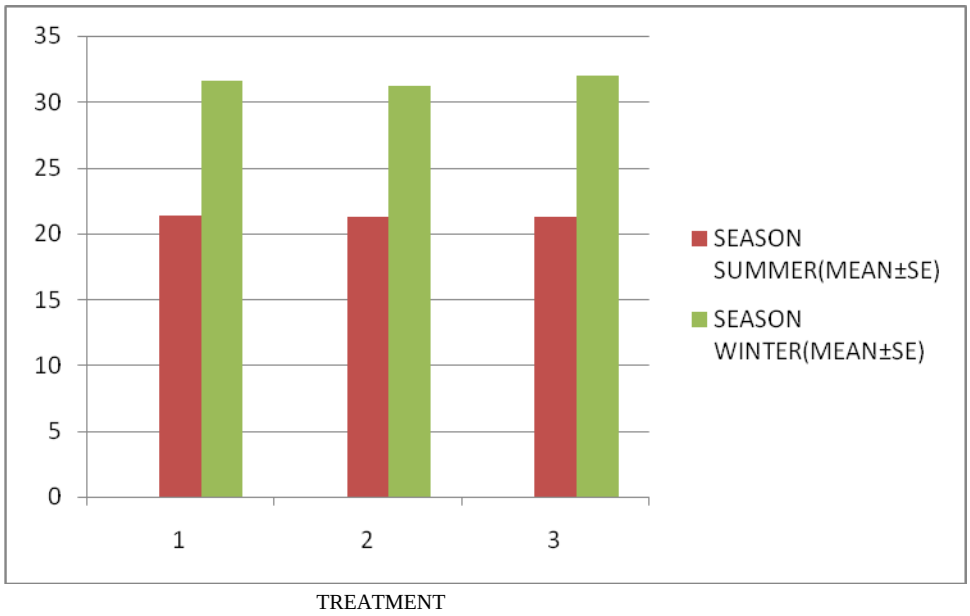

Fig. 4.13. SERUM T_4 (ng/ml) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

The present findings are in close association with the findings obtained by Djurdjevic *et al.* (1992). He studied the effect of different dietary levels on serum level of T_3 and T_4 in Swedish X Big Yorkshire X German Landrace gilts and found that normal level of T_3 and T_4 as 1.48 ± 0.28 and 46.57 ± 11.37 respectively at 4 months of age which were remained unchanged until first oestrus (about 6.5 months of age) in all gilts with a significant lower concentration of T_3 and T_4 only at 1-3 days before parturition. On the other hand Kallfelz and Erali (1973) demonstrated a fluctuating T4 and T3 value in suckling, young adult and mature pigs and reported that the serum T_4 concentration decreased significantly with age. They also found that the T_3 values were highest in young adult animals. The respective values for T_3 (%) and T_4(µg / 100 ml of blood) were 30.1 ± 2.52 and 8.40 ± 0.54, 3.17 ±1.18 and 4.70 ± 0.45 and 32.6 ±2.20 and 2.10 ± 6.42 in suckling, young adult and mature pigs. A slight bifurcating values in comparison to the present findings were also demonstrated by Reap *et al.* (1978). He reported the normal serum T_4 and T_3 values in pigs as 3.32±0.80 and 1.70± 4.68 µg/dl respectively whereas Anderson *et al.* (1988) reported that the concentration of total T_4, free T_4, total T_3 and free T_3 in pig serum as 53 ng/ml,21.7pg/ml,760pg/ml and 2.74 pg/ml respectively. The present findings were similar to that reported by Deka *et al.* (2011)

who reported a increasing trend of TT3 and TT4 concentration along with the advancement of age and treatment periods.

Blood thyroid hormones are considered to be good indicators of metabolic status of an animal (Magdub *et al*., 1982). Appropriate thyroid gland function and activity of thyroid hormones are considered crucial to sustain productive animals performance in domestic animals (Todini, 2007). Based on the metabolic and physiological status of the animals the level of thyroid hormones varies. As such season, breed and age of animals has significant effect on plasma concentration of 3-3-5 –triiodothyronine (T3) and thyroxine (T4) (Bhattacharya *et al*., 1994;Bhattacharya *et al*., 1995; Dutta *et al*., 2002; Bhooshan *et al*., 2010). The thyroid gland is highly sensitive to the ambient temperature variation (Rasooli *et al*., 2004) and thyroid hormones are good indicators of heat stress, as exposure of animals to heat stress activates the hypothalamo-pituitary-adrenal axis (Abilay *et al*., 1975), and estimation of thyroid hormones could be one of the important indicators for assessment of stress in animals.

T3 and T4 concentrations in all the treatment groups during summer was found to be lower compared to winter. In summer the mean T3 concentration was lowest in treatment 2 (melatonin supplemented) followed by treatment 1 (control) and treatment (Vitamin E supplemented). Increased secretion of thyroid hormones increases body metabolism and hence heat production. Therefore decreased thyroid hormone levels during heat stress were an adaptive response and also might be an attempt to reduce metabolic rate and heat production (West *et al*., 1999). When the animal starts to suffer due to heat food ingestion is reduced and metabolism slows down, causing a hypo-function of the thyroid (McManus *et al*., 2009). Similar findings were observed by Prakash and Rathore (1999) in goats. They found significant decrease in thyroid hormones during summer months. On the other hand T3 and T4 concentration are found higher in all the treatment groups in winter compared to summer. Similar finding was reported by Hasin (2015) in goats. The T3 and T4 concentration during winter may be attributed to the fact that there is increased body metabolism contributed by increase in the food intake to maintain the body equilibrium which may be affected by the low environmental temperature leading to stimulative function of the thyroid gland.

4.6.3 Serum Cortisol (ng/ml)

The mean cortisol values in the three treatment groups during summer and winter are presented in Table 4.27 and 4.28 Fig 4.14. The mean cortisol values during summer was found to be 7.08±0.06 in treatment group 1, 6.70±0.03 in treatment group 2 and 6.79±0.03 in treatment group 3. On the other hand the mean cortisol value was comparatively low during winter in the three treatment groups and it was found to be 3.49±0.05 in treatment group 1, 3.39±0.04 in treatment group 2 and 3.43±0.04 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean cortisol values between treatment and between season. There was also significant difference ($P<0.01$) between treatment and season.

TABLE 4.27. SERUM CORTISOL (ng/ml, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREAT

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	7.08±0.06[a]	3.49±0.05[c]	5.29±0.22[a]
2	6.70±0.03[b]	3.39±0.04[c]	5.05±0.20[b]
3	6.79±0.03[b]	3.43±0.04[c]	5.11±0.20[b]
AGGREGATE	6.86±0.03[**a**]	3.44±0.02[**b**]	5.15±0.12

Values having same superscript do not differ significantly

TABLE 4.28. ANOVA FOR SERUM CORTISOL CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	df	Mean Square	F	P Value
Replication	0.834	5	0.167	2.613	0.026
Treatment	2.199	2	1.099	17.219	<0.001
Error (Treatment)	0.792	10	0.079		
SEASON	632.119	1	632.119	9901.588	<.001**
Treatment × SEASON	0.862	2	0.431	6.753	0.001**
Error (Season)	12.449	195	0.064		
Total	649.255	215			

In the present study the mean cortisol concentration during summer was 7.08±0.06 in treatment group 1, 6.70±0.03 in treatment group 2 and 6.79±0.03 in treatment group 3. A comparatively lower trend was observed during winter in the three treatment groups and it was found to be 3.49±0.05 in treatment group 1, 3.39±0.04 in treatment group 2 and 3.43±0.04 in treatment group 3.

