IMPACT OF SEASONAL VARIATIONS ON PHYSIO-BIOCHEMICAL PROFILES, BIOCHEMICAL AND HORMONAL STRESS MARKERS IN BLACK BENGAL GOATS

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By

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Dedicated To My Teachers and

My Beloved Family

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Certificate-I

This is to certify that the thesis entitled, "***Impact of seasonal variations on physio-biochemical profiles, biochemical and hormonal stress markers in Black Bengal goats***" submitted for the degree of **Master of Veterinary Science** in the subject of **Veterinary Biochemistry (Minor subject Veterinary Physiology)** of the Bihar Animal Sciences University, Patna, Bihar is the bonafide research work carried out by **Dr. Komal, Registration No- VM0012/2018-19,** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of this investigation have been fully acknowledged.

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Certificate- II

This is to certify that the thesis entitled, "**Impact of seasonal variations on physio-biochemical profiles, biochemical and hormonal stress markers in Black Bengal goats**" submitted by the Bihar Animal Sciences University, Patna, in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** in the discipline of **Veterinary Biochemistry (Minor subject Veterinary Physiology)** has been approved by the Students Advisory Committee after an oral examination of the student in collaboration with an external Examiner.

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ABSTRACT

The influence of different seasons i.e. summer, monsoon, post monsoon and winter had been studied on ten Black Bengal female goat. The data on meteorological variables like temperature and relative humidity was obtained from meteorological station Patna and temperature humidity index (THI) was calculated. Rectal temperature (°C), respiration rate and Pulse rate of each animal was recorded.

Blood sample was collected two times in month at 15 days interval in each season for estimation of hematological parameter such as Haemoglobin (Hb), total leucocyte count (TLC), total erythrocyte count (TEC), differential count (DLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Reduced glutathione (GSH) and Malondialdehyde (MDA) concentration, Superoxide dismutase (SOD) and Catalase (CAT) activity was estimated in erythocytic hemolysate. Serum was used for estimation of total protein, albumin, globulin albumin: globulin ratio, glucose, blood urea nitrogen (BUN), creatinine and serum enzyme like Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH) and electrolyte and mineral like Na, K, Cl, Ca, P, Cu, Zn, Mg. Serum was also used for estimation of hormone like T3, T4, TSH, cortisol and also for estimation of GSH, MDA concentration and SOD, CAT, DPPH and FRAP activity in serum. THI value indicating that goats were in stress during summer and monsoon season.

The levels of Hb, PCV, TEC, TLC neutrophils and monocytes were observed to be higher during winter and lower during summer. There was no significant difference observed in MCH and MCHC, while MCV was significantly lower during summer season. The season had no significant effect on the concentrations of serum total protein, albumin and globulin concentrations There was increased in level of BUN, creatinine concentration and activities of AST, ALT, LDH during summer season.

Concentration of sodium, potassium and chloride was varied with season, while level of Ca and P decreased during summer season. The magnesium concentration was gradually decreased from summer to winter season. There was no significant difference was observed in serum copper concentration. Serum zinc concentration was significantly lower during summer season as compare to other seasons. elevated level of serum hormone T3, T4, TSH and cortisol were observed during summer season.

Elevated GSH, MDA concentration and activates of SOD, CAT found during summer as compare to other season in both serum and erythrocyte. DPPH and FRAP activity in serum was significantly higher during summer season.

Abbreviations

µg	Microgram
µl	Microlitre
ALT	Alanine amino transferase
ANOVA	Analysis of variance
AST	Aspartate transaminase
BUN	Blood urea nitrogen
CAT	Catalase
DMSO	Dimethyl sulfoxide
DTNB	5,5' dithiobis (2- nitrobenzoic acid) -diphenyl tetrazolium bromide
EC	Enzyme commission
EDTA	Ethylene diamine tetra acetic acid
GPx	Glutathione peroxidase
GSH	Glutathione (reduced)
HB	Hemoglobin
IU	International units
LDH	Lactate dehydrogenase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
mg	Milligram
mM	Millimolar
MTT	3-(4, 5-Dimethyl thiazol-2-yl)-2, 5
nm	nanometer
nM	Nanomolar
PBS	Phosphate buffer saline
PUFA	Polyunsaturated fatty acids
PVC	Packed cell volume
RBC	Red blood cell
ROS	Reactive oxygen species

rpm	Revolutions per minute
SE	Standard error
SOD	Superoxide dismutase
T3	Triiodothyronine
T4	Thyroxine
TBA	Thiobarbituric acid
TCA	Trichloro acetic acid
TEC	Total erythrocyte count
TLC	Total leukocyte count
TSH	Thyroid stimulating hormone

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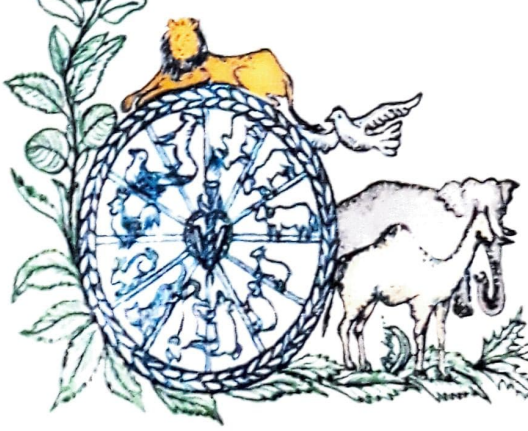


1. INTRODUCTION

About 80% of goat in world is reared in undeveloped and developing countries predominantly in tropical Africa and Asia (Morand-Fehr *et al*. 2004). In developing countries of the world, goats are recognized as the poor man's cow (Haldar and Ghosh 2014). India has 24.49 % goat population out of total livestock population. Bihar contribute 8. 99 % of goat population in India (19^{th} livestock census). In tropical countries like India, goats are raised commonly on open grazing system by the landless farmers and affected by adverse climatic condition. (Dangi *et al.* 2012). The Black Bengal goat is a local breed found in Bangladesh and eastern India including Bihar and characterized by its smaller physique and it is preferred by farmers due to high reproductive performance and good quality of meat and skin (Chowdhury *et al*. 2010).

Goats (Capra hircus) experience a diversity of environmental challenges resembling extensive variation in temperature, humidity and pathogenic invasion (Kaushalendra and Haldar,2012). The tropical climate of Indian sub-continent provides several environmental indications to different animal species to develop adaptive strategies to cope up with ecological stress. Being short-day breeder goats are mostly affected by temperature and humidity (Ghosh *et al.* 2013). The goats of tropical zone are capable enough to tolerate heat stress but during the months of monsoon and winter they get infected with several season-dependent diseases and may even succumb to death (Kumar *et al.* 2011). Conversely, animal's adaptation to ecological stress improve survivors of animal for which the metabolic strength of blood is highly important. (Ghosh *et al.* 2013).

Blood being the most important specialized tissue of the body creates an open channel system to provide the equal amount of nutrients, hormones and other important factors to different organs. Thus, blood biochemistry is not only the vital marker for the general health and basic metabolic pattern of an animal but also for adaptive modifications to different geographical distributions/conditions (Ghosh *et al.* 2013). Numerous biochemical markers are recognized to assess the health status of goat

exposed to stress. The monitoring of blood constituents at regular interval can predict the unnatural physiological condition and to formulate strategies which may prevent massive loss in goat husbandry due to pathogens or climatic factors (Bhatta, *et al.*2014).

Although goats are known to be adapted to harsh environments but their productivity is affected adversely by extreme climatic condition. Lowering of food intake and decrease in meat as well as milk production are commonly observed in heat stressed goat. In the present scenario of global climate changes, the physiological response of goat to elevated temperature is a major focus to maintain the goat rearing a sustainable venture. Goat undergo various kinds of stress, i.e. physical, nutritional, chemical, psychological and heat/thermal stress. Among all, heat stress is the most concerning issue nowadays in the ever-changing climatic scenario. Stress causes production of reactive oxygen species (ROS). Under normal physiological conditions, ROS are counterbalanced by the antioxidant defense system present in animal body (Omidi, *et al.* 2017). Any inequity between production of ROS and antioxidant defense system induces oxidative stress. Oxidative stress and antioxidant defense status depends upon disease seasonal variations (Maibam, *et al.* 2017). Mammalians cell possesses both nonenzymatic and enzymatic antioxidants defenses to up cope with damage caused by ROS. The nonenzymatic defenses include vitamin C, A, E β-carotene, glutathione (GSH) etc. Enzymatic ones include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Kumar, *et al.* 2011).

The levels of thyroid hormone may fluctuate according to the physiological stage, growth, breed, age, environmental temperature, and sex. The thyroid gland is prone to the variation of environmental temperature since its hormones are related to thermogenesis (Ribeiro *et al*. 2018b).The another hormone cortisol synthesized by the adrenal cortex stimulates the degradation and release of glucose, amino acid, and fat in the liver, muscle and adipose tissue and its concentrations changes according to several factors, such as circadian rhythm, season, photoperiod and diet composition (Sejian *et al*. 2010).

The susceptibility of red blood cells (RBCs) to oxidative damage is due to the presence of poly unsaturated fatty acid (PUFA), haem iron and oxygen which may produce oxidative changes in RBC (Kumar, et al. 2011). Fully mature RBC, lacking a

nucleus cannot produce new proteins in response to stress. They have to rely on proteins synthesized earlier in development to protect themselves from damage by ROS and thus ensure their own survival. The activities of antioxidant enzymes and lipid peroxidation are significantly increased during oxidative stress. So, they can be used as markers of oxidative stress (Agarwal and Sengupta,2020).

Considering these facts, the present study was conducted in goat to accomplish the following objectives-

- ✓ **To study the impact of seasonal variations on physio-biochemical profiles in Black Bengal goats**
- ✓ **To study the impact of seasonal variations on biochemical and hormonal stress markers in Black Bengal goats**

Review of Literature

2.REVIEW OF LITERATURE

Animal health and production are affected by changing climatic condition and it involves multifarious interaction between climatic and animal factors. Animal having powerful mechanisms of thermoregulation, but still they are unable to maintain body temperature strictly under thrilling climatic condition (Lu, 1989). The deviation in some climatic parameter like temperature and humidity are possible factors that negatively influence the growth and performance of domestic livestock. Once high ambient temperature is coupled with elevated air humidity, the animals become under stress and reduces their production, health and wellbeing (Ghavi Hossein-Zadeh et al. 2013; Alhussien and Dang 2017; Lakhanin et al 2018).

In small ruminant like sheep and goat the responses to thermal stress involves a number of metabolic, physiological and behaviour factor, for example elevated respiration frequency, rectal temperature, panting, sweating, increased water consumption and reduction in feed intake (Marai *et al*., 2007; Pardo and del Prado, 2020). However, sheep and goats are less prone to thermal stress than other domesticated animals (Lu, 1989; Pardo and del Prado, 2020), which might be offer them a competitive advantage over other domesticated animals under changing climatic scenarios (Pardo and del Prado, 2020). Ribeiro *et al.* (2016) found in his research that climatic variations affect directly on animals by changing their physiology. The climatic variation may adversely affect livestock and its production.

Goat has a considerable economic and ecological role and plays a significant role in the lives of farmers of the developing countries (Oluwatayo and Oluwatayo, 2012). Goats are integral part of the rural production system. Goats have exclusive biological attributes like small gestation period, quick growth rate, soaring prolificacy, high feed conversion efficiency and high diseases resistance capacity with easy market ability (Lebbie, 2004; Sejian, 2013; Adams and Ohene-Yankyera, 2014). Goats also play crucial role in contributing valuable animal proteins like meat and milk protein over and above food security in the varying climate scenario (Oluwatayo and Oluwatayo, 2012).

Temperature humidity index (THI) is used as indicator to measure the heat stress and it is based on air temperature and relative humidity (RH) (Lallo et al, 2018). The stress due to seasonal variation in animal can also be predicted with the help of THI (Akyuz et al., 2010). Temperature humidity index (THI) values have been established to differentiate between comfortable zone and heat tolerance thresholds in dairy animals (El-Tarabany and El-Tarabany 2015). The THI allows the integration of temperature and humidity into one value, which allows an objective comparison of environmental conditions (El-Tarabany 2016). THI of 70 or less is considered comfortable, 75 –78 is stressful, while greater than 78 shows that animals are under stress and are unable to maintain homeothermy (Silanikove, 2000; Dikmen and Hansen, 2009; Banerjee, *et al*, 2015; Ocheja *et al*., 2017).

2.1. Adaptive response of goat in different environmental conditions

The aptitude of each species and breed for adaptation in different environmental condition are central characteristics for building the finest method of raising and the good managing approach to surge the production output from animals (Mirkena *et al*. 2010). The animal's capacity to sustain the equilibrium of its body has been challenged by elevated environmental temperature in tropical and arid zone and low environmental temperatures in temperate zone (Silanikove, 2000). Goat has typical capability to thrive well in diverse environmental situations because of their great disease resistance ability, dexterous grazing behavior, good feed conversion efficiency, and drought tolerance capacity (Shilja *et al*. 2016). In addition to that goat rearing comprises little investment with best output chiefly due to its small size, fruitful breeding, low feed and housing management requirements. The inborn goat breeds in specific area are resilient with high thermo-tolerance and disease resistance capability, which help them to survive in harsh environmental circumstances.

Adaptation ability of animal tohot or cold climate involves several physiological systems for example respiratory, circulatory, excretory, endocrine, nervous, and enzymatic systems. But the change observed every time is not only between species, but also between breeds and also between individuals within breed in the harmonization of entire systems to retain the production performance under thermal stress (Marai and Haeeb 2010; Banerjee et al. 2015). Adaption ability of animals to hot or cold climatic

condition may be assessed on the basis of variations in morphological and physiological features indispensable for their existence **(**Marai and Haeeb 2010; Banerjee *et al*. 2015). The breed variations suggest different responses to the different stressors in a precise environmental condition and that rely on adaptation ability in that environmental condition (Jindal 1980).

Black Bengal goats are indigenous breed of Eastern and North-Eastern region of India and popular for its great quality chevon and skin worldwide. Goats are raised under open grazing system in most of the India. So, they are more prone to heat stress during summer (Mondal *et al*. 2019). Indigenous breeds have better adapting capability to their native environments where they have evolved then exotic and crossbred animals (Pragna *et al*. 2018a). Indigenous goat breeds are also more adjusted to abrupt environmental variations and disease outbreaks than other exotic and crossbred goats (Pragna *et al*. 2018b). It has been commonly recognized that diverse indigenous breeds display superior adaptive aptitudes in their relevant ecological zones, but only few breeds hold the capacity to bloom well in different ecological zones due to their higher genetic merit (Helal *et al*. 2010). There are numerous exclusive adaptive machineries in indigenous breeds which help them to live in a precise environment. Fluctuation of metabolic activities is common adaptive response in the indigenous breeds to cope up with external hot environment (Wheelock *et al*. 2010).

2.1.1. Physiological response

Seasonal variations exhibited by differences in meteorological circumstances, like temperature of environment and relative humidity, which considerably affect the animals' physiology and may cause heat stress in animal (Habibu *et al*. 2016). Physiological parameters like respiratory rate (RR), rectal temperature (RT), pulse rate (PR) and skin temperature (ST) are most common variables to assess adaptive capability in small ruminants. Usually season, sex, age, day time, exercise, physiological stage of animal, food intake capacity, water consumption and digestion are accountable for the alteration in cardinal physiological parameters (Ribeiro et al. 2018b). Respiration rate is one of the furthermost sensitive physiological sign to thermal stress and acts as one of the most valuable animal based indicator for heat stress in livestock (Gaughan *et al.* 2000). This may be due to variations in RR, in most of the time RR

alter the other cardinal physiological signs for instance RT and PR during heat stress (Singh *et al*. 2016). Small ruminants like sheep and goat survives in stressful environment by activating the evaporative cooling machinery and increasing the RR which ultimately leads to reduction in additional heat burden from them (Singh *et al*. 2016; Kumar *et al*. 2017).

Blood is considered as most effective means in the animal body which expedites heat dissipation process in the course of heat stress through its involvement in heat loss via the skin and respiratory tract. Some animal physiological and genetic machineries are meant for improvement of heat tolerance capacity in livestock for loss of heat from the blood via the skin surface and respiratory tract (Dalcin *et al*. 2016). Despite the fact that there is a substantial deviation in different parts of the body core at different times of the day but still rectal temperature is considered as a good indicator of body temperature. (Srikanda kumar *et al*. 2003). In several studies rectal temperature was found to be elevated with high environmental temperature and increased RT suggested the incompetence of the animal to retain the body temperature normal while exposing to heat stress in summer. Consequently, increase in RR and RT are usual physiological reactions of small ruminants to stress caused by heat (Marai *et al*. 2007). The PR along with the general metabolic status primarily reflects the homeostasis of circulation (Sejian *et al.* 2010). According to Devendra (1987) alteration in metabolism and muscle action in goats also alter the RR and PR. Increase in heart rate (HR) and PR may be due to either increase in movement of muscle which control the RR when RR is increasing or due to decreased resistance of peripheral vascular beds.