The present findings are in close proximity with the results opined by Gyo-Moon Chu and Young-Min Song (2013). They reported the cortisol concentration in fattening pigs during summer and winter seasons as 5.67µg/dL and 2.57 µg/dL which differed significantly. Also present findings are in close association with the results obtained by Fagundes *et al.* (2008) who reported that pigs in high temperature showed significantly higher average cortisol level 7.06mg/dL ($P<0.01$) than those at comfort temperature 4.82mg/dL. He also found that the increase in serum concentration was continuous and linear during the experimental period. Averos *et al.* (2007) demonstrated the seasonal variation in the pigs transported to slaughter under commercial conditions. The seasonal variation were 2.83±0.24 in winter and 3.85±0.19 in summer and variation in sex was 3.00±0.21 in female and 3.68±0.22 in males.

The lower level of serum cortisol was observed in treatment 2 during summer. Melatonin inhibits the responsiveness of the adrenal glands to ACTH (Konakchieve *et al*., 1997; Torres-Farfan et al., 2003). Ishida et al. (2005) observed the stimulatory effect of light via suprachiasmatic nucleus on the gene expression in adrenal gland causing plasma corticosterone surge independent of activation of hypothalamic-pituitary-adrenal axis. Also they speculated that melatonin might inhibit gene expression leading to reduced cortisol secretion. Furthermore, the cortisol concentration treatment 2 differed significantly ($P<0.01$) to control i.e., treatment 1 but not with treatment 3 reflecting the effectiveness of melatonin in reducing the heat stress. On the other hand the cortisol concentration during winter was reduced reflecting the comfortable state of the animals during the winter season and therefore the values did not differ significantly in all the treatment groups.

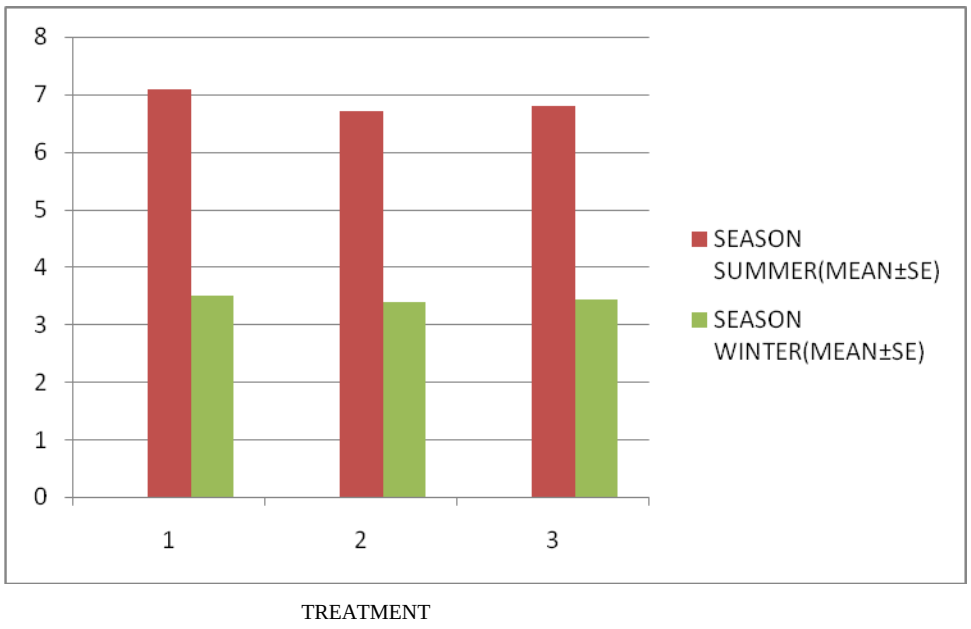

Fig. 4.14. SERUM CORTISOL (ng/ml) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.6.4 Serum Growth Hormone (pg/ml)

The mean GH values in the three treatment groups during summer and winter are presented in Table 4.29 and 4.30 Fig 4.15. The mean GH values during summer was

found to be 1.87±0.10 in treatment group 1, 1.91±0.10 in treatment group 2 and 1.92±0.10 in treatment group 3. On the other hand the mean GH value was comparatively low during winter in the three treatment groups and it was found to be 2.11±0.12 in treatment group 1, 2.18±0.13 in treatment group 2 and 2.16±0.13[a] in treatment group 3.Statistical analysis revealed significant difference (P<0.01) in the mean GH values between season.

TABLE 4.29. SERUM GROWTH HORMONE (pg/ml, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	1.87±0.10[a]	2.11±0.12[a]	1.99±0.08[a]
2	1.91±0.10[a]	2.18±0.13[a]	2.05±0.08[a]
3	1.92±0.10[a]	2.16±0.13[a]	2.04±0.08[a]
AGGREGATE	1.90±0.06[b]	2.15±0.07[a]	2.02±0.05

Values having same superscript do not differ significantly

TABLE 4.30. SERUM GROWTH HORMONE (pg/ml, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	0.070	5	0.014	0.028	1.000
Treatment	0.153	2	0.076	0.154	0.857
Error (Treatment)	0.079	10	0.008		
Season	3.330	1	3.330	6.734	0.010*
Treatment × Season	0.010	2	0.005	0.010	0.990
Error (Season)	96.432	195	0.495		
Total	100.075	215			

In the present study the mean growth hormone concentration in summer and winter under three different treatment regimes averaged 1.90±0.06 in summer and 2.15±0.07 in winter.

The present findings show a similar trend with the findings reported by Mili (2012) who reported that the mean (±SE) concentration of growth hormone in the piglets(2 to 6 months of age) prior to supplementation ranged between 1.44± 0.13 and 1.84± 0.21 pg/ml. There was significant ($p<0.01$) variation of growth hormone among different experimental (different amount of Zn and Cu supplementation) groups (T_1,T_2 and T_3) and also amongst the day under study. Similar findings has been illustrated by Gogoi (2012).