Seasonal variation in RT, RR and energy expenditure in goats under tropical conditions reported by Shinde *et al*. (2002). Increased PR in goat (Sirohi, Barbari and Gaddi) in the course of summer and winter has been reported by Banerjee *et al*. (2015) and its increase during summer season may be due to direct temperature effect on goats. The increased PR increases the flow rate of blood from core area to surface area that enhance body heat loss in sensible and insensible means. Banerjee *et al*. (2015) also reported in summer season, there is significant rise in RT and RR in goat as compared to spring season and fall in winter season in hot (Sirohi and Barbari) and cold (Gaddi and Chegu) environment adopted breeds of goat. Shilja *et al.* (2016) reported Osmanabadi

goats showed better adaptive response to heat stress, nutritional stress and combined stress raised in the Indian semi-arid regions by varying their physiological responses. Rathwa*et al*. (2017) reported that higher value of physiological parameters for example RT, RR, PR, and ST during summer season than winter season in goat.

Bhan *et al.* (2012) found that there was significant rise in RT in hot humid season than hot dry, spring and winter seasons in growing and adult Sahiwal cattle. RT, RR and PR exhibited significant increase during summer season as compared to winter season in dairy cattle (Kumar *et al*. 2017; Grewal and Aggarwal, 2018). Study of Sailo *et a.l* (2017) in Sahiwal and Karan Fries cow revealed that increase in RR and RT during summer compared to other seasons.

2.1.2. Haematological response:

Like other tissues of the animal body, the blood could experience sequence of adaptive transformation on exposure to different seasons environment. Blood cells are sensitive to temperature variation and acts as key indicator of physiological responses to stressors (Casella *et al*. 2013). Haematological variables are usually used for monitoring and evaluating the health status of small ruminants (Fazio *et al*. 2016). Several elements for example species, breed, sex, age, nutrition, diseases, physiological stage of animal and seasonal variations can affect the pattern of haematological parameters (Zumbo *et al*. 2011; Habibu *et al*. 2017b).

The central nervous system (CNS) modulates hematological variations in livestock due to heat stress and seasonal variation through hypothalamic-pituitary-adrenal (HPA) axis. CNS modulates erythrocyte attributes by influencing respiration, circulation, sweating and water intake so as to enhance heat loss during heat stress and conserve heat during cold stress. **(**Habibu *et al*. 2018**).** The leucocyte attributes are largely influenced by the effects of neuroendocrine products from the HPA axis on trafficking, proliferative and cytolytic activities of leucocytes (Lara and Rostagn, 2013). Generally, hematological profile is an important indicator of physiological changes in animals and may serve as an animal-based indicator for evaluating heat stress (Singh *et al.* 2016). Individually, interpretation of each hematological parameter gives meaningful insight into the responses of animals to heat stress; however, the integration

of many hematological parameters is important for maximum understanding (Casella *et al*. 2013).

Quantitative and morphological variations in blood cells are connected with heat stress. These results show the variations in hematocrit values, mean erythrocytes count and hemoglobin (Ribeiro *et al*. 2016). Erythrocytes vary in diameter and thickness according to the breed and the nutritional status of the animal; however, they are capable of being modified in form as they pass through capillaries. During physical exercise, the release of oxygen is processed more quickly, helping to increase the rate of oxygen consumption and, consequently, to increase hemoglobin. Poor nutrition, which occurs in animals under long-term heat stress, reduces the number of erythrocytes and hemoglobin level, resulting in a decrease of red blood cells in the bloodstream (Swenson and Reece 2006).

Study of Ghosh *et al.* (2013) in goat revealed that significant variation in total leucocyte count (TLC) among seasons. He also reported that higher eosinophil, neutrophil and lymphocyte count during winter season, while monocyte decreases during monsoon and winter seasons. Results of Habibu *et al.* (2017b) show that total elevated erythrocyte count (TEC) and packed cell volume (PCV) along with low mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were observed in kids during the hot dry season as compare to cold dry and rainy seasons. While Values of mean corpuscular volume (MCV) in both breeds were significantly lower in the summer compared to the other seasons. Variations in hemoglobin (Hb), PCV, TEC and TLC have been observed between seasons by Banerjee *et al*. (2015). Blood Hb, PCV and TEC levels decreased during summer and increased during winter in goats. He also observed increased neutrophil percentage and decreased lymphocyte percentage during summer. Hb, PCV, MCV, MCH and MCHC concentration is higher in pre-monsoon than in post-monsoon on the other hand TEC count is much lower in pre-monsoon than post-monsoon in goat (Bhatta *et al*. 2014).

Shibu *et al.* (2008) reported significantly lower Hb during heat compared to cold temperature. Naik *et al*. (2013) observed apparently higher level of Hb in Kankrej cattle during monsoon and summer than that of winter while apparent rise in PCV during

summer and monsoon due to the high temperature and humidity, respectively. Hot and humid environment provokes sweating causing loss of water leading to hemo-concentration and increase in TEC and thereby PCV value (Piccione *et al*. 2010). Shrikhande*et al*. (2008) reported the higher average Hb level during summer season. However, there was no significant difference in hemoglobin levels of rainy and winter season. Singh *et al*. (2016) reported significant decrease in Hb and PCV in all the three sheep (Chokla, Marwari and Magra) breeds due to heat stress.

Bhan *et al. (*2013) found variation in Hb, PCV and TLC concentration among different season in adult Karan Fries cattle, but no significant variation was observed by him in TEC concentration. Lateef *et al*. (2014) reported no significant changes in Hb and TLC concentration, while TEC, MCV, MCH and MCHC were higher during summer in kankrej cattle. He also found higher level of monocyte during summer, while other white blood cell shows non-significant difference.

2.1.3. Biochemical response

The knowledge of biochemical blood variables is essential to define the biochemical profile, the energy metabolism, metabolism disorders, liver function, bone abnormalities and, based on them to access the adaptation level of animals to climatic adversities (Gupta and Mondal, 2019).The interpretation of biochemical profiles is complex due to both the mechanisms that control the blood level of various metabolites and to the considerable variation in these levels promoted by several factors such as breed, age, physiological stage, diet, and management of the animal and the climate (Ribeiro *et al*. 2018b), when failures in glucose requirements occur.

Physiologically, blood glucose and total serum cholesterol levels are an adaptation mechanism that can be affected by high ambient temperatures. Their level shows greater variations during heat stress condition than in the comfort zone. Some researchers reported that heat stress decrease blood glucose and total serum cholesterol levels in goats (Pandey *et al*. 2012; Hooda and Upadhyay 2014; El-Tarabany *et al*. 2017). Decrease in glucose and cholesterol was observed in hot dry and hot humid season as compared to winter season by Chaudhary*et al*. (2015) and decrease in glucose and cholesterol may be due to decrease in feed intake (Scharf *et al.* 2010). The

level of total plasma protein, albumin and globulin decreases in goats due to increase in plasma volume as a result of heat stress. (Helal *et al*. 2010; El-Tarabany *et al*. 2017; Shaji *et al*. 2017; Inbaraj *et al*. 2017). Abdelatif *et al*. (2009) and Al-Eissa *et al*. (2012) observed higher levels of globulin in the rainy period, resulting in a low ALB/globulin ratio. Increased level of blood urea nitrogen(BUN), uric acid and creatinine during summer season in goat due to reduced blood flow toward kidney during heat stress condition (Suhair 2012; Ghosh *et al* 2013; Chaudhary*et al*. 2015; Rathwa *et al* 2017). Increase in urea in the hot dry and hot humid season also reported due to catabolism of protein to maintain the metabolic needs of the body (Chaudhary*et al*. 2015). Increase in urea is also an indication of dehydration as reported by Scharf *et al.* (2010).

Metabolic regulators are important determinants of physiological mechanisms during stressed conditions and are best assessed by determining the level of these enzymes in plasma or serum (Gupta and Mondal, 2019). Climatic stress lowers the alkaline phosphatase (ALP) and lactic dehydrogease (LDH) activity (Sevi *et al*. 2001; Helal *et al*. 2010). Decrease in these enzymes during heat stress is due to decrease in thyroid activity during heat stress (Helal *et al*. 2010;Ribeiro *et al*. 2018b). In other reports increase in LDH enzyme activity during the summer season due to an increase muscular activity involve in cellular respiration through catalization by which pyruvate from glucose was converted into usable energy as lactate in the cells (Bhan *et al*. 2013; Chetia *et al*. 2017). Serum level of aspartate transaminase (AST) and alanine transaminase (ALT) is helpful in assessing the animal welfare. The serum level of these enzymes increases during summer seasons due to their higher adaptive capability (Banerjee *et al*. 2015; Rathwa *et al*. 2017). On the contrary, no significant changes were observed in AST level of heat-stressed goats (Sharma and Kataria 2011).

Electrolytes are a critical element in cellular metabolism, muscle contraction, nerve transmission, and enzyme reactions (Piccione *et al*. 2007). Sodium is the primary ion involved in every metabolic process, from glucose transport to neural transmission, and its change influences the chloride concentration, as an increase or a decrease (Piccione *et al*. 2007). The seasonal variation in sodium and chloride content is consistent with that occurring in forages and in soil mineral content, that just in spring–summer seasons are richer in minerals than during the rest of the year (Xin *et al*. 2010).

Seasonal rhythmicity has been demonstrated for calcium, sodium, and chloride in the serum of goats (Piccione *et al*. 2012;Rathwa *et al.* 2017).Increase in sodium concentration during summer season due to dehydration (Chaudhary*et al*. 2015). Calcium is required for the normal functioning of a wide variety of tissues and physiological processes (Horst *et al*. 1994). Dietary content of phosphorus can influence calcium absorption and utilization (Huber *et al*. 2002). Inbaraj *et al*. 2017 reported that increase in serum phosphorus levels during monsoon compared to summer in goats. Magnesium supports the conversion of blood sugar to energy and is also considered as an anti-stress mineral, regulating HR and blood clots (Ribeiro *et al*. 2018a). Magnesium concentration in serum was significantly lower in the rainy season (Kaneko *et al*. 2009;Ribeiro *et al*. 2018a).

2.1.4. Endocrine response

Thyroid hormones triiodothyronine (T4) and thyroxine (T3) act on most of the tissues of the body. The key effect of thyroid hormones is to increase the metabolic activity in tissues, which result in to increasing the rate of oxygen consumption and heat production in the body cells. The overall effects are an increase in basal metabolic rate, making more glucose available to the cells, with stimulation of protein synthesis and increased lipid metabolism, stimulating cardiac and neural functions (Todini *et al*. 2007).

The thyroid gland is highly sensitive to the ambient heat variation **(**Rasouli *et al*. 2004: Ribeiro *et al.* 2018b). Appropriate thyroid gland function and activity of thyroid hormones are considered crucial to sustain productive performance in domestic animals (Todini *et al*. 2007). When the animals start to suffer due to heat, food ingestion is reduced and metabolism slows down, causing a hypo-function of the thyroid gland (Mc Manus *et al*. 2009). One of the principal functions of glucocorticoid under thermal stress is the stimulation of hepatic gluconeogenesis, which involves conversion of non-carbohydrate sources into glucose. The net result is an increase in hepatic glycogen and a tendency to increase blood glucose level. The insulin concentration in the blood directly reflects the energy status of the animal to sustain production under such extreme environmental conditions (Sejian *et al*. 2008). Hence, measuring metabolic

hormones such as thyroid hormones and insulin will provide clues as to how an animal adapts itself to changing environments by altering its metabolic activity.

The intensity of the adverse effects of high environmental temperatures on the animals depends on the efficiency of thermoregulatory mechanisms. The thyroid gland activity is reduced when the animal is exposed to hot conditions and increased when exposed to cold. Well-adapted animals respond quickly to environmental changes and thus can make the necessary physiological adjustments (Ribeiro *et al*. 2018b). The thyroid and adrenal glands play important roles in the adaptation of animals by controlling heat production in the organism. In animals exposed to high temperatures, the decrease in the secretion of thyroxine occurs due to decreased need for thermogenesis, as an important step to heat stress (Nazifi *et al*. 2003; Rasouli *et al*. 2004; Saber *et al*. 2009: Ribeiro *et al*. 2018b). The reduction of heat stress is due to the effect of heat on the hypothalamic-pituitary-adrenal (HPA) axis, thus decreasing the release of thyroid hormones, causing the animal to reduce basal metabolism. Likewise, the increased level of T3 and T4 during cold stress results in increased oxygen consumption and heat production by cells to increase basal metabolism (Bernabucci *et al*. 2010).

Seasonal variation in serum concentrations of thyroxine (T4) and triiodothyronine (T3) of goats has been widely reported in the literature (Todini *et al*. 2007; Ribeiro *et al*. 2016). In these studies, variation in the behaviour of the thyroid hormones was observed, and it was due to changes in temperature. Thyroid hormones, specifically triiodothyronine (T3) and thyroxine (T4), play a vital role in metabolic adaptation and growth performance of animals (Aleena *et al*. 2017). During heat stress, serum or plasma concentrations of T3 and T4 in three indigenous Osmanabadi, Malabari and Salem Black goat breeds are reduced in an effort to limit the basal metabolism with an intention to produce less metabolic heat (Pragna *et al*. 2018b). The TSH is an important pituitary hormone that enhances the production of thyroid hormones and its blood level is synchronized by the negative feedback mechanisms of thyroid hormones (Lalsangpuii *et al*. 2015).

The association between thermal stress and increased secretion of cortisol—the principal glucocorticoid hormone in small ruminants is well documented (Ali and Hayder 2008; Sejian *et al*. 2008a; Sejian and Srivastava 2010). Further cortisol is thermogenic in nature and its action will contribute to additional heat load (Alvarez and Johnson, 1973), and hence these combined stressed animals have the capacity to adjust the cortisol level to the minimum possible increase to elicit the thermal stress relieving effects.

Cortisol is the primary stress hormone that acts as an important biological marker for stress in goats (Binsiya *et al*. 2017; Shaji *et al*. 2017). The association between heat stress and increased secretion of cortisol in small ruminants is well documented (Shilja *et al*. 2016; Tajik *et al*. 2016; Chergui *et al*. 2017; Sejian *et al*. 2018). Further, cortisol is the principal stress reliever in ruminant species and hence its higher level is beneficial to cope up with heat stress condition (Shaji *et al*. 2017). The main function is to increase protein metabolism to convert protein into amino acids, supporting gluconeogenesis. The levels of cortisol increase during the initial phase of the active cycle of animals, according to the circadian rhythm and also during stress. During heat stress, one of the main functions of cortisol is to promote protein catabolism, converting the protein into amino acids to support gluconeogenesis (Sejian *et al*. 2010). The higher concentrations of plasma cortisol in summer and monsoon seasons is due to the activation of the hypothalamic–pituitary–adrenal axis which results in more cortisol secretion, thus help the heat stressed animals to maintain homoeostasis (Lakhani *et al* . 2020).

2.1.5. Antioxidant response

Cells continuously generates minute quantity of free radicals during their normal metabolism (Simioni *et al*. 2018; Abdelnour *et al.* 2019). Although, low levels of free radicals are essential in many biochemical processes and involved in homeostasis but excessive production of free radical like reactive oxygen species (ROS) leads to oxidative stress. The most important ROS includes hydroxyl radicals, superoxide anions, and hydrogen peroxide; these are the by-products of oxidative metabolism. Some other sources of ROS are NADPH oxidase and xanthine oxidase (Mahmood *et al.* 2020). Heat stress is similar to oxidative stress (Belhadj Slimen *et al*. 2016) and induces reactive oxygen species (ROS) production, especially the superoxide anion

(Mujahid *et al*. 2005; Verma *et al*. 2020). Highly reactive superoxide anion is the precursor of most ROS and a mediator in oxidative chain reactions (Belhadj Slimen *et al*. 2016). This superoxide anion is primarily produced in the mitochondrial electron transport chain in complex I and III (Verma *et al*. 2020).

Physiologically active ROS plays a pivotal role in cell signaling, gene expression, protein expression, intrinsic apoptosis, etc. to stabilize the cellular environment (Duhig *et al.* 2016). ROS are scavenged by antioxidant enzyme superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Celi, 2011; Kurutas, 2016; Simioni *et al*. 2018). Elevated environmental temperatures increase the production of reactive oxygen species (ROS), with subsequent induction of oxidative damage to lipids, proteins, and DNA (Maibam *et al*. 2018;Abdelnour *et al*. 2019).Heat stress increased lipid peroxidation which was associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids (Sunil Kumar *et al*. 2011). The unsaturated lipids are more prone to free radicals damage and hence, lipid peroxidation is considered as the biomarker of free radical load (Wagner *et al*. 1994). As oxidative stress is an indicative imbalance between oxidants and antioxidants, methods for quantifying oxidative stress mostly include direct or indirect estimation of oxidants and antioxidants. Malonaldehyde (MDA) is a low molecular weight end product which is generated as the free radical damages the lipids (Wong-Ekkabut *et al*. 2007).

CAT detoxifies H_2O_2 produced during different metabolic processes and also in stressful conditions by reducing it to H_2O and O_2 (Fridovich, 1978; Kalmath and Swamy, 2020). SOD in conjugation with catalase and GPx scavenges both intracellular and extracellular superoxide radicals and prevents lipid peroxidation (Agarwal and Prabhakaran, 2005). SOD is one of the major cellular enzymes that play a vital role in protecting the cells against the toxic effects of superoxide radicals produced during stress conditions like high temperature and humidity. SOD is a major antioxidant enzyme responsible for protecting and maintaining the oxidative/antioxidative balance in different compartments of cell and extracellular space. It catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide (Khan *et al*. 2020).

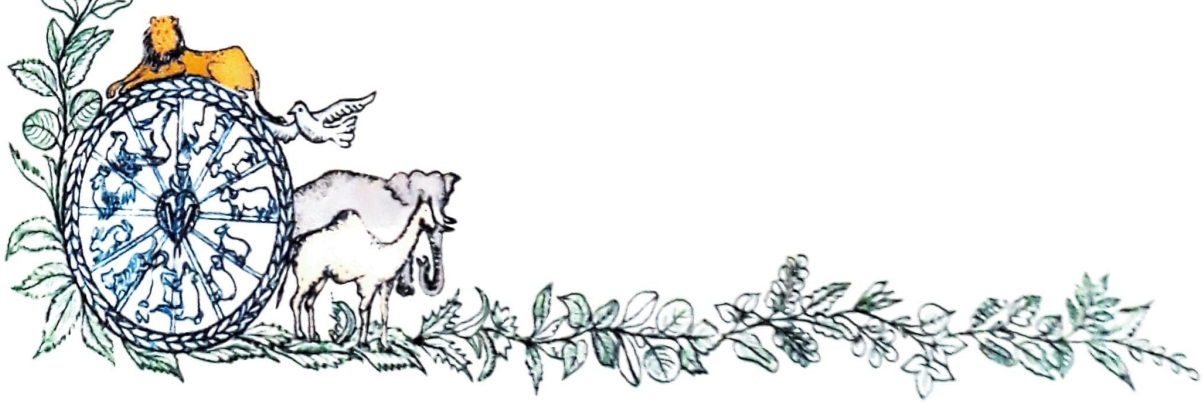
Ghosh *et al*. (2013) reported that during monsoon, anti-oxidant enzyme like CAT, SOD levels in blood were higher than summer and lowest during winter. Gupta *et al*. (2013) reported that SOD, CAT and GPx activity were higher during summer season than winter and moderate cold season in barbari goat, white in pashsmina goat SOD, Cat and GPX activity was higher during winter compare to summer and moderate cold seasons.CAT, SOD, GSH and lipid peroxidation significantly elevated in both cattle and buffalo during hot summer and cold winter than during spring season indicating stress due to climatic stress (Yatoo *et al*. 2014;Lakhani *et al*. 2018; Lakhani *et al*. 2020; Kalmath G. and Swamy, 2020).Kumar *et al*. (2019) reported increase in FRAP, DPPH and CAT activity due to high altitude stress in goat. Chaudhary*et al*. (2015) found that elevated level of SOD, GPx, MDA and total antioxidant capacity (TAS) during summer and monsoon seasons in surti buffalo.

The main function of erythrocytes is to transport oxygen into the tissues of the body. Hence, any condition that affects the erythrocyte alters its functions which may be detrimental to the body. Being the carrier of oxygen in every part of the living tissues, erythrocytes are exceedingly exposed to different forms of ROS (Mohanty *et al*. 2014). There is a firm association between erythrocyte oxidative stress and hemolytic anemia (Fibach and Rachmilewitz, 2008) heat stress (Kumar 2011) has also been depicted. Disintegration of RBC structure has also found as an integral part of ROS mediated stress (Banerjee *et al*. 2020). Haemoglobin present in erythrocytes also acts as a potent promoter of oxidative processes (Radwan *et al*. 2013). It is reported that the erythrocytic antioxidant enzyme activity (SOD, GPx, CAT activities) were higher during summer in cattle (Chetia *et al*. 2017; Verma *et al*. 2020; Kalmath and Swamy, 2020).

Polyunsaturated fatty acids (PUFA), the most vulnerable substrate of lipid peroxidation, are abundant in the membrane of erythrocytes (Bolchoz *et al*. 2002; Fazio *et al*. 2016). Malondialdehyde, an important product of lipid peroxidation, reacts with elements of cell membrane and thereby leads to increased permeability of cells and enzyme activities and ultimately lead to destruction of erythrocytes, hence malondialdehyde (MDA) is a good indicator of oxidative damage of erythrocyte (Banerjee *et al*. 2020). GSH is cornerstone to the cellular antioxidant defences as it acts

as an essential cofactor for antioxidant enzymes such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase (Dhanasree *et al*. 2020).

Materials and Methods



3.1 MATERIALS:

3.1.1 Chemicals and Reagents

All The chemicals, buffers and reagents used in this study were of analytical grade obtained from reputed manufacturers viz. Himedia (India), Sigma (USA), Sisco Research Laboratories (India), Merck Research Laboratries (USA), Thermo Fisher Scientific (USA). Details and composition of different reagents, solutions and buffers used in the present study are given in the appendix or at appropriate places.

3.1.2 Plasticware, glassware and other consumables

All the plasticwares like centrifuge tubes of various capacities, micropipette tips, micro- centrifuge tubes of 1.5 ml and 2.0 ml, syringes and needles, EDTA coated blood collecting vials were procured from different reputed firms viz. Tarsons (India), Dispovan HMD (India) and B.D (India).

The glasswares used for the study viz. Pipette, slide, test tubes were procured from Borosil (India) and neubauer chamber from Rohem (India). All these were thoroughly washed and sterilized in hot air oven before being used for the analysis.

3.1.3 Equipments:

Air displacement micropipettes (Thermo Scientific, Finland), -20 degree Celsius Deep freezer (Vestfrost, Denmark), Spectrophotometer (Systronics, India), Water bath (B.R Biochem, India), Micro-centrifuge machine (Beckman coulter, India), Vortex (Laboquest, India), Centrifuge machine (Remi, India), Semi-automated analyser (SS MED, India), pH meter (Systronics, India), Weighing balance (Mettler Toledo, India), Hot plate (Tarson,India), Binocular microscope (Magnus,India), ELISA reader (Biorad, US).

3.1.4Experimental animals:

The experiments were performed on ten Black Bengal female goat. The mean body weight of animals was 55.0 Kgs (approx.). Deworming was done for both ecto and endoparasites on regular basis. The animals were maintained in the goat shed of

University farm. All the animals were reared under strict management and proper hygienic condition throughout the period of study. This experiment was carried out for four different seasons which includes summer (April, May, June), monsoon (July, August, September), post monsoon (October, November) and winter (December, February).

3.2 Methods:

3.2.1 Meteorological parameters:

The data on meteorological variables like temperature and relative humidity were obtained from meteorological station Patna. Temperature Humidity Index (THI) was calculated from dry and wet bulb temperature using following formula (Johnson et al. 1963).

THI = 0.72 (Dbt + Wbt) + 40.6

where, Dbt = dry bulb temperature in °C

Wbt = wet bulb temperature in °C

3.2.2 Physiological responses:

Rectal temperature (°C) was recorded with a digital thermometer by keeping the thermometer in contact of rectal mucosa for about 2 min. Respiration rate per minute of each animal was recorded by visual observations of inward and outward abdominal movement. Pulse rate per minute was recorded from femoral artery of goat.

3.2.3 Schedule of Blood collection

Blood sample was collected two times in month at 15 days' interval in each season from jugular vein in tube containing anticoagulant EDTA and tube without anticoagulant. Blood with anticoagulant was used for hematological study and blood without anticoagulant was centrifuge at 3000 RPM for 15 minutes to separate the serum. The separated serum was stored at -20 °C till further use.

3.2.4 Haematological estimation in blood

All haematological study was performed within 24 hours of blood collection. Following haematological parameter were taken in to consideration during this study.

3.2.4.1 Estimation of haemoglobin:

Haemoglobin (Hb) concentration was estimated by cyanmethemoglobin method by using Drabkin's solution. In this method 5 ml of Drabkin's solution taken into a test tube then adding 20 µl of EDTA blood and then leave for 10 mint. Now after 10 min absorbance readings were taken at 540 nm in a spectrophotometer.

$$\text{Haemoglobin (gm/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 15.06$$

3.2.4.2 Estimation of total leucocyte count (TLC):

TLC was done manually using haemocytometer (Neubauer's chamber). Each sample was counted thrice, and the mean was calculated.

3.2.4.3 Estimation of total erythrocyte count (TEC):

TEC was done manually using haemocytometer (Neubauer's chamber). Each sample was counted thrice, and the mean was calculated.

3.2.4.4 Estimation of differential count (DLC):

DLC was done manually by putting a drop of blood from sample on a clear glass slide and spread into a thin blood smear. Then, blood smear was stained with a dye that helps to differentiate the types of white blood cells in the sample. After staining the DLC was counted under oil immersion microscopy.

3.2.4.5 Estimation of packed cell volume (PCV):

PCV was determined using capillary tubes in microhaematocrit centrifuge based on the technique described by Jain (1993) in which EDTA blood was taken in a capillary tube (microhematocrit tube) and then sealed from one end with wax and then centrifuge at 10000 RPM for 5 mint which separates the blood into plasma and packed cells and then PCV

$$\text{PCV (\%)} = \frac{\text{Volume of packed red blood cells}}{\text{Total volume of blood samples}} \times 100$$

3.2.4.6 Estimation of mean corpuscular volume (MCV):

MCV was calculated by using standard formulae (Jain, 1986). i.e.

$$\text{MCV (fL)} = \frac{\text{PCV (\%)}}{\text{RBC count per cmm}} \times 10$$

3.2.4.7 Estimation of mean corpuscular haemoglobin (MCH): MCH was

MCH was calculated by using standard formulae (Jain, 1986). i.e.

$$\text{MCH (pg)} = \frac{\text{Hb (gm/dl)}}{\text{RBC count per cmm}} \times 10$$

3.2.4.8 Estimation of mean corpuscular haemoglobin concentration (MCHC) :

MCHC was calculated by using standard formulae (Jain, 1986).i.e.

$$\text{MCHC (g/dl)} = \frac{\text{Hb (gm/dl)}}{\text{PCV}} \times 100$$

3.2.5 Serum biochemical estimation:

Following biochemical parameter has been taken in to consideration during this study.

3.2.5.1 Total protein:

Total protein was estimated by Biuret method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.2 Albumin:

Albumin was estimated by BCG method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.3 Globulin:

Globulin was calculated by subtracting albumin from total protein.

3.2.5.4 Albumin: Globulin Ratio:

AG Ratio was calculated by dividing albumin to globulin.

3.2.5.5 Glucose:

Glucose was estimated by GOD/POD method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.6 Blood Urea Nitrogen (BUN):

BUN was estimated by GLDH Kinetic method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.7 Creatinine:

Creatinine was estimated by Modified Jaffe's Kinetic method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.8 Alanine Transaminase (ALT):

ALT was estimated by Modified IFCC method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.9 Aspartate Aminotransferase (AST):

AST was estimated by Modified IFCC method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.10 Lactate Dehydrogenase (LDH) (E.C 1.1.1.2.7):

LDH was estimated by Modified, IFCC method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.11 Sodium:

Sodium was estimated by Na^+ Colorimetric method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.12 Potassium:

Potassium was estimated by K^+ Colorimetric method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.13 Chloride:

Chloride was estimated by Thiocyanate method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.14 Calcium:

Calcium was estimated by OCPC method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.15 Phosphorus:

Phosphorus was estimated by molybdate U.V. method using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.16 Magnesium:

Magnesium was estimated by Colorimetric method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.17 Copper:

Copper was estimated by Colorimetric method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.5.18 Zinc:

Zinc was estimated by Colorimetric method by using commercial kit coral clinical system as per manufacturer's protocol.

3.2.6 Serum hormone estimation:

T3, T4, (Thyroid stimulation hormone) TSH and Cortisol hormone was estimated in serum by using commercial ELISA kit Bioassay Technology Laboratory, UK as per manufacturer's protocol

3.2.7 Serum antioxidant estimation:

3.2.7.1 Reduced glutathione (GSH):

Reduced glutathione level in serum was estimated using the method given by Prins and Loos (1969). Serum sample (0.2 ml) was added to 4 ml of H_2SO_4 and mixed carefully. After 10 min of standing at room temperature, 0.5 ml of tungstate solution

was added. The tube was vortexed and then wait for 5 min in order to avoid crust formation on top of the supernatant. The mixture was then centrifuged for 15 min at 2000 rpm at room temperature. After centrifugation, 2 ml of the supernatant was added to 2.5 ml of Tris-buffer, 0.2 ml of DTNB (Appendix II) reagent was added and mixed well. Within a minute, absorbance was measured at 412 nm against blank in which 2 ml of distilled water was substituted for the supernatant.

GSH levels were conveniently calculated by using the extinction co-efficient (EC) 13100 M^{-1} cm^{-1} and results are expressed as mM GSH per ml of serum (mM/ml), using the following formula**.**

GSH (mM/ml of serum) =

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added}} \times \text{dilution factor}$$

3.2.7.2 Malondialdehyde (MDA)

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thoibarbuturic acid (TBA) as per modified method of Stock and Dormandy (1971) as described by Jain (1988). 0.2 ml of serum sample was suspended in 0.8 ml of phosphate buffer saline (PBS) (Appendix II) followed by addition of 0.5 ml of Tri choloro acetic acid (Appendix II). Mixture was vortexed and incubated for 2 hours at 37^0C. Now suspension was centrifuged at 2000 rpm for 15 minutes. 1ml of each supernatant was transferred in to another tube and to this 0.075 ml EDTA and 0.025 ml of thiobarbituric acid (Appendix II) was added. The mixture was heated in a boiling water bath for 15 minutes and then allows cooling at room temperature. Absorbance was measured at 535 nm.

Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, i.e., 1.56 x 10^5 $mmol^{-1}$ cm^{-3}. The amount of LPO was expressed as nano mol of MDA formed per ml of serum by employing following formula**.**

MDA produced (µmoles/ml) = Absorbance X dilution factor/ (1.56 X 10^5). MDA produced (n moles/ml of serum) =

OD of test Total volume of reaction mixture

---------- x ---------- x dilution factor

EC Volume of supernatant added

3.2.7.3 Superoxide dismutase (SOD) activity (E.C.1.15.1.1):

Serum superoxide dismutase (SOD) activity was measured using the method as described by Madesh and Balasubramanian (1997) with some modifications. In the microtitre plate method, the assay mixture in a total volume of 300µl per well consisted of 120µl PBS, 10µl serum sample, 5µl of 1.25 mM MTT and 15µl of freshly prepared 1mM pyragallol solution (Appendix II) to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150µl DMSO was added and absorbance was taken in ELISA reader at 570 nm.

The percent inhibition by the presence of SOD was calculated from the reduction of the MTT colour formation as compared to the MTT formazan formed in the absence of SOD which was taken as 100%. One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%.

SOD activity (units/ml) = 2 X 100 X A_T/ A_B

Where, A_T= Absorbance for test.

And A_B= Absorbance for blank.

3.2.7.4 Catalase (CAT) (E.C.1.11.1.6):

Serum catalase activity was estimated spectrophotometrically as per the method given by Cohen *et al.* (1970). The reaction commences with the addition of 50µl of serum to 2.95ml of phosphate buffer- H_2O_2 solution (Appendix II). In the blank, sample was substituted by same amount of PBS. Phosphate buffer- H_2O_2 gives an absorbance of 0.5-0.6 at 240 nm. The decrease in absorbance was measured for every 20 seconds up to 1 minute. Since a decrease in absorbance of 0.05 at 240 nm corresponds to the disappearance of 3.45 µmoles of H_2O_2, the units of catalase activity per ml serum was calculated as below.

0.05 change in absorbance = 3.45 µmoles of H_2O_2 disappeared

'A' change in absorbance in 50µl sample = 3.45 X A/ 0.05

So, 'A' change in absorbance in 1ml sample = 3.45 X A/ (0.05 X 0.05)

= 1380 A

3.2.7.5 Ferric reducing ability of plasma (FRAP) assay:

FRAP assay was performed as describe by Benzie and Strain (1996). Briefly 100 µl of plasma was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute after vortexing. Thereafter, samples were placed at 37°C in water bath and absorbance was measured after 4 minutes. Ascorbic acid standards (100 µM-1000 µM) were processed in the same way.

$$\text{FRAP value } (\mu\text{mol/l}) = \frac{\text{A} - \text{B}}{\text{X} - \text{Y}} \times 100$$

Where; A is reading of sample at 0 minute, B is reading of sample at 4 minute, X is reading of standard at 0 minute, Y is reading of standard at 4 minute and is FRAP value of 100 µM standard.

3.2.7.6 2,2-Diphenyl-1-picryl-hydrazyl (DPPH):

DPPH was estimated spectrophotometrically as per the method given by Chrzczanowicz et al (2008). The assay is based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine.In first step serum was deproteinization by mixing serum sample (0.2 ml) was mixed with 0.2 ml of acetonitrile and incubate for 2 min at room temperature. After incubation centrifuged for 10 min at 4^{O}C and then supernatant (deproteinized serum) was separated. Then 5 µl of 10 mmol DPPH in methanol was taken in a tube and then 5 µl of methanol was mixed and incubated for 3 min. Thereafter, 25 µl of supernatant was mixed and again incubated for 30 min. After incubation absorbance was taken at 517 nm.

Scavenging effect (Sc %) = [(A0-A1)/A0] × 100

Where A0 is the absorbance of the control; A1 is the absorbance of test samples.