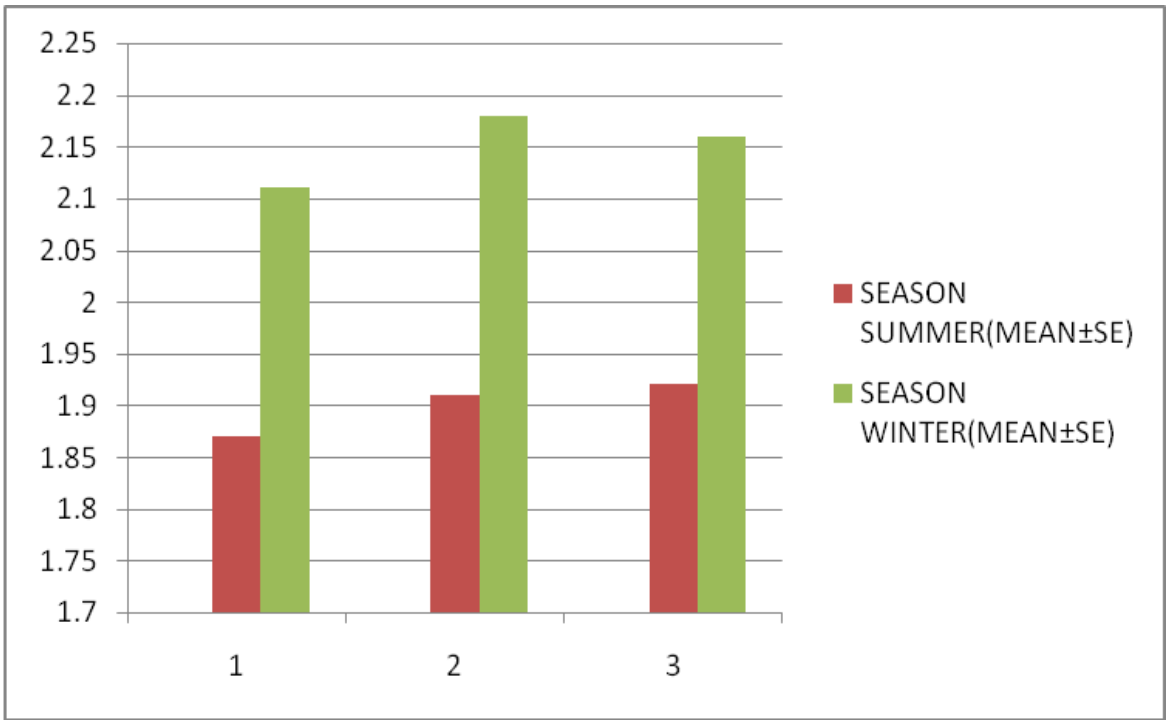

Fig. 4.15. SERUM GROWTH HORMONE (PG/ML) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.6.5 Serum Progesterone (ng/ml)

The mean progesterone concentration in the three treatment groups during summer and winter are presented in Table 4.31 and 4.32 and Fig 4.16. The mean progesterone concentration in the three treatment groups during summer was found to be 22.95 ±0.77 ^{A} whereas it was 25.60± 0.93^{B} during winter. The mean body weight values during summer and winter (aggregate) was found to be 24.09±1.05^{B} for treatment group 1, 24.38±1.06^{A} for treatment group 2 and 24.35±1.05^{A} for treatment group 3. Statistical analysis revealed significant difference (P<0.01) in the mean progesterone concentration between treatment and between season. There was also significant difference (P<0.01) between day and season

TABLE 4.31. SERUM PROGESTERONE (ng/ml, MEAN±SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Treatment	Season		
	Summer	Winter	Total
	Mean± SE	Mean± SE	Mean± SE
1	0.398±0.003	0.401±0.004	0.400±0.002^{b}
2	0.424±0.005	0.431±0.006	0.427±0.004^{a}
3	0.406±0.004	0.410±0.004	0.408±0.003^{b}
Total	0.409±0.003	0.414±0.003	0.412±0.002

Values having same superscript do not differ significantly

TABLE 4.32. ANOVA FOR SERUM PROGESTERONE CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P value
Replication	0.002	5	0.000	0.529	0.754
Treatment	0.028	2	0.014	21.164	<0.001**
Error Treatment	0.006	10	0.001	0.859	0.573
Season	0.001	1	0.001	1.725	0.191
Treatment X Season	0.000	2	8.10E-005	0.121	0.886
Error	0.131	195	0.001		
Total	0.168	215			

** (P <0.001)

The present findings are in close association with the findings reported by Saikia (2007). He reported the mean serum progesterone concentration on day 14 days before treatment of probiotics, 7 day before treatment of probiotics, day of probiotic treatment and the day of estrus as 0.47 ±0.04, 0.51±0.05, 0.49±0.04 and 0.42±0.05 ng/ml; 0.46±0.05, 0.50±0.06, 0.48±0.05 and 0.42±0.05 ng/ml; and 0.47±0.06, 0.51±0.04, 0.48±0.06 and 0.40±0.06 ng/ml, in gilts of group A, B and C respectively. The present findings are also in close proximity with the findings reported by Callaghan (1978) who indicated that plasma progesterone level in prepubertal gilts averaged 0.5 ng/ml when gilts were induced to oestrus by treating 400 IU PMSG and 200 IU HCG. Similarly Esbenshade *et al*. (1982) reported that the mean concentration of plama progesterone concentration ranged from 0.1 to 0.3 ng/ml between 6 day before to the day on which gilts exhibited first oestrus. The low progesterone concentration in all the treatment groups during summer and winter may be attributed to the absence of functional corpus luteum. The significant difference seen in the mean progesterone concentration in treatment group 2 may validate the fact that compared to implants melatonin administered orally is effective in overcoming the seasonal inhibition of puberty in domestic gilts (Paterson *et al*., 1992).

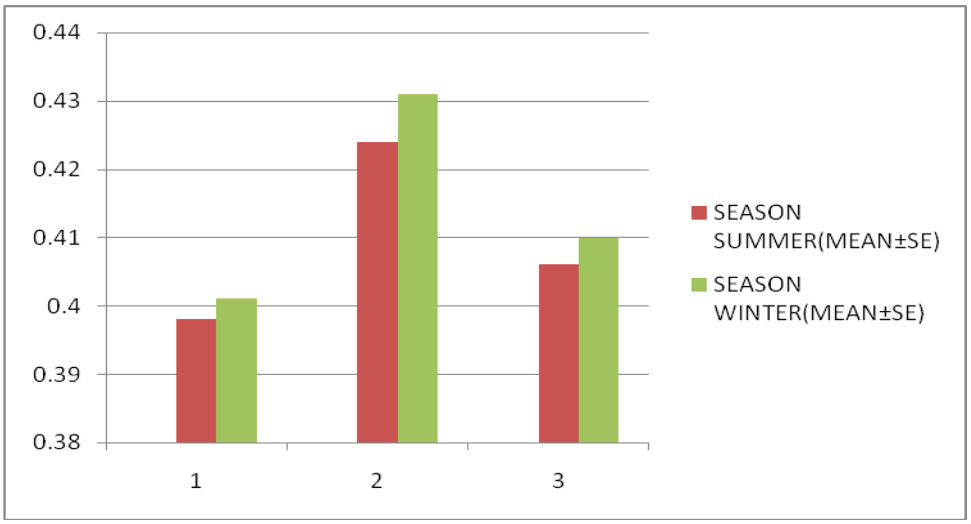

Fig. 4.16. SERUM PROGESTERONE (ng/ml) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.6.6 Serum Melatonin (pg/ml)

The mean melatonin concentration in the three treatment groups during summer and winter are presented in Table 4.33 and 4.34 and Fig 4.17 The mean melatonin concentration in the treatment group 2 during summer was found to be 167.09±3.05^{a} which is significantly different (P<0.01) from treatment group 1 and 3.Similarly in winter, the mean melatonin concentration in treatment group 2 is significantly different (P<0.01) from treatment group 1 and 3 respectively.

TABLE 4.33. SERUM MELATONIN (pg/ml, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Treatment	Season		Total
	Summer	Winter	
	Mean±SE	Mean± SE	Mean± SE
1	126.02±0.49^{c}	141.6±00.31^{b}	133.81±0.97^{b}
2	167.09±3.05^{a}	170.92±2.75^{a}	169.01±2.05^{a}
3	126.20±0.53^{c}	141.72±0.39^{b}	133.96±0.98^{b}
Total	139.77±2.13^{b}	151.41±1.62^{a}	145.59±1.40

Values having same superscript do not differ significantly

TABLE 4.34. ANOVA FOR SERUM MELATONIN CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	26.975	5	5.395	0.048	0.999
Treatment	59215.007	2	29607.503	261.480	<0.001**
Error Treatment	126.507	10	12.651	0.112	1.000
Season	7320.979	1	7320.979	64.655	<0.001**
Treatment X Season	1646.706	2	823.353	7.271	<0.001**
Error	22079.964	195	113.231		
Total	90416.137	215			

***P* (<0.001)

Melatonin is a hormone of the pineal gland responsible for the regulation diurnal and seasonal activity rhythms in animals. The action of the pineal gland hormone requires the activity of specific receptors located primarily in the brain and in the peripheral tissues thereby affecting the metabolism directly or indirectly. Pineal gland melatonin is a known antioxidant hormone which functions via a number of pathways to reduce oxidative stress. It acts as a direct free radical scavenger, as an indirect antioxidant and has ability to augment the activities of other antioxidants. It plays an important role in relieving heat stress by influencing cardiovascular system and evaporative heat loss (Harlow, 1987). It also interacts with other hormones to alleviate heat stress possibly with thyroxine and successfully modify adrenal function to relieve thermal stress (Seijan and Srivastava, 2010a).

In the present investigation the melatonin concentration in the melatonin treated group i.e., treatment 2 is significantly higher (P<0.01) than in treatment 1 and 3 because of the supplementation of exogenous melatonin in treatment group 2. On the other hand the melatonin concentration was significantly higher in treatment 1 and 3 in winter in comparison to summer, which may be due to natural melatonin level (Singh *et al.*, 2014). In case of the melatonin fed group in winter the concentration is higher though not significant is due to supply of exogenous melatonin. The higher level of melatonin in

winter compared to summer in treatment 1 and 3 may be due to the diurnal variations for melatonin secretion in winter that may give a natural advantage to the animals for ameliorating cold stress. This is substantiated by the report of Singh *et al.* (2014), who reported that the clinically immune parameters such as lymphocyte count and stimulation ratio of T lymphocytes presented a day/night rhythm prominently in winter. They also observed that the oxidative load in terms of malonedialdehyde was always low during night while antioxidants such as superoxide dismutase catalase and total antioxidant status were high during nighttime.

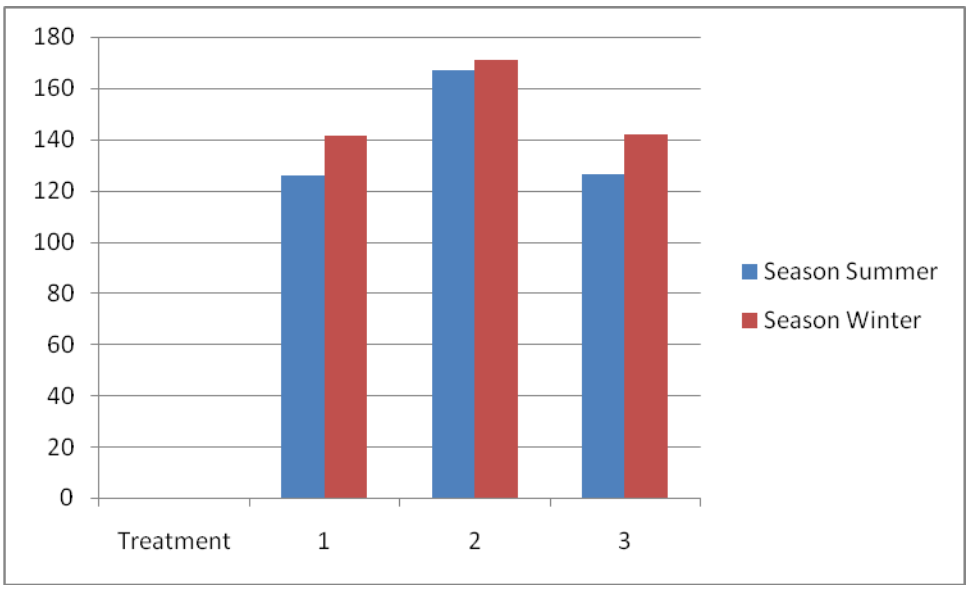

Fig. 4.17. SERUM MELATONIN (pg/ml)CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.7 SERUM VITAMIN-E (mg)

The mean Vitamin E concentration in the three treatment groups during summer and winter are presented in table 4.35 and 4.36 and fig 4.18. The mean Vitamin E concentration in the three treatment groups during summer was found to be 1.74±0.04 in treatment group 1, 1.76±0.05 in treatment group 2 and 4.17±0.09 in treatment group 3 . On the other hand the mean Vitamin E concentration in the three treatment groups during winter was found to be 1 .80±0.05 in treatment group 1, 1.82±0.05 in treatment group 2

and 4.27±0.10 in treatment group 3. The aggregate mean vitamin E concentration reveals that treatment 3 (4.22±0.06[a]) is significantly different (P<0.01) from treatment 1 (1.77±0.03[b]) and treatment 2 (1.79±0.04[b]) respectively.

TABLE 4.35. SERUM VITAMIN E (mg, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Treatment	Season		
	Summer	Winter	Aggregate
	Mean± SE	Mean± SE	Mean± SE
1	1.74±0.04	1.80±0.05	1.77±0.03[b]
2	1.76±0.05	1.82±0.05	1.79±0.04[b]
3	4.17±0.09	4.27±0.10	4.22±0.06[a]
Aggregate	2.56±0.12	2.63±0.12	2.59±0.08

Values having same superscript do not differ significantly

TABLE 4.36. ANOVA FOR SERUM VITAMIN E CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	1.844	5	0.369	2.306	0.046
Treatment	285.205	2	142.603	891.846	<.001**
Error Treatment	0.789	10	0.079	0.494	0.893
Season	0.282	1	0.282	1.762	0.186
Treatment X Season	0.014	2	0.007	0.043	0.958
Error	31.180	195	0.160		
Total	319.313	215			

**P (<0.001)

The present findings are in close association with the findings reported by Mitssioulis and Judson (2000). They reported that on the day of collection (0 day), the plasma vitamin E concentration (mg/l) in the animals sampled from each species ranged from 1.5 to 2.8 in pigs, 6.7 to 10.2 in cattle and 0.2 to 1.7 in sheep. The present findings are also in close proximity with the findings reported by Niculita *et al*. (2007). They reported that the serum from Vitamin E supplemented pigs had the highest concentrate ion of alpha-tocopherol throughout the 1st 4 weeks of the experiment. They also reported that the blood levels of vitamin E were 2.7 to 3.5 times higher in the vitamin E supplemented pigs than those in the basal diet. Therefore the significant difference ($P<0.01$) in the vitamin E supplemented group i.e., treatment 3 in both the seasons is supportive of the fact that supplementation of Vitamin E increases its concentration in the circulation.