3.2.8 Antioxidant enzyme estimations in erythrocytes (RBCs):

Blood samples were collected with anticoagulant and were centrifuged at 2000 rpm for 15 min. Then plasma and buffy coat were removed and then separate from erythrocyte. Now this erythrocyte pellet was diluted by using cold water to 1:10

dilution. During this process erythrocyte was hemolysed and haemolysate was prepared. This haemolysate was stored at -20 till further use and used for estimation of following antioxidant enzyme.

3.2.8.1 Reduced glutathione (GSH)

Reduced glutathione level in erythrocyte was estimated using the method given by Prins and Loos (1969). 0.2 ml of hemolysate was added to 4 ml of H_2SO_4 and mixed carefully. After 10 min of standing at room temperature, 0.5 ml of tungstate solution was added to clear the brown haemolysate. The tube was stoppered and the mixture was shaken vigorously for 5 min. The stopper was removed and suspension was allowed to stand for 5 min in order to avoid crust formation on top of the supernatant. The suspension was then centrifuged for 15 min at 2000 rpm at room temperature. After centrifugation, 2 ml of the supernatant was added to 2.5 ml of Tris-buffer, 0.2 ml of DTNB (Appendix II) reagent was added and mixed well. Within a minute, absorbance was measured at 412 nm against blank in which 2 ml of distilled water was substituted for the supernatant.

GSH levels were conveniently calculated by using the extinction co-efficient (EC) 13100/M/cm and results are expressed as mM GSH per ml of blood (mM/ml), using the following formula**.**

GSH (mM/g Hb) =

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added x Hb}} \times \text{dilution factor}$$

3.2.8.2 Lipid peroxide (LPO):

Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thoibarbuturic acid (TBA) as per modified method of Stock and Dormandy (1971) as described by Jain (1988). 0.2 ml of RBC suspension was suspended in 0.8 ml of phosphate buffer saline (PBS) (Appendix II) followed by addition of 0.5 ml of Tri choloro acetic acid (Appendix II). Mixture was vertexed and incubated for 2 hours at 37^0C. Now suspension was centrifuged at 2000rpm for 15 minutes. 1ml of each supernatant was transferred in to another tube and to this 0.075 ml EDTA and 0.025 ml of thiobarbituric acid (Appendix II) was added. The mixture was

heated in a boiling water bath for 15 minutes and then allows cooling at room temperature. Absorbance was measured at 535nm.

Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, i.e., 1.56×10^5 $mmol^{-1}$ cm^{-3}. The amount of LPO was expressed as nano mol of MDA formed per ml of whole blood by employing following formula**.**

MDA produced (n moles/g Hb) =

$$\frac{\text{OD of test}}{\text{EC}} \times \frac{\text{Total volume of reaction mixture}}{\text{Volume of supernatant added} \times \text{Hb}} \times \text{dilution factor}$$

3.2.8.3 Superoxide dismutase (SOD) activity (E.C.1.15.1.1):

Erythrocytic superoxide dismutase (SOD) activity was measured using the method as described by Madesh and Balasubramanian (1997) with some modifications. It involves generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2, 5 diphenyl tetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. In the microtitre plate method, the assay mixture in a total volume of 300µl per well consisted of 120µl PBS, 10µl RBC heamolysate, 5µl of 1.25 mM MTT and 15µl of freshly prepared 1mM pyragallol solution (Appendix II) to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150µl DMSO was added and absorbance was taken in ELISA reader at 570 nm.

The percent inhibition by the presence of SOD was calculated from the reduction of the MTT color formation as compared to the MTT formazan formed in the absence of SOD which was taken as 100%. One unit of SOD was defined as the amount of protein required to inhibit the MTT reduction by 50%.

SOD activity (units/g Hb) = 2 X 100 X A_T/ A_B X dilution factor X1/g Hb

Where, A_T= Absorbance for test.

And A_B= Absorbance for blank.

3.2.8.4 Catalase (CAT) (E.C.1.11.1.6) :

Activities of catalase enzymes were estimated by spectrophotometric method as described by Aebi *et al*. (1983). The principle of the assay based on the determination of the rate constant (S^{-1} K) of hydrogen peroxide decomposition by catalase.

2 ml of heamolysate (1:400 dilution) was directly taken in to cuvette followed by addition of 1ml of phosphate buffer- H_2O_2 solution (Appendix II). Decrease in absorbance recorded at 0 second and after 15 seconds against blank (1ml phosphate buffer and 2 ml sample). The activity of catalase per gram of heamoglobin was calculated as below.

Catalase (K/g Hb) = $2.3/\Delta t \times \log A_0/A_t \times$dilution factor 1/ Hb

Where A_0 = Absorbance at 0 second.

And A_t = Absorbance after 15 seconds.

Results

To delineate the influence of the environmental condition on animal wellbeing during different climatic seasons in Black Bengal goats, the meteorological data, physiological response, hematological, biochemical, hormonal attributes and oxidative stress related markers were studied. The blood from all the experimental goats were withdrawn during the four seasons viz. summer, monsoon, post-monsoon and winter and all samples were analyzed. The experimental values for all the study parameters were statistically analyzed, and the means were statistically compared between different seasons. All the statistical results are being presented both in table and graphical form of representation. The detailed output of the study results is described in following sections.

4.1 Meteorological Observations during different seasons

For observing the seasonal influence, the environmental temperature (ET), relative humidity (RH), and Temperature Humidity Index (THI) were recorded and represented in Table.1 and Fig. 1. The mean comparison shown a significant ($p<0.05$) difference in ET, RH and THI. The ET were recorded lowest in the winter and highest during summer. The RH were observed lowest during the summer and highest in monsoon season, however, it was statistically non-significant during the winter and post monsoon. The THI were observed higher than 72 during the summer, monsoon and post-monsoon and it was highest during the summer season. The meteorological observations were suggestive of the environmental stress.

4.2 Effect of seasonal variation on physiological response

The mean ± SE values in physiological responses like Rectal temperature (RT), Pulse Rate (PR) and Respiratory Rate (RR) were represented in Table.2 & Fig.2a,2b. The environmental condition had been shown to significantly($p<0.05$) influence the RT, PR and RR. From the results it was attributed that with the increase in environmental temperature, a corresponding increase in RT, PR and RR occurred. The significant ($p<0.05$) variation in RT were found in tandem with the environmental temperature i.e.

Table:1 Meteorological condition during experiment. Values are Mean ± SE

Season	Maximum Temperature (°C)	Relative humidity (%)	THI	Rain fall
Summer	$39.19^{d} \pm 0.34$	$46.75^{a} \pm 1.48$	$76.92^{c} \pm 0.33$	6.8
Monsoon	$33.84^{c} \pm 0.31$	$73.45^{c} \pm 1.12$	$80.87^{d} \pm 0.20$	361.96
Post-Monsoon	$30.49^{b} \pm 0.27$	$63.99^{b} \pm 1.36$	$72.83^{b} \pm 0.48$	4.4
Winter	$22.38^{a} \pm 0.39$	$65.85^{b} \pm 1.51$	$60.70^{a} \pm 0.45$	20.63

Mean bearing different superscripts (a, b, c , d) in a column differ significantly ($P<0.05$) between seasons.

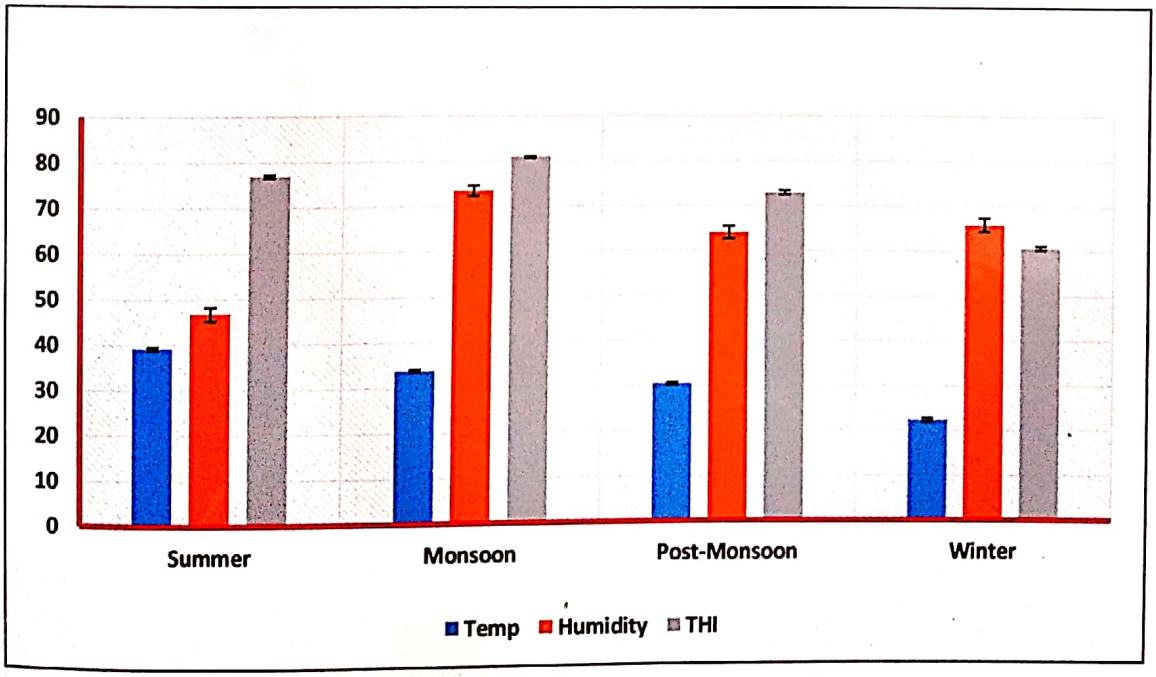


Fig.1: Meteorological condition during experiment

Table:2 Influence of seasons on physiological parameter during experiment. Values are Mean ± SE

Season	Rectal Temperature (°C)	Pulse Rate (Per Min)	Respiration Rate (Per Min)
Summer	$39.22^{d} \pm 0.03$	$88.60^{b} \pm 0.39$	$50.38^{d} \pm 0.31$
Monsoon	$37.97^{c} \pm 0.03$	$87.87^{ab} \pm 0.09$	$38.65^{c} \pm 0.33$
Post-Monsoon	$37.68^{b} \pm 0.03$	$87.68^{a} \pm 0.04$	$30.70^{b} \pm 0.30$
Winter	$37.32^{a} \pm 0.04$	$87.08^{a} \pm 0.12$	$23.41^{a} \pm 0.22$

Mean bearing different superscripts (a, b, c, d) in a column differ significantly ($P<0.05$) between seasons.

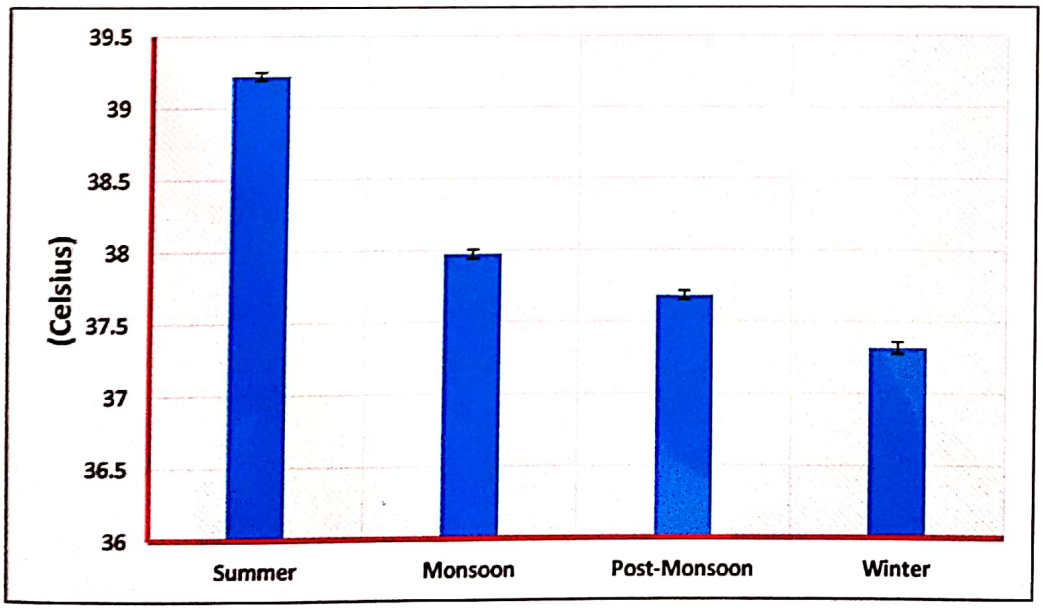


Fig. 2a: Influence of seasons on rectal temperature during experiment

Table: 3 Influence of seasons on haematological profile during experiment. Values are Mean ± SE

Season	Haemoglobin (g/dl)	TEC (10^6/mm^3)	TLC (10^3/mm^3)	PCV (%)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Summer	$8.56^{a} \pm 0.10$	$8.53^{a} \pm 0.14$	$11.87^{a} \pm 0.23$	$21.42^{a} \pm 0.25$	$37.36^{b} \pm 0.62$	$2.25^{b} \pm 0.20$	62.68 ± 1.16	$1.35^{b} \pm 0.16$
Monsoon	$9.10^{b} \pm 0.15$	$9.39^{b} \pm 0.18$	$12.40^{ab} \pm 0.22$	$27.41^{b} \pm 0.43$	$35.85^{ab} \pm 0.86$	$2.08^{ab} \pm 0.11$	65.01 ± 1.07	$1.20^{ab} \pm 0.12$
Post-Monsoon	$9.49^{b} \pm 0.31$	$9.86^{b} \pm 0.24$	$12.18^{a} \pm 0.24$	$28.44^{b} \pm 0.61$	$35.30^{a} \pm 0.76$	$1.97^{ab} \pm 0.19$	63.85 ± 1.57	$1.15^{ab} \pm 0.11$
Winter	$10.60^{c} \pm 0.17$	$11.03^{c} \pm 0.15$	$12.95^{b} \pm 0.22$	$32.34^{c} \pm 0.53$	$34.26^{a} \pm 0.68$	$1.55^{a} \pm 0.18$	63.85 ± 0.82	$0.83^{a} \pm 0.10$

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

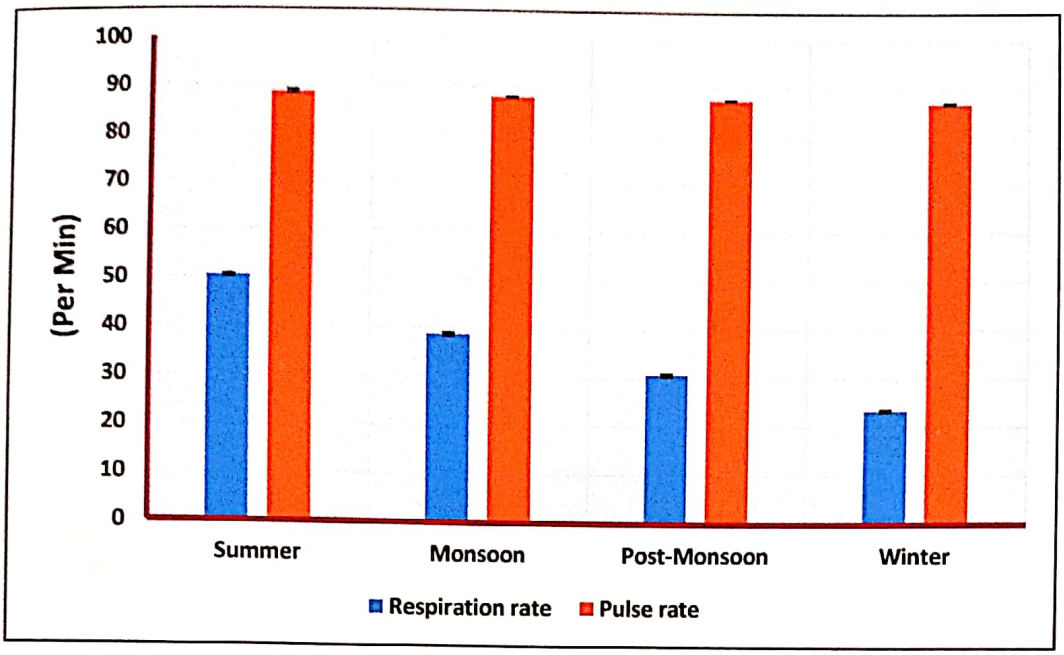


Fig. 2b: Influence of seasons on respiration rate and pulse rate during experiment

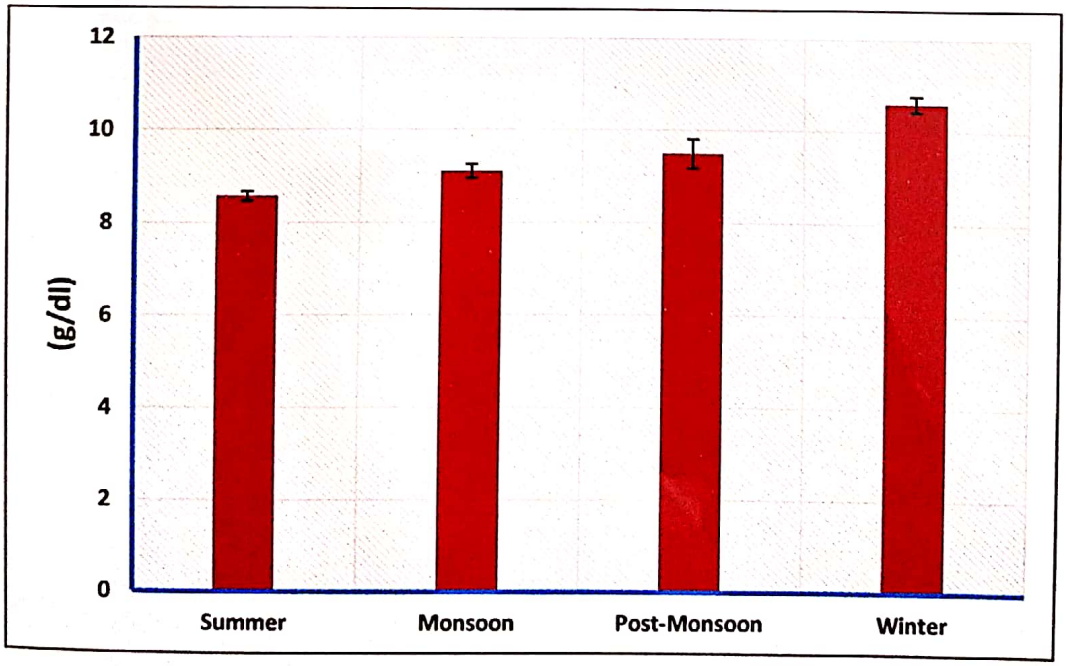


Fig. 3a: Influence of seasons on haemoglobin during experiment

Lowest during winter and highest during summer. A similar trend was also observed for the PR and RR.