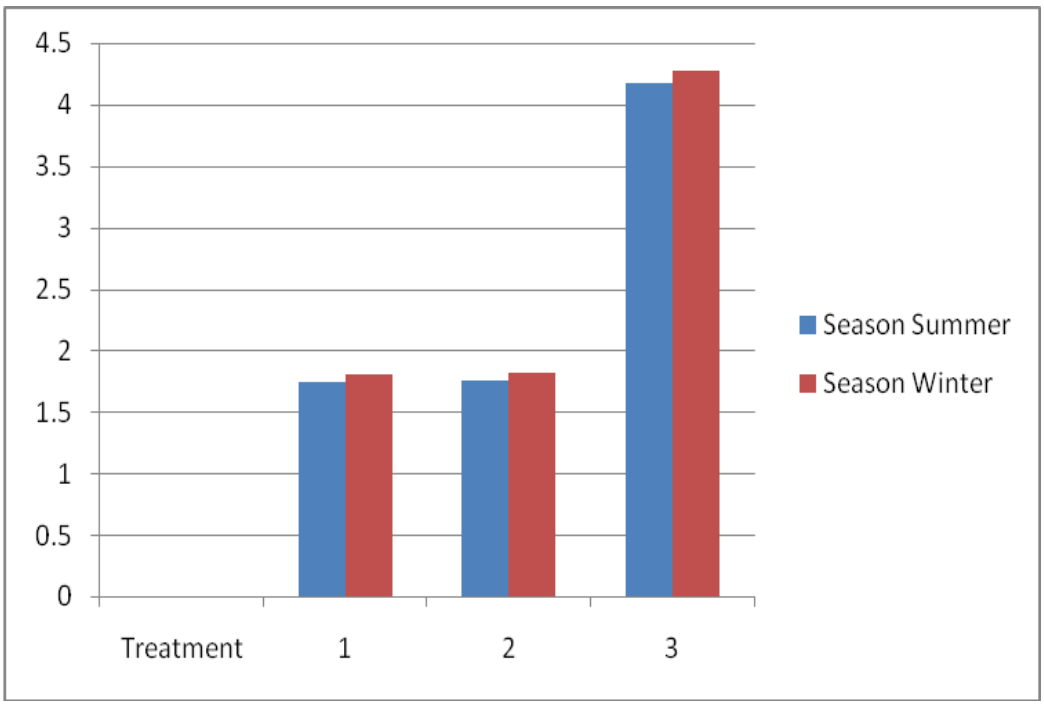

FIG. 4.18. SERUM VITAMIN E (mg) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.8 ENZYME ACTIVITY

4.8.1 LACTATE DEHYDROGENASE (LDH) (U/L)

The mean LDH values in the three treatment groups during summer and winter are presented in Table 4.37 and 4.38 Fig 4.19. The mean LDH values during summer was found to be 940.16±2.73 in treatment group 1, 941.18±2.08 in treatment group 2 and 948.56±1.81 in treatment group 3. On the other hand the mean LDH value was comparatively low during winter in the three treatment groups and it was found to be 799.00±4.55 in treatment group 1, 800.78±4.59 in treatment group 2 and 794.41±6.00 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean LDH values between season.

TABLE 4.37. SERUM LDH (UL^{-1}, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

TREATMENT	SEASON		AGGREGATE
	SUMMER (MEAN±SE)	WINTER (MEAN±SE)	
1	940.16±2.73[a]	799.00±4.55[b]	869.58±8.78[a]
2	941.18±2.08[a]	800.78±4.59[b]	870.98±8.70[a]
3	948.56±1.81[a]	794.41±6.00[b]	871.49±9.66[a]
AGGREGATE	943.30±1.33[**a**]	798.06±2.92[**b**]	870.68±5.21

Values having same superscript do not differ significantly

TABLE 4.38. ANOVA FOR SERUM LDH CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	14112.918	5	2822.584	7.885	<0.001
Treatment	140.329	2	70.165	0.196	0.822
Error (Treatment)	33043.366	10	3304.337		
SEASON	1139108.606	1	1139108.606	3182.200	<0.001**
Treatment × Season	2150.585	2	1075.292	3.004	0.052
Error (Season)	69802.702	195	357.963		
Total	1258358.505	215			

In the present study the mean LDH values during summer was 940.16±2.73 in treatment group 1, 941.18±2.08 in treatment group 2 and 948.56±1.81 in treatment group 3. Whereas it was low during winter in the three treatment groups and it was found to be 799.00±4.55 in treatment group 1, 800.78±4.59 in treatment group 2 and 794.41±6.00 in treatment group 3.

The present findings are in very close proximity with the findings of Gyp-Moon Chu and Young-Min Song (2013) who reported that the plasma LDH concentration in the fattening pigs was significantly higher ($P<0.05$) in summer ($937.70 UL^{-1}$) than in winter ($798.70 UL^{-1}$). Similar seasonal trend was demonstrated by Korzeniowska *et al.* (2012) who reported that LDH concentration in the fattening pigs in the winter season were : Mean-2106.25,SD-292.29 in the 1st stage and Mean-1706.60 and SD-293.64 in the 2nd stage whereas in the summer season it was, Mean-2178.64 and SD-427.65 in the 1st stage and Mean-1831.08 and SD-376.98 in the 2nd stage. On the other hand, Prasad and Dilip Kumar (2002) reported LDH values in pigs at 60, 90 and 120 days of age as 710.29, 390.71 and 350.00 IU/L which differed significantly ($P<0.01$) indicating a decreasing trend as the age increases.

LDH plays an important role in cellular respiration through catalyzation by which pyruvate from glucose is converted into usable energy as lactate in the cells. When animals or animal tissues are subjected to heat stress which causes damage to or injury to them, more LDH is released into the bloodstream. Moreover, conditions that can cause increase LDH in the blood include liver disease, heart attack, anaemia, muscle trauma, bone fractures etc. (Joseph *et al*. 2002)

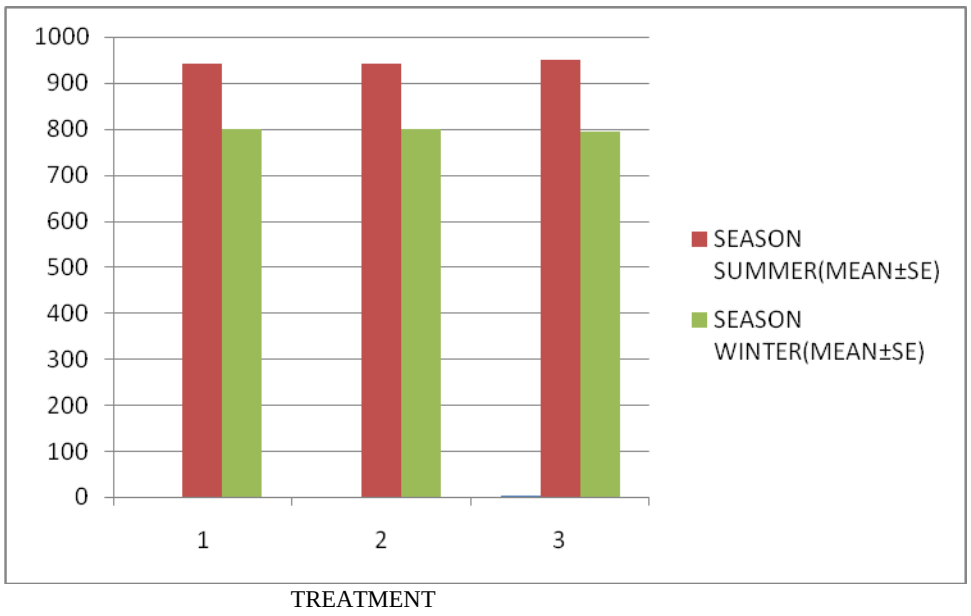

Fig. 4.19. SERUM LDH (U/L) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.8.2 Superoxide Dismutase (SOD) (U/ml)

The mean SOD values in the three treatment groups during summer and winter are presented in Table 4.39 and 4.40 Fig 4.20. The mean SOD values during summer was found to be 1.87±0.10 in treatment group 1, 1.80±4.67E-03 in treatment group 2 and 1.84±0.01 in treatment group 3. On the other hand the mean SOD value during winter in the three treatment groups was found to be 1.18±4.69E-03 in treatment group 1, 1.18±4.00E-03 in treatment group 2 and 1.19±3.95E-03 in treatment group 3.Statistical analysis revealed significant difference ($P<0.01$) in the mean SOD values between treatment and between season. There was also significant difference ($P<0.01$) between treatment and season.