4.3 Effect of seasonal variation on blood profile and erythrocyte indices

4.3.1 Effect of seasonal variation on blood profile

The results for hemoglobin (Hb), total erythrocyte count (TEC), Total leucocyte count (TLC), Packed cell volume (PCV), neutrophils, eosinophils, lymphocytes and monocyte are represented in Table 3 and Fig. 3a-h. A statistically significant ($p<0.05$) influence of season was observed on the Hb, TEC, TLC, PCV, neutrophils, monocytes and lymphocytes. An increasing trend was observed for Hb, TEC, TLC and PCV from summer to winter season. Furthermore, a higher value of neutrophils and monocytes and lower value lymphocytes were found during summer as compared to monsoon post-monsoon and winter season. The eosinophils were non-significantly ($p>0.05$) differ in different seasons.

4.3.2. Effect of seasonal variation on erythrocyte indices

The result for Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin concentration (MCHC) were represented in Table. 4 and Fig. 4 a-c. The MCV value was significantly ($p<0.05$) lower during summer season as compare to monsoon, post-monsoon and summer. However, there was a non-significant difference between the monsoon, post-monsoon and winter season. The other two erythrocytic indices i.e. MCH and MCHC were statistically non-significant ($p>0.05$) seasonal influence.

4.4 Effect of seasonal variation on serum metabolites, electrolytes and minerals concentration and activity of serum enzymes

4.4.1 Effect of seasonal variation on serum metabolites

The mean±SE values of metabolic parameters viz. total protein (TP), Albumin (Alb), Globulin (Glb), A: G ratio, glucose, blood urea nitrogen (BUN) and creatinine were depicted in Table 5 and Fig. 5 a-e.

The mean values of metabolic parameters i.e. glucose, A: G ratio, BUN and creatinine were found statistically significant ($p<0.05$) during different climatic seasons

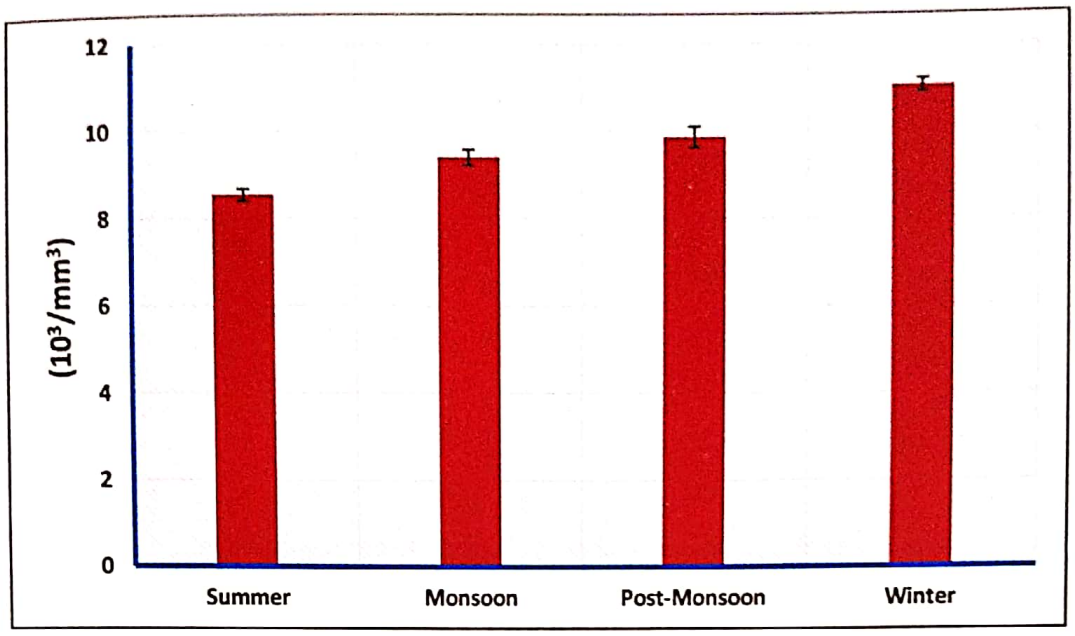


Fig. 3b: Influence of seasons on total erythrocyte count (TEC) during experiment

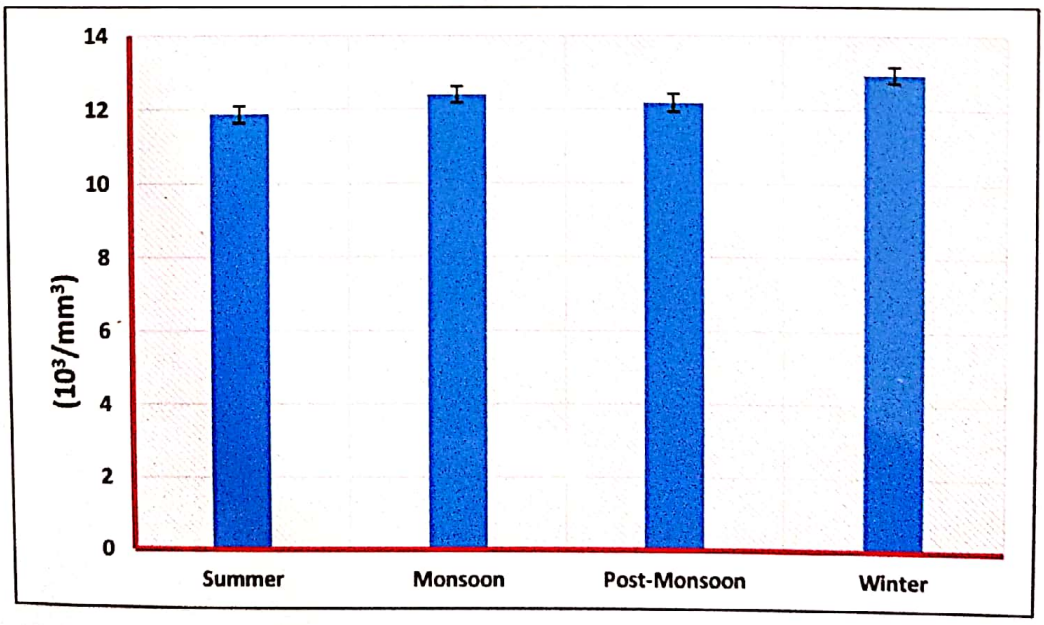


Fig. 3c: Influence of seasons on total leukocyte count (TLC) during experiment

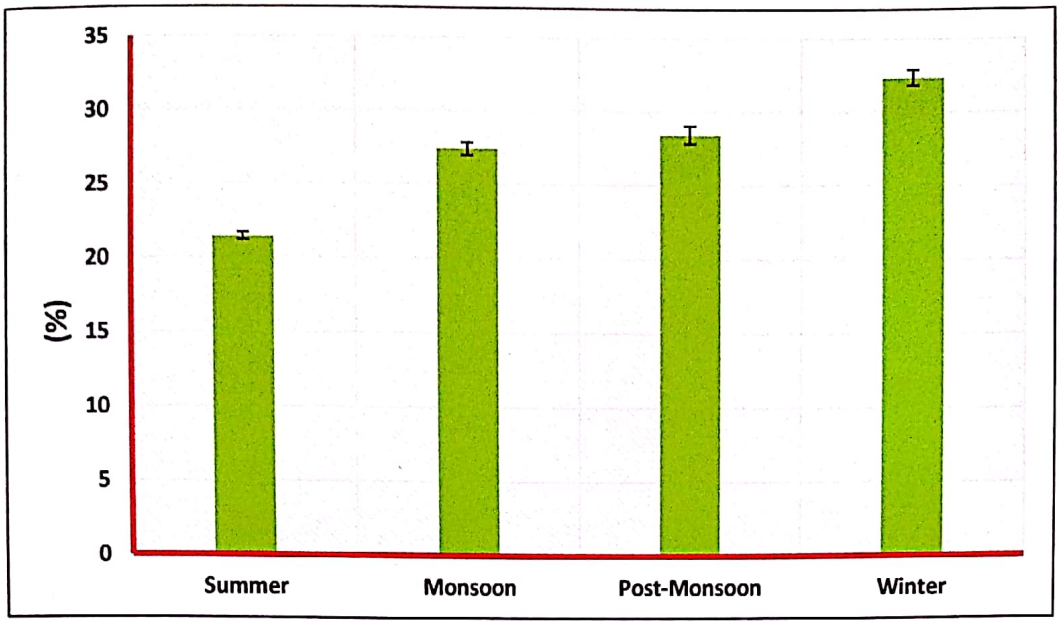


Fig. 3d: Influence of seasons on packed cell volume (PVC) during experiment

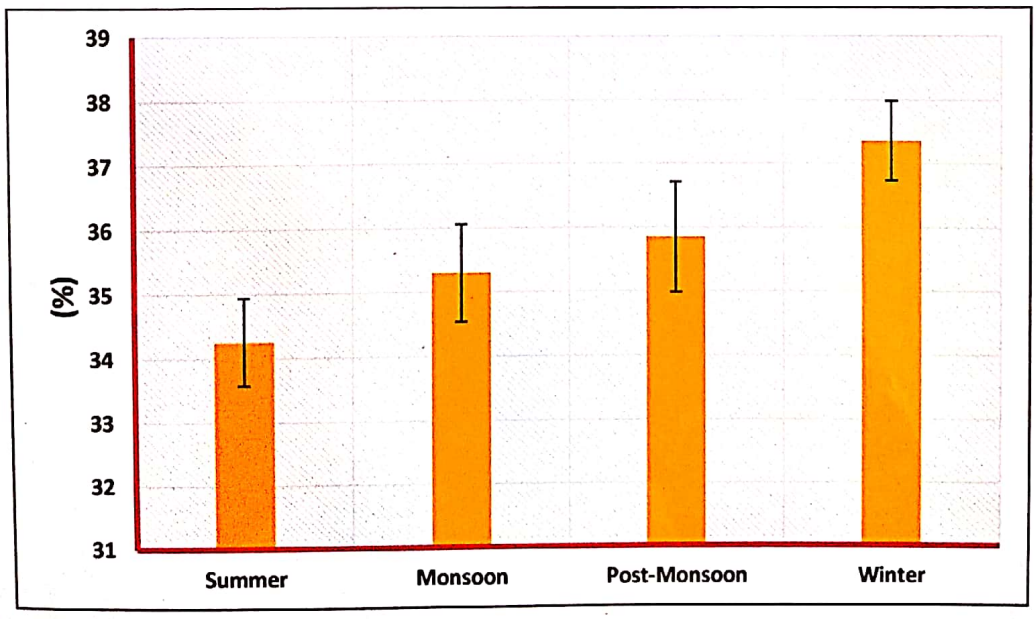


Fig. 3e: Influence of seasons on neutrophils count during experiment

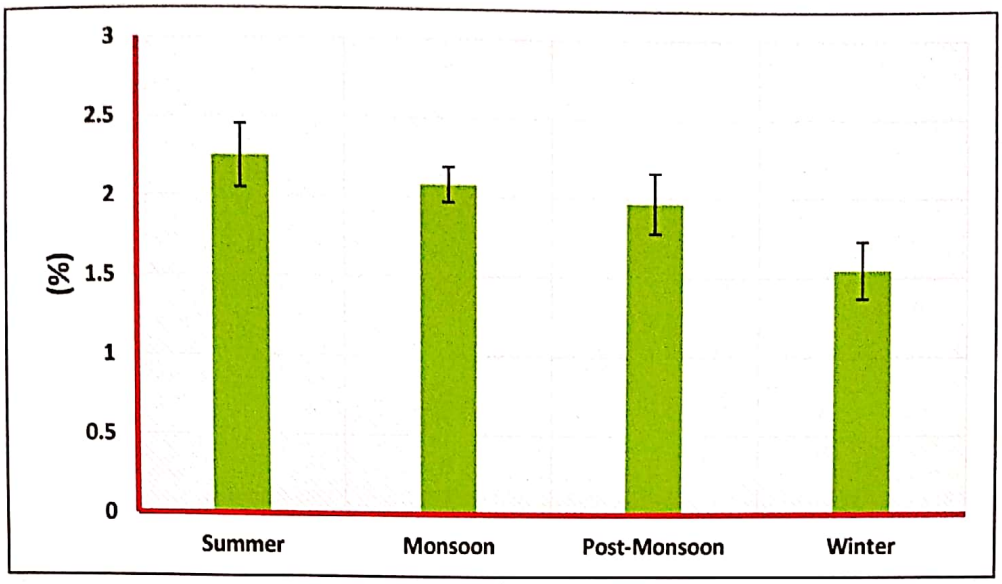


Fig. 3f: Influence of seasons on eosinophils count during experiment

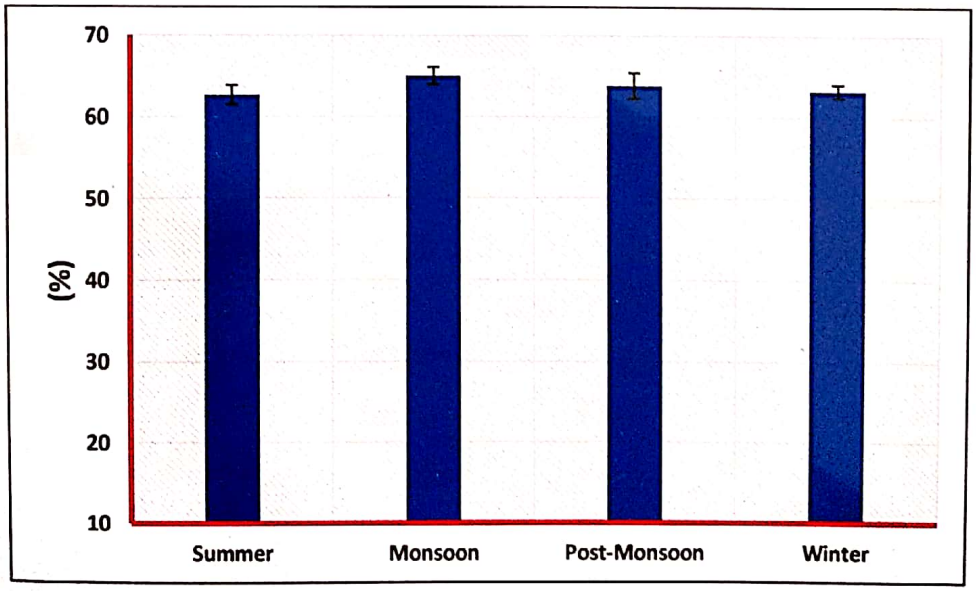


Fig. 3g: Influence of seasons on lymphocyte count during experiment

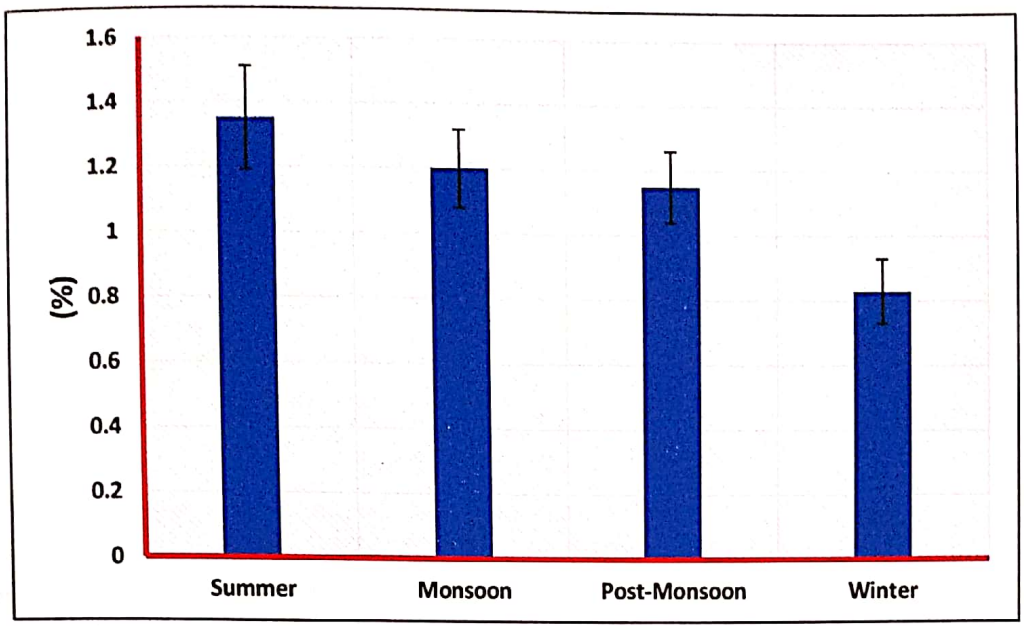


Fig. 3h: Influence of seasons on monocyte count during experiment

Table:4 Influence of seasons on erythrocytic indices during experiment. Values are Mean ± SE