TABLE 4.39. SERUM SOD (U/ml**, MEAN± SE) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON**

TREATMENT	SEASON		AGGREGATE
	SUMMER(MEAN±SE)	WINTER(MEAN±SE)	
1	1.87±0.01[a]	1.18±4.69E-03[d]	1.53±0.04[a]
2	1.80±4.67E-03[b]	1.18±4.00E-03[d]	1.49±0.04[b]
3	1.84±0.01[c]	1.19±3.95E-03[d]	1.51±0.03[c]
AGGREGATE	1.83±0.01[**a**]	1.18±2.45E-03[**b**]	1.51±0.02

E stands for 10 and figure after E is power of 10
Values having same superscript do not differ significantly

TABLE 4.40. ANOVA FOR SERUM SOD CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

Source	Sum of Squares	Df	Mean Square	F	P Value
Replication	0.042	5	0.008	4.963	<0.001
Treatment	0.414	2	0.207	122.309	<0.001**
Error (Treatment)	0.061	10	0.006		
Season	19.147	1	19.147	11325.117	<0.001**
Treatment * Season	0.487	2	0.243	144.000	<0.001**
Error (Season)	0.330	195	0.002		
Total	20.480	215			

In the present study mean SOD values during summer was found 1.87±0.10 in treatment group 1, 1.80±4.67E-03 in treatment group 2 and 1.84±0.01 in treatment group 3 with an aggregate of 1.78±0.01 whereas in winter it was 1.18±4.69E-03 in treatment group 1, 1.18±4.00E-03 in treatment group 2 and 1.19±3.95E-03 in treatment group 3 with an aggregate of 1.18±2.45E-03[**b**].

The present findings are in close proximity with the findings reported by Hasin (2015). She reported the mean activity of serum SOD during summer in pre and post sunshine Beetal goats as 1.81±0.01 and 1.72±0.01 U/ml respectively. The corresponding activity in the melatonin fed group was 1.83±0.01 and 1.77±0.01 /ml respectively. On the other hand during winter season the mean activity of serum SOD in pre and post sunshine exposed Beetal goats was 1.15±0.01 and 1.17±0.01 /ml, respectively. The corresponding values in the melatonin fed group was 1.17± 0.01 and 1.20±0.01 U/ml, respectively.

There is excessive production of reactive oxygen species (ROS) such as superoxide anion, hydroxyl ion and hydrogen peroxide during thermal. They are continuously produced in the course of normal aerobic metabolism. If these free radicals are not eliminated they can damage the healthy cells. This may result in disturbed physiology and altered biochemical profile of the animal (Bernabucchi *et al*., 2002). Superoxide dismutase, catalase and glutathione peroxidase are the major defense in the detoxification of superoxide anion and hydrogen peroxide (McCord *et al.,* 1969 and Chance *et al*., 1979). Superoxide dismutase along with catalase and glutathione peroxidise scavenges both intracellular and extracellular superoxide radicals and prevents lipid peroxidation (Agarwal and Prabhakaran, 2005). Superoxide dismutase that catalyzes dismutation of superoxide becomes important in the defense mechanisms against oxidative stress (Halliwel and Chirico, 1993). The SOD activity in summer was lowest in the melatonin supplemented group i.e., treatment 2. Al-Badwi *et al*., (2013) suggested decrease SOD activity is due to consumption of SOD to overcome the oxidative stress. This might also be due to the antioxidant effects of melatonin that scavenges the free radicals generated during heat stress (Ahmed *et al*., 2005). In winter little variation in the SOD activity was witnessed and they did not differ significantly which is suggestive of the comfortable environment the animals were in.

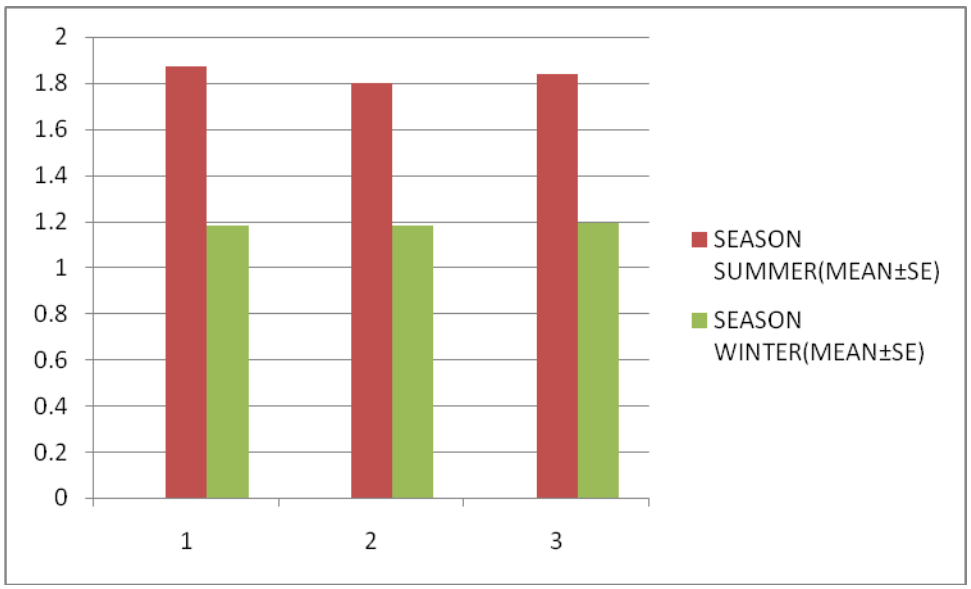

Fig. 4.20. SERUM SOD (u/ml) CONCENTRATION IN THE DIFFERENT TREATMENT GROUPS DURING SUMMER AND WINTER SEASON

4.9 HEAT SHOCK PROTEIN 70 (HSP-70) GENE EXPRESSION

The relative expression of HSP 70 gene in summer and winter in the three treatment groups are presented in Table 4.41 & 4.42 and Fig 4.21 & 4.22.

The normalized expression for HSP70 during summer shows that the animals with Melatonin treatment i.e., treatment 2 have 1.98 fold lower expression than the control i.e., treatment 1 animals. Likewise, animals with Vitamin E treatment i.e., treatment 3 shows 0.56 fold lower expression than control animals i.e., treatment 1, during summer season.

TABLE 4.41. RELATIVE EXPRESSION OF HSP70 GENE DURING SUMMER

Season	Treatment 1	Treatment 2 (ΔΔCt)	Treatment 3 (ΔΔCt)
Summer	0	0.99	0.28
Normalized expression for Melatonin = $2^{-\Delta\Delta Ct} = 2^{-(0.99)} = -1.98$			
Normalized expression for Vitamin E = $2^{-\Delta\Delta Ct} = 2^{-(0.28)} = -0.56$			

During winter, the animals with Melatonin treatment shows 0.70 fold higher expression compared to control animals. Similarly, animals with Vitamin E treatment have 1.28 fold higher expressions than control animals.

TABLE 4.42. . RELATIVE EXPRESSION OF HSP70 GENE DURING WINTER

Season	Treatment 1	Treatment 2(ΔΔCt)	Treatment 3(ΔΔCt)
Winter	0	- 0.35	- 0.64
Normalized expression for Melatonin = $2^{-\Delta\Delta Ct} = 2^{-(-0.35)} = 0.70$			
Normalized expression for Vitamin E = $2^{-\Delta\Delta Ct} = 2^{-(-0.64)} = 1.28$			

In mammalian cells, non-lethal heat shock produces changes in gene expression and in the activity of expressed proteins, resulting in what is referred to as a cell stress response (Jaattela, 1999; Lindquist, 1986). This response characteristically includes an increase in thermo tolerance that is temporally associated with increased expression of HSPs. Heat induced changes in gene expression occur both during hyperthermia as well as after return to normothermia.

The results obtained in the present investigation in summer is in close proximity with the findings of Mishra *et al.* (2011). They reported a significant increase ($P<0.05$) in mRNA expression of HSP70 in tropical goats during summer season in comparison to winter season. However, no significant difference was observed in HSP70 expression between summer and winter season in the temperate region goats. Sharma *et al.* (2013) also documented significant increase ($P<0.05$) in mRNA expression of HSP60, HSP70, HSP90 and ubiquitin in Barbari goats when they were exposed to 35-40^{o}C in a psychrometric chamber @ 6 h/day for 11 days. Dangi *et al.* (2012) also recorded significantly higher HSP 70 mRNA expression in summer season in all age groups of tropical region goats in comparison to winter. However, in the present investigation the expression of HSP 70 during summer shows that the animals with Melatonin treatment ie., treatment 2 have 1.98 fold lower expression than the control animals i.e., treatment 1.Similarly, animals with Vitamin E treatment i.e., treatment 3 shows 0.56 fold lower expression than control animals i.e., treatment 1, during summer season. These expressions reflect the antioxidant role of melatonin and vitamin E in ameliorating the heat stress in animal upto an extent which is evident with the extent of expression of HSP 70 gene in comparison to control animals. On the other hand, the extent of expression of HSP 70 gene dictates a relatively null effect of antioxidants during the winter season indicating signs of cold stress, making it a topic of interest in the future studies.