Season	MCV (fl)	MCH (pg)	MCHC (%)
Summer	$25.56^{a} \pm 0.56$	10.22 ± 0.22	33.20 ± 0.70
Monsoon	$29.70^{b} \pm 0.80$	9.95 ± 0.28	32.97 ± 0.54
Post-Monsoon	$29.53^{b} \pm 0.99$	9.87 ± 0.43	33.24 ± 1.10
Winter	$29.68^{b} \pm 0.57$	9.70 ± 0.19	33.24 ± 0.56

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

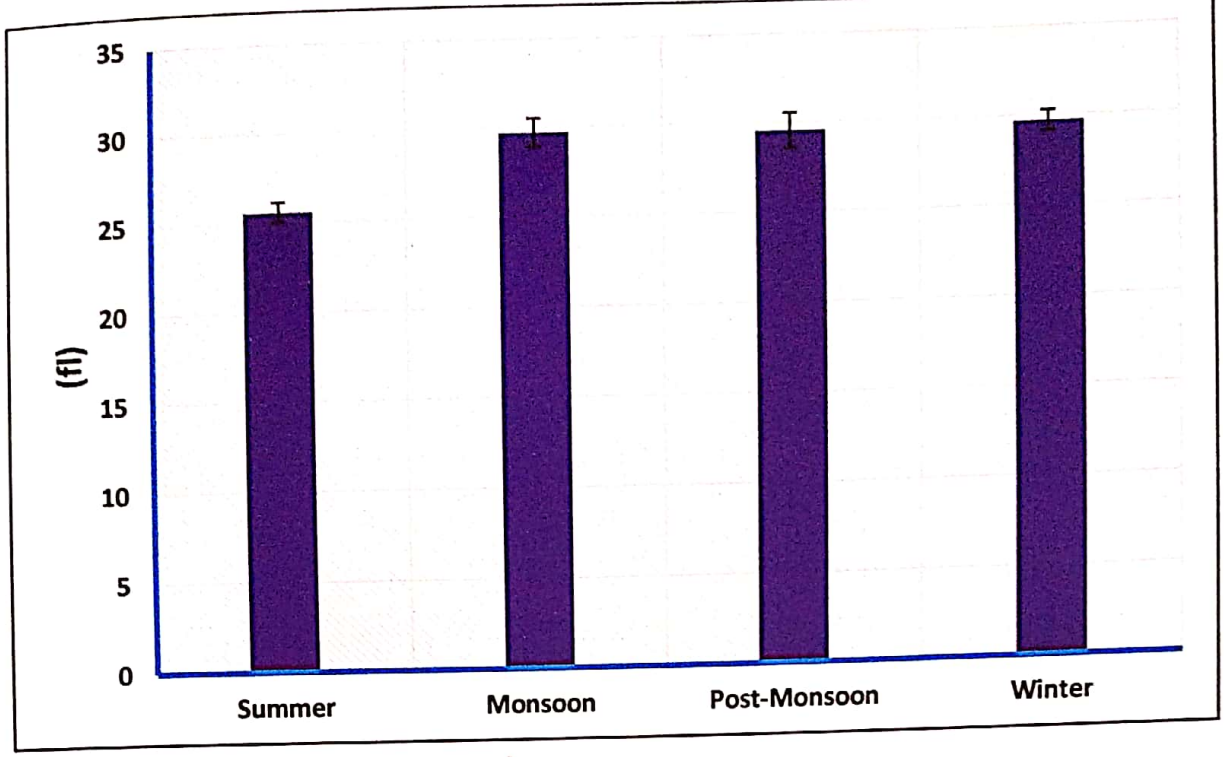


Fig. 4a: Influence of seasons on mean corpuscular volume (MCV) during experiment

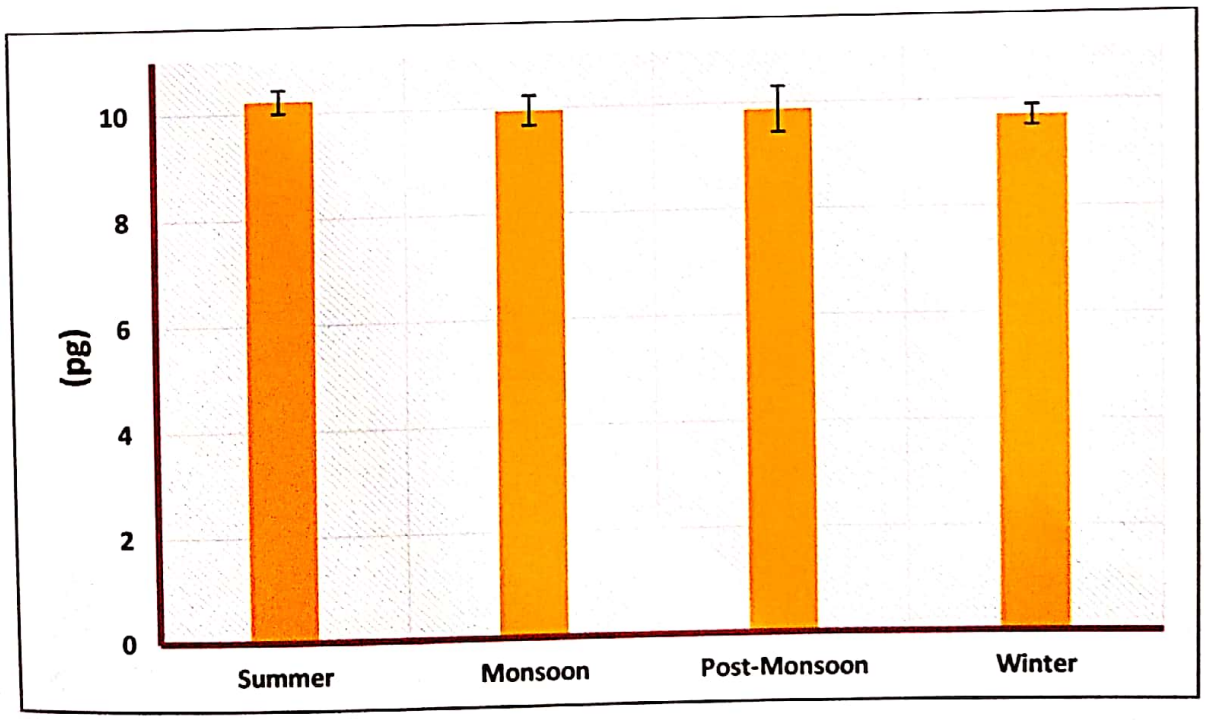


Fig. 4b: Influence of seasons on mean corpuscular haemoglobin (MCH) during experiment

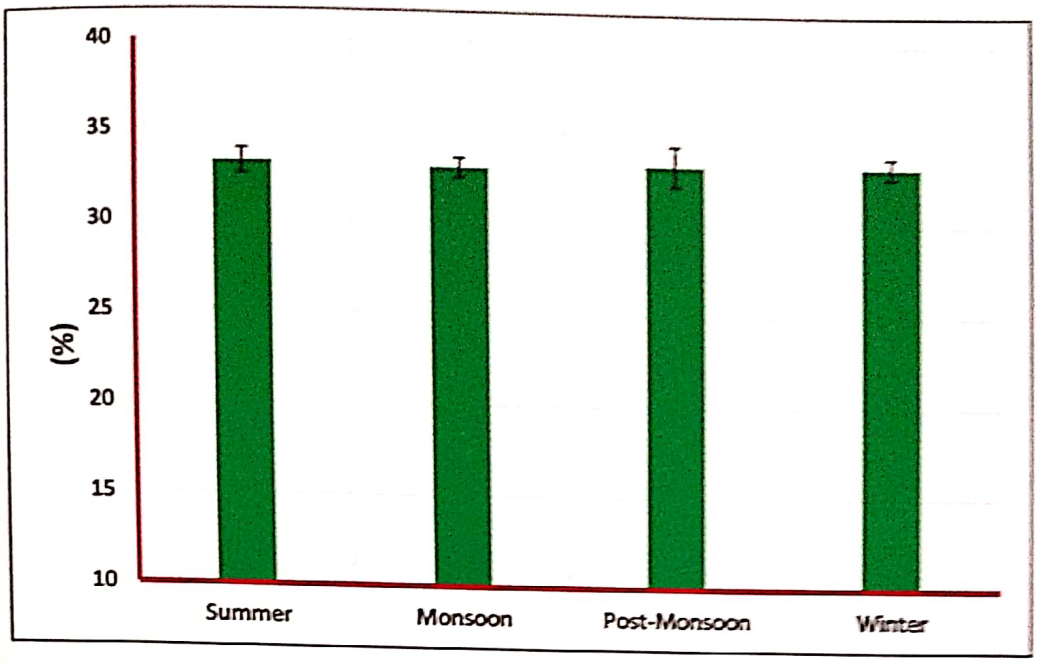


Fig. 4c: Influence of seasons on mean corpuscular haemoglobin concentration (MCHC) during experiment

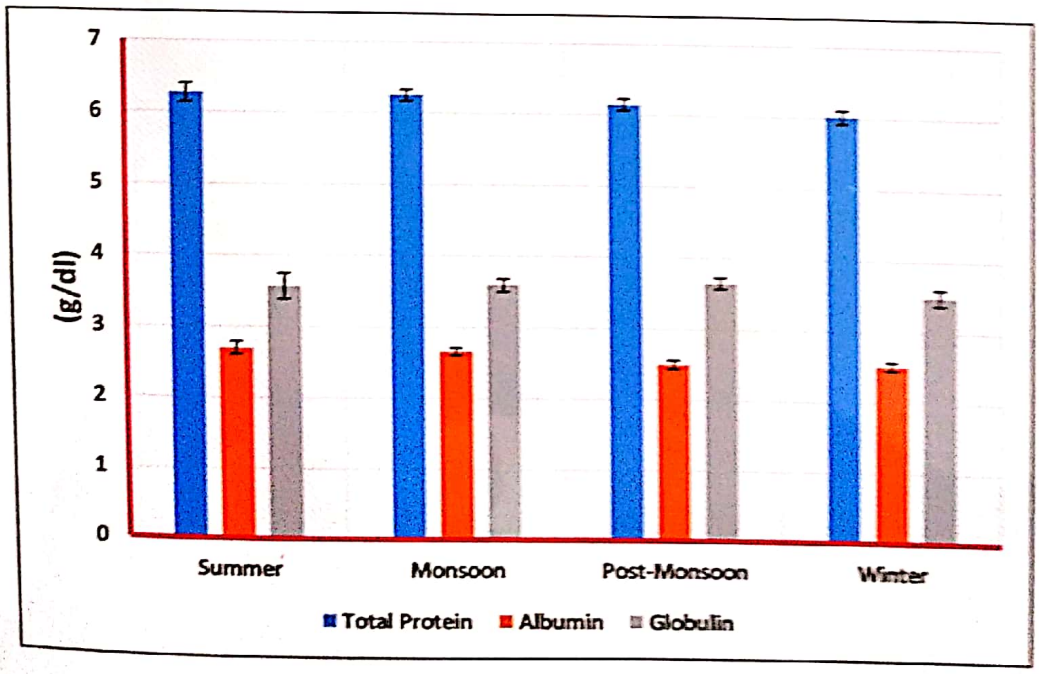


Fig. 5a: Influence of seasons on protein profiles during experiment

and the mean ± SE values of total protein, albumin and globulin were observed statistically non-significant ($p>0.05$) during different seasons.

A gradual decrease in TP concentration was observed from summer to winter season, and the average value of TP was found highest during summer and lowest during winter. For the albumin and globulin concentration, such pattern was not seen. Albumin concentration was found lowest during post-monsoon and highest during summer season whereas, globulin highest during post-monsoon and lowest during winter season. A: G ratio average value were maximum during summer and minimum during post-monsoon.

Mean values of blood glucose concentration shown a gradual increase from summer to winter season, it was recorded maximum during winter and minimum during summer.

Season has significant effect on BUN concentration except between summer and monsoon. Creatinine show significant variation only between winter season and other season. the maximum values for BUN and creatinine was observed during summer season and minimum during winter season.

4.4.2 Effect of seasonal variation on activity serum enzyme

The enzyme profile constitutes of Alanine Aminotransferase (AST, EC 2.6.1.2), Aspartate Aminotransferase (AST, EC 2.6.1.1) and Lactate Dehydrogenase (LDH. EC 1.1.1.27). The mean ± SE values of all three enzymes were shown in Table 6 and Fig. 6a and 6b.

Average value of ALT and LDH activity were found significant ($p<0.05$) and AST were non-significant ($p>0.05$) in respect to climatic season. A gradual decreasing seasonal response over ALT and LDH activity were observed from summer to winter season. The maximum value of ALT and LDH activity were found during summer and minimum during winter and moderate during monsoon and post-monsoon season.

4.4.3 Effect of seasonal variation on serum electrolytes:

The mean ± SE values of electrolyte viz. Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-)during different environmental seasons have been presented in Table.7 and depicted graphically in Fig.7a and 7b. The statistical analysis (ANOVA) showed that there was significant ($p<0.05$) seasonal effect on sodium, potassium and chloride.

Table: 5 Influence of seasons on serum metabolite during experiment. Values are Mean ± SE

Season	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G Ratio	Glucose (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Summer	6.27 ±0.13	2.70 ± 0.09	3.57 ± 0.18	0.98^{b} ±0.09	38.59^{a}±2.14	44.14^{c}±1.47	0.78^{b}±0.02
Monsoon	6.25 ±0.08	2.66 ± 0.05	3.59 ± 0.09	0.78^{ab}±0.03	46.61^{ab}±3.23	44.53^{c}±2.09	0.75^{b}±0.02
Post-Monsoon	6.15 ±0.08	2.50 ± 0.06	3.65 ± 0.08	0.70^{a} ±0.02	49.05^{b} ±2.84	36.03^{b}±1.15	0.74^{b}±0.02
Winter	6.02 ±0.09	2.52 ± 0.06	3.50 ± 0.11	0.80^{ab}±0.05	60.03^{c} ±1.86	27.73^{a}±1.04	0.65^{a}±0.01

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

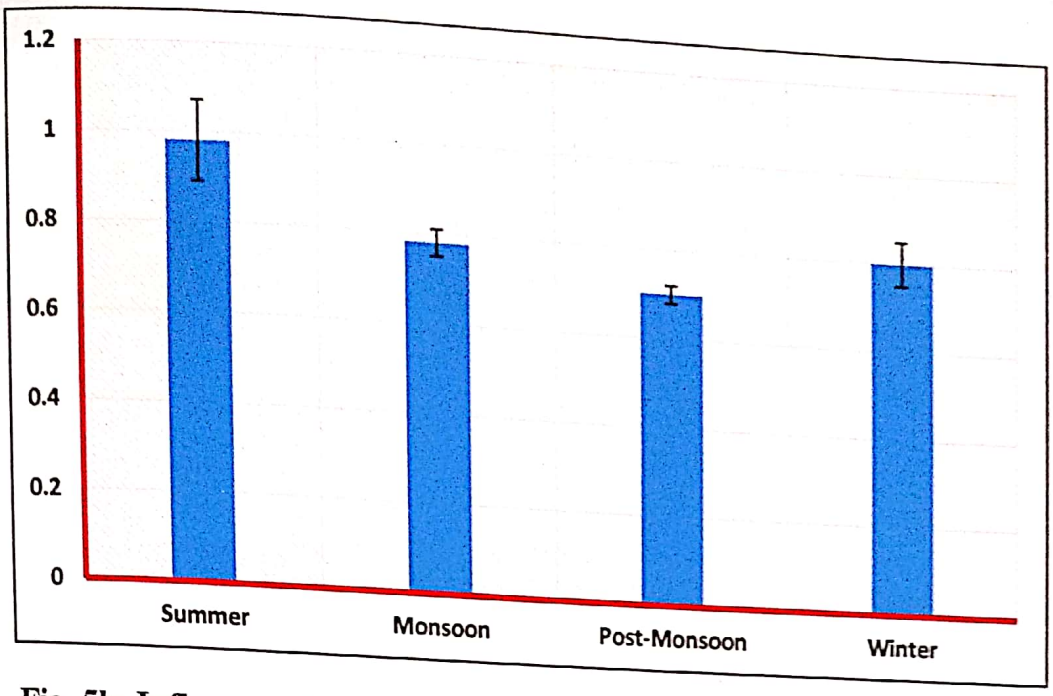


Fig. 5b: Influence of seasons on albumin: globulin ratio during experiment

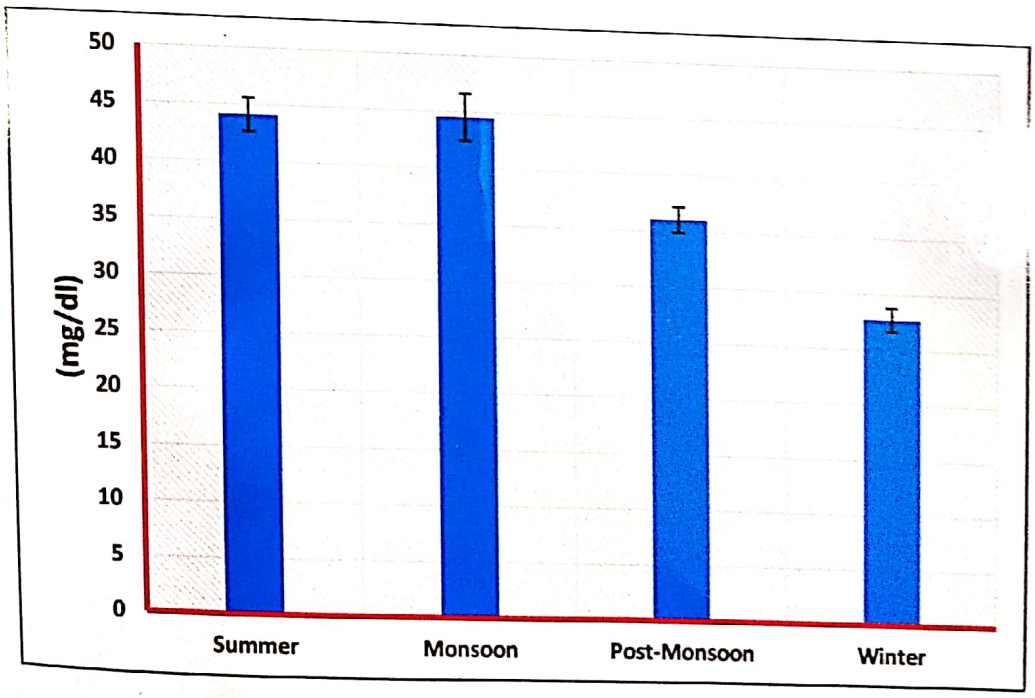


Fig. 5c: Influence of seasons on blood urea nitrogen concentration during experiment

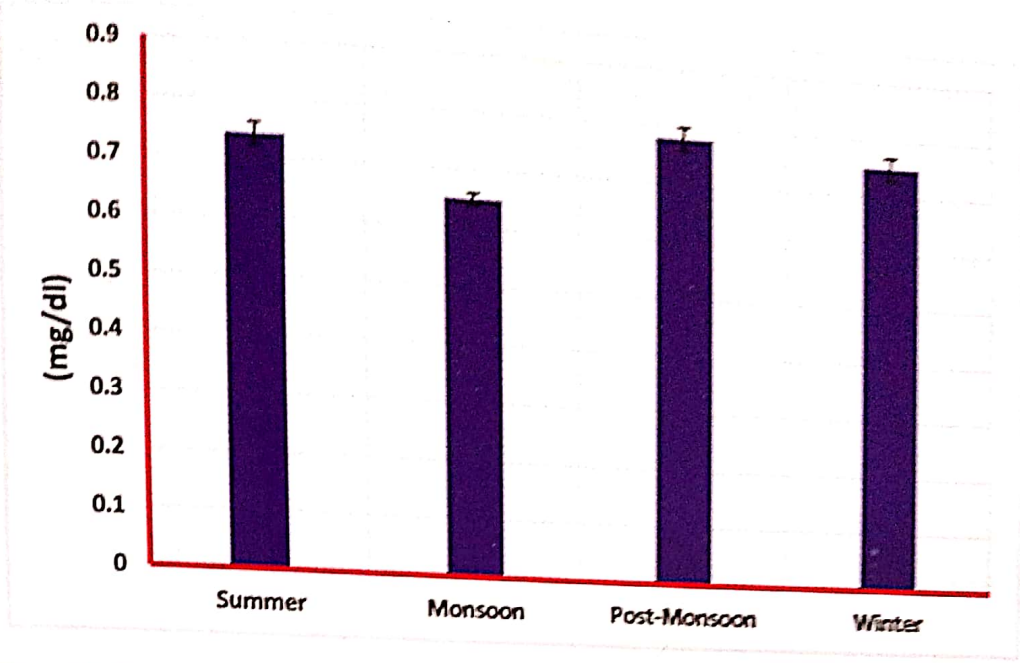


Fig. 5d: Influence of seasons on creatinine concentration during experiment

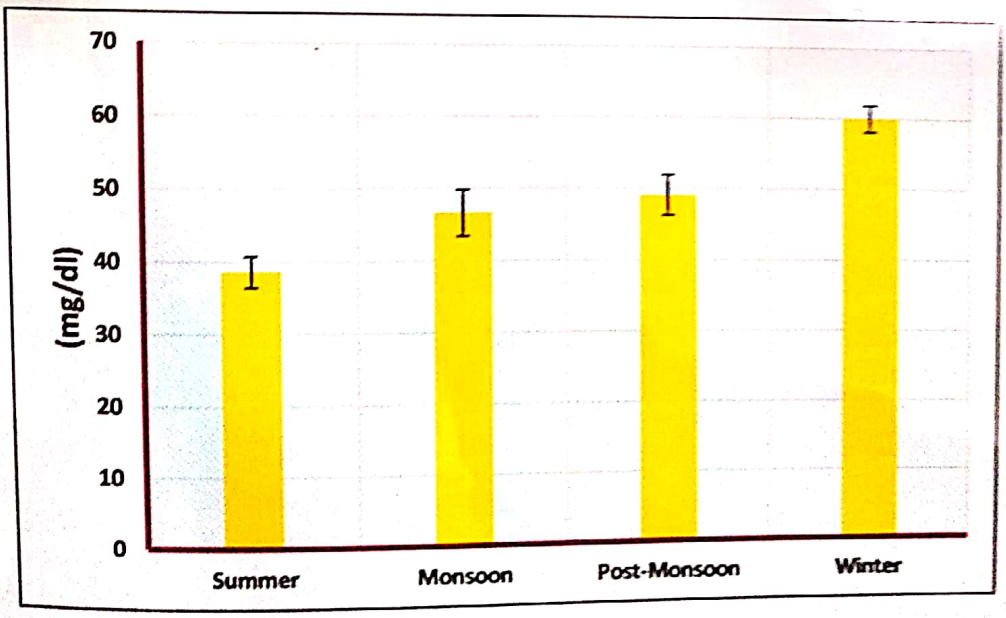


Fig. 5e: Influence of seasons on glucose concentration during experiment

Table:6 Influence of seasons on serum enzymatic profile during experiment. Values are Mean ± SE

Season	ALT (U/L)	AST (U/L)	LDH (U/L)
Summer	30.21^{c}±0.81	92.29±2.40	197.30^{c}±3.72
Monsoon	27.28^{b}±0.74	89.73±2.40	156.28^{b}±3.66
Post-Monsoon	22.23^{a}±1.10	93.17±2.75	112.97^{a}±3.68
Winter	21.18^{a}±0.69	88.56±2.05	101.29^{a}±3.34

Mean bearing different superscripts (a , b , c) in a column differ significantly (P<0.05) between seasons.

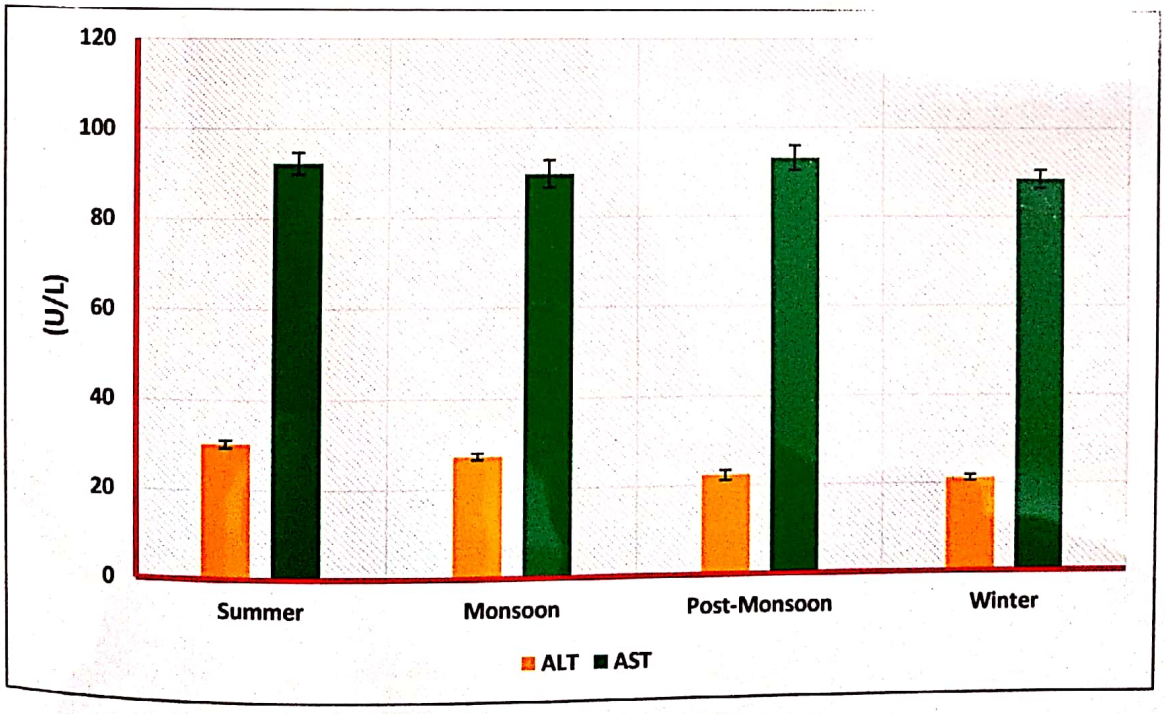


Fig. 6a: Influence of seasons on alanine amino transferase and aspartate transaminase activity during experiment

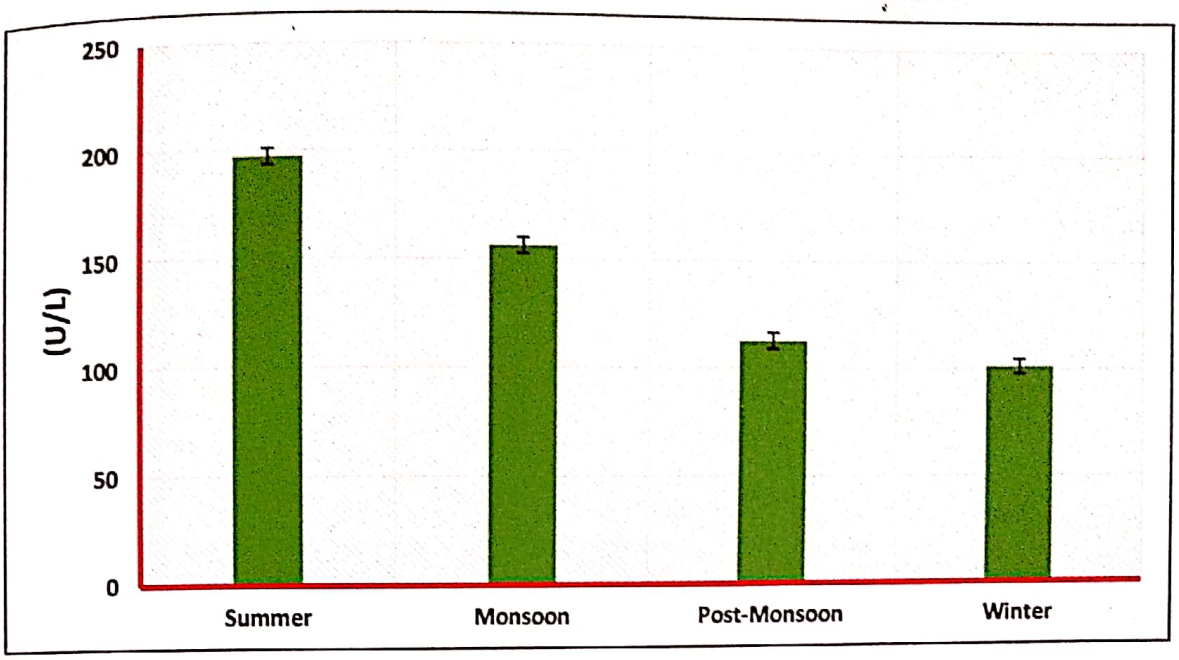


Fig. 6b: Influence of seasons on lactate dehydrogenase activity during experiment

Table.7 Influence of seasons on electrolyte profile during ( ) are Mean ± SE

Season	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Summer	$150.86^{b} \pm 1.38$	$6.14^{b} \pm 0.10$	$115.28^{b} \pm 2.10$
Monsoon	$139.72^{a} \pm 3.77$	$4.95^{a} \pm 0.11$	$114.31^{b} \pm 1.62$
Post-Monsoon	$134.86^{a} \pm 1.86$	$4.95^{a} \pm 0.15$	$110.34^{b} \pm 1.06$
Winter	$132.56^{a} \pm 2.51$	$4.82^{a} \pm 0.14$	$102.09^{a} \pm 1.47$

Mean bearing different superscripts (a, b , c) in a column differ significantly ($P<0.05$) between seasons.

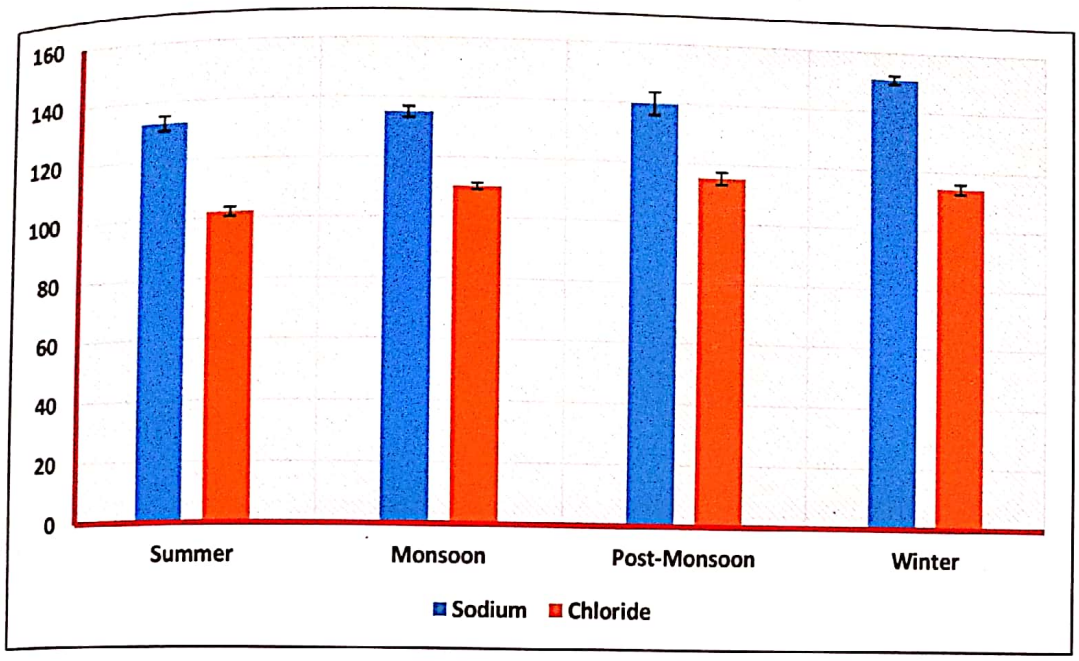


Fig. 7a: Influence of seasons on sodium and chloride concentration during experiment

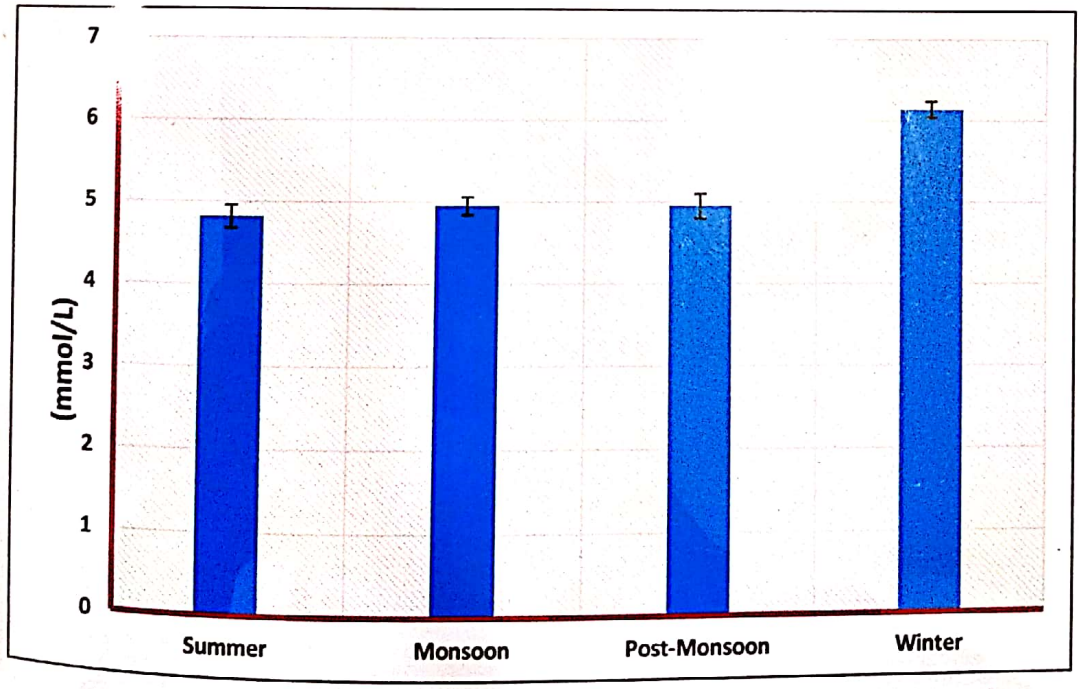


Fig. 7b: Influence of seasons on potassium concentration during experiment

For all the three electrolytes, the lowest concentration was observed during the winter season. However, during the monsoon, post-monsoon and winter the mean values of sodium and potassium were statistically non-significant ($p>0.05$). The mean concentration of sodium and potassium were observed maximum during summer.