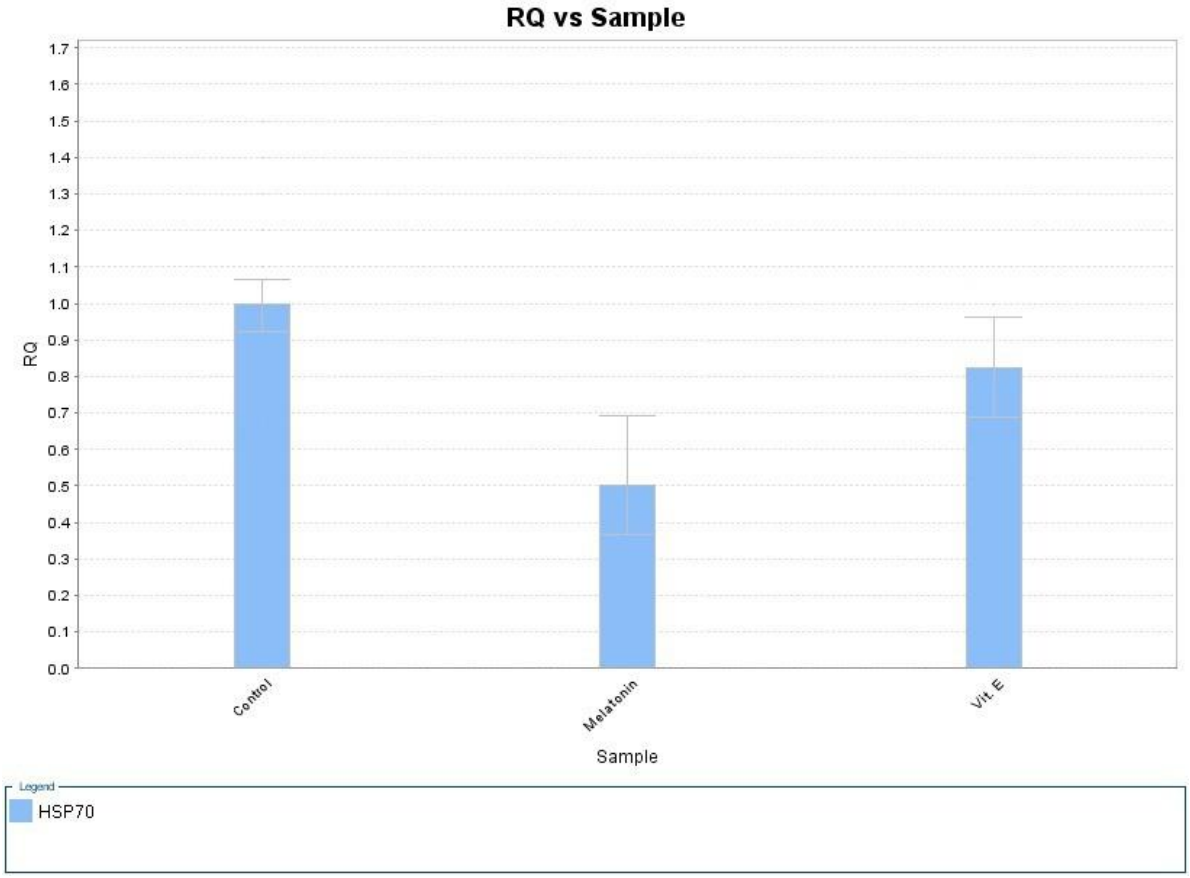

Fig. 4.21. RELATIVE EXPRESSION OF HSP70 GENE DURING SUMMER

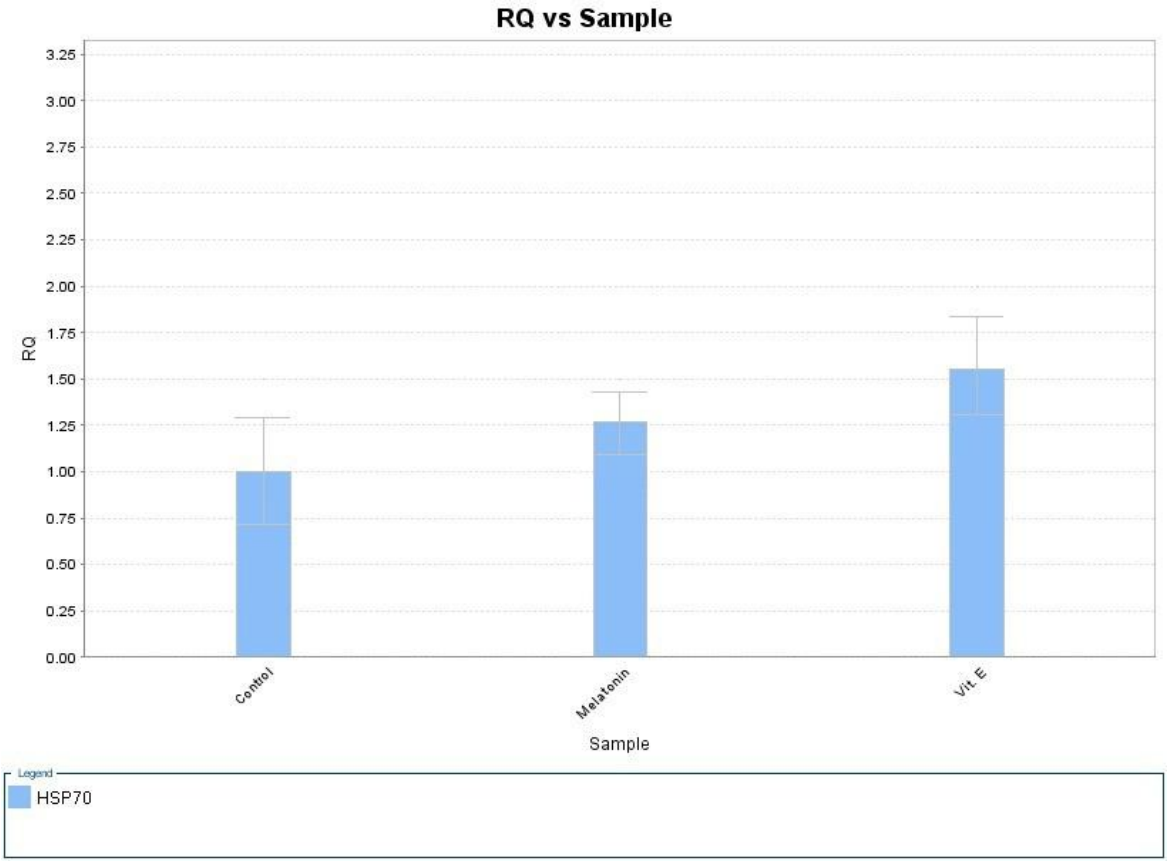

Fig. 4.22. RELATIVE EXPRESSION OF HSP70 GENE DURING WINTER

CHAPTER V

SUMMARY AND CONCLUSION

The present experiment was carried out to study the effect of thermal stress on various physiological, haematological, biochemical and hormonal parameters including expression profiles of HSP70 in the experimental pigs during summer and winter season with special reference to the antioxidative effects of melatonin and vitamin E.

The experimental study was carried out during two different seasons viz., Summer (June, July & August, 2014) and winter (December, 2013 & January and February 2014). The present experiment included 36 nos. of weaned, healthy and uniform sized crossbred (Hampshire X Assam local). Eighteen (18) animals were subjected to treatment separately during summer and winter. The selected animals were divided into three groups with six pigs in each group consisting of a control group (Treatment 1), one group was fed melatonin @ 3 mg per animal (Treatment 2) and the other group was fed Vitamin E @ 100 mg per animal (Treatment 3) for both the seasons. The animals were fed as per standard feeding practices of the farm. About 5 ml of blood was collected from each experimental animal aseptically at 15 days interval for the whole experimental period.

After the end of three months of treatment period for both summer and winter season the animals were observed till furrowing to determine the litter size.

Temperature humidity Index (THI) was calculated out by using standard formula. It was then correlated with the other parameters. All the three parameters except relative humidity were found significantly different ($P<0.01$) between seasons ie., temperature and the temperature humidity index was more in summer than in winter, whereas within seasons they were not significant.

The mean rectal temperature showed significant difference ($P<0.01$) between seasons. There was also significant difference ($P<0.01$) between the treatment and season. Statistical analysis revealed significant difference ($P<0.01$) in the mean

respiration rate between seasons. Also the mean pulse rate showed significant difference ($P<0.01$) in the mean pulse rate between treatment and between seasons. There was also significant difference ($P<0.05$) in the mean pulse rate between treatment and season.

In the present investigation the mean Hb (g/dl), PCV (%) and TEC (million/cmm) was found to be significantly lower ($P<0.01$) in summer compared to winter season. These variations may be attributed to the fact that high environmental temperature during summer season increases the oxygen consumption of animals through increased respiration rate. The higher oxygen intake increases the partial pressure of oxygen in the blood, decreases erythropoiesis, which in turn reduces the number of circulating RBCs and thus PCV and Hb values. The present investigation reveals higher TLC values during summer compared to winter. The increase in the TLC values during summer may be due to the state of stress that stimulates the anterior pituitary gland to secrete ACTH. The circulating ACTH in turn induces the adrenal cortex to produce glucocorticoids, involved in the mobilisation of neutrophils from the pool into the peripheral circulation.

Statistical analysis revealed significant difference ($P<0.01$) in the mean body weight values between treatment and between season. There was also significant difference ($P<0.01$) between day and season. The average body weight was higher in winter than in summer season.

The mean age at puberty revealed significant difference ($P<0.01$) in between treatment. There was also significant difference ($P<0.01$) in the mean age at puberty between season. The melatonin supplemented group showed early puberty followed by vitamin E supplemented group and control group. Statistical analysis revealed significant difference ($P<0.05$) in the litter size between treatment. The melatonin supplemented group showed more litter size followed by vitamin E supplemented group and control group. Also the litter size was more during winter than in summer.

A higher T3 value was obtained during winter in the three treatment groups. On the other hand the mean T4 value was comparatively high during winter in the three treatment groups. Statistical analysis revealed significant difference ($P<0.01$) in the mean

cortisol values between treatment and between season. There was also significant difference (P<0.01) between treatment and season. The mean GH value was comparatively low during winter in the three treatment groups Statistical analysis revealed significant difference (P<0.01) in the mean GH values between seasons. Statistical analysis revealed significant difference (P<0.01) in the mean progesterone concentration between treatment and between season. There was also significant difference (P<0.01) between day and season. The mean melatonin concentration in the treatment group 2 during summer was found to be higher which is significantly different (P<0.01) from treatment group 1 and 3.Similarly in winter, the mean melatonin concentration in treatment group 2 is significantly higher (P<0.01) from treatment group 1 and 3 respectively.

The mean vitamin E concentration in the treatment group 3 during summer was found to be significantly higher (P<0.01) from treatment group 1 and 2. Similarly in winter, the mean vitamin E concentration in treatment group 3 is significantly higher (P<0.01) from treatment group 1 and 2 respectively.

The mean LDH values during summer was found to be higher in the three treatment groups. On the other hand the mean LDH value was comparatively low during winter in the three treatment groups. Statistical analysis revealed significant difference (P<0.01) in the mean LDH values between season. The mean SOD values during summer was found to be higher in the three treatment group. On the other hand the mean SOD value during winter in the three treatment groups was low in the three treatment group .Statistical analysis revealed significant difference (P<0.01) in the mean SOD values between treatment and between season. There was also significant difference (P<0.01) between treatment and season.

The normalized expression for HSP70 during summer shows that the animals with Melatonin treatment i.e., treatment 2 have 1.98 fold lower expression than the control i.e., treatment 1 animals. Likewise, animals with Vitamin E treatment i.e., treatment 3 shows 0.56 fold lower expression than control animals i.e., treatment 1, during summer season. During winter, the animals with Melatonin treatment shows 0.70

fold higher expression compared to control animals. Similarly, animals with Vitamin E treatment have 1.28 fold higher expressions than control animals.

From the present findings, the following conclusions could be drawn :

1. THI during summer and winter were found to be 82.01 and 63.16 respectively
2. Supplementation of melatonin and vitamin-E during summer can reduce the expression of HSP-70 gene in pig.
3. Supplementation of melatonin and vitamin-E is effective in reducing the age at puberty during summer and winter in pig.
4. Supplementation of melatonin and vitamin-E can alter the biomolecular concentration during summer and winter in pig.

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