In case of chloride concentration, the mean values were significantly ($p<0.05$) higher during summer, monsoon and post monsoon as compared to winter season. Chloride concentration showed maximum value in summer and monsoon and minimum during winter.

4.4.4 Effect of seasonal variation on serum minerals:

The mean ± SE values of serum minerals i.e. Calcium, phosphorus, magnesium, copper and zinc during different seasons is represented in Table 8 and depicted graphically in Fig.8a,8b and 8c. The statistical analysis (ANOVA) shown significant ($p<0.05$) effect of season on zinc, magnesium, calcium and phosphorus concentration, whereas there was a non-significant effect was observed on serum copper concentration.

A gradual increase on the Zinc concentration was observed from summer to winter season. However, the concentration during the monsoon, post monsoon and winter season were statistically similar.

The magnesium concentration was gradually decreased from summer to winter season. Further, during the summer and monsoon season its concentration was statistically non-significant ($p>0.05$).

However, for calcium and phosphorus concentration any such season pattern was not observed. The average value of calcium was observed to be maximum during winter and minimum during summer and moderate during monsoon and post-monsoon whereas, the average value of phosphorus was observed to be maximum during post-monsoon and minimum during winter and moderate during monsoon and summer.

4.5 Effect of seasonal variation on serum hormone status

The influence of the climatic season on the fuel metabolism, the endocrine function was evaluated. This include the thyroid hormone profile and stress hormone i.e. cortisol. The mean ± SE values of (ng/ml) thyroid hormones (T_3, T_4 and TSH) and cortisol have been presented in Table.9 and depicted graphically in Figure.9a,9b 9c and 9d.

Table: Influence of seasons on Mineral profile during experi... Values are Mean ± SE

Season	Copper (mg/dl)	Zinc (mg/dl)	Magnesium (mmol/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
Summer	1.75 ± 0.08	1.66^{a} ± 0.09	2.48^{b} ± 0.12	7.47^{a} ± 0.21	5.28^{a} ± 0.16
Monsoon	1.85 ± 0.13	2.45^{b} ± 0.09	2.38^{b} ± 0.19	8.21^{a} ± 0.27	5.46^{ab} ± 0.13
Post-Monsoon	1.76 ± 0.13	2.45^{b} ± 0.09	2.17^{ab} ±0.29	8.17^{a} ± 0.31	5.91^{b} ± 0.19
Winter	1.66 ± 0.09	2.58^{b} ± 0.09	1.69^{a} ± 0.07	9.23^{b} ± 0.20	5.25^{a} ± 0.13

Mean bearing different superscripts (a, b, c) in a column differ significantly (P<0.05) between seasons.

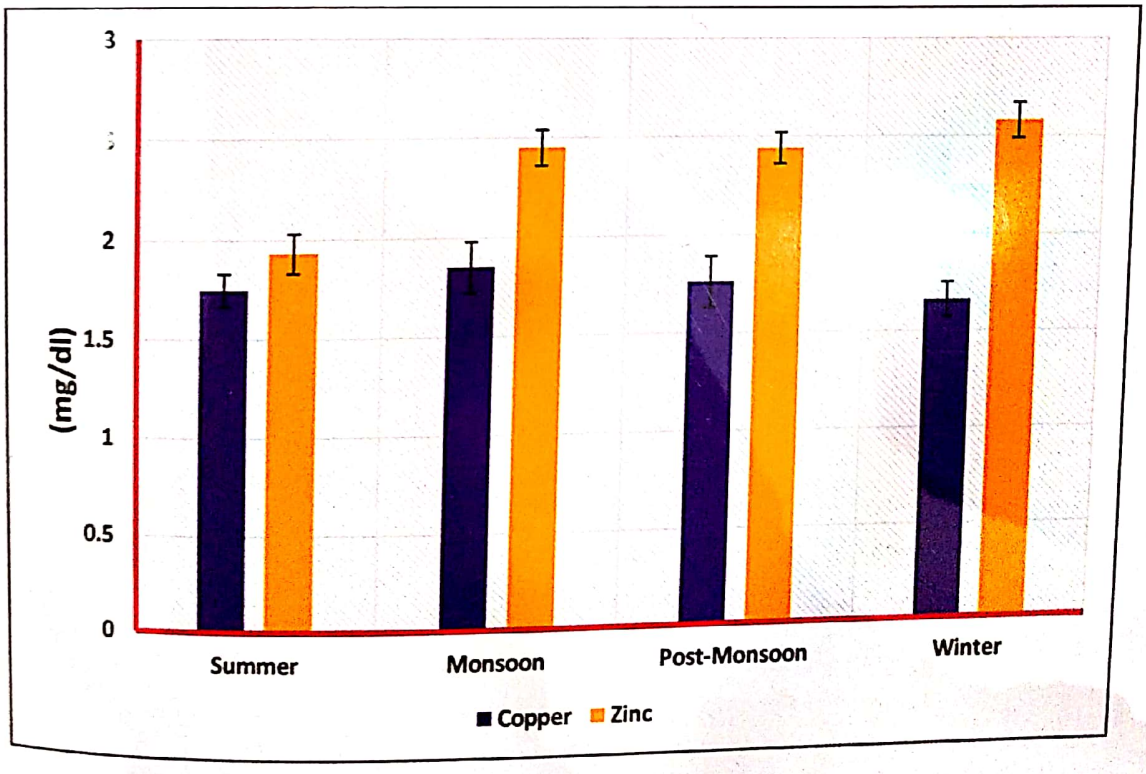


Fig. 8a: **Influence of seasons on copper and zinc concentration during experiment**

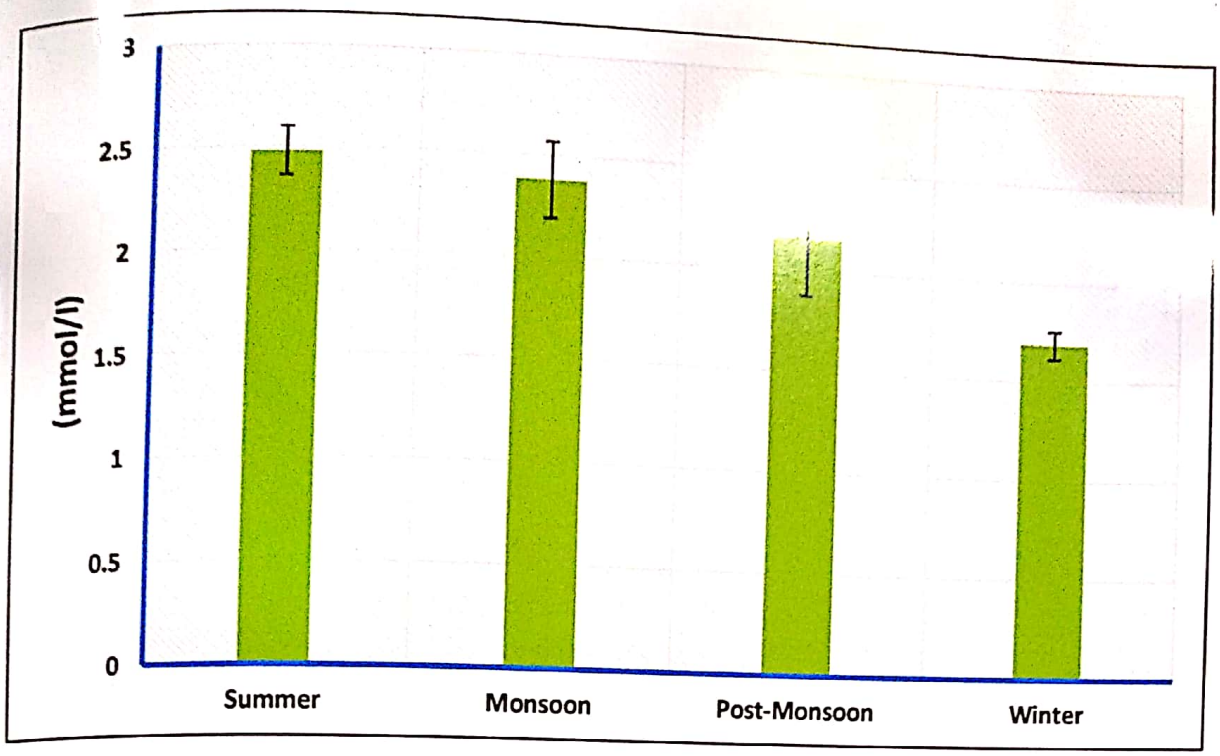


Fig. 8b: Influence of seasons on magnesium concentration during experiment

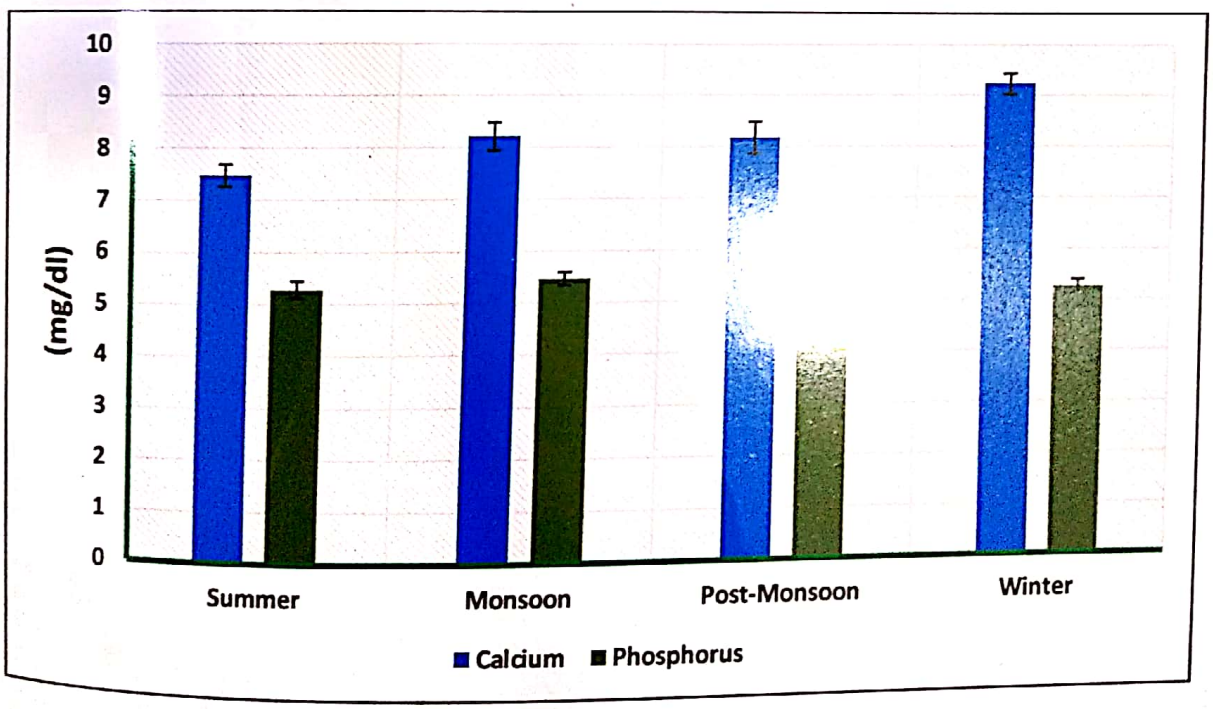


Fig. 8c: Influence of seasons on calcium and phosphorus concentration during experiment

Table:9 Influence of seasons on serum hormone profile during experiment. Values are Mean ± SE

Season	T3 (ng/ml)	T4 (ng/ml)	TSH (μ IU/ml)	Cortisol (ng/ml)
Summer	$1.82^{a} \pm 0.10$	$29.84^{a} \pm 1.04$	$2.53^{a} \pm 0.12$	$20.66^{b} \pm 0.83$
Monsoon	$1.87^{a} \pm 0.23$	$38.81^{b} \pm 1.89$	$2.90^{a} \pm 0.10$	$16.65^{a} \pm 0.95$
Post-Monsoon	$2.21^{ab} \pm 0.29$	$37.78^{b} \pm 2.20$	$2.90^{a} \pm 0.15$	$15.14^{a} \pm 0.69$
Winter	$2.75^{b} \pm 0.26$	$41.66^{b} \pm 1.99$	$3.51^{b} \pm 0.18$	$14.60^{a} \pm 0.83$

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

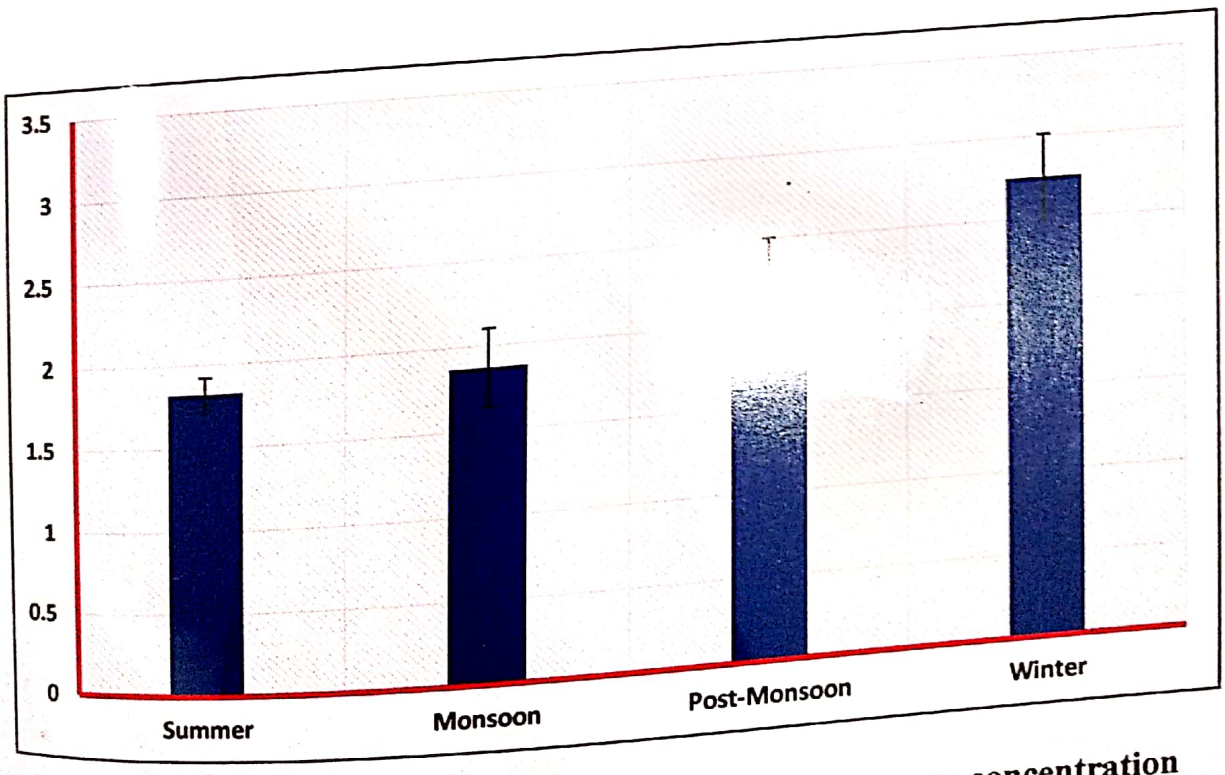


Fig. 9a: Influence of seasons on triiodothyronine (T3) hormone concentration during experiment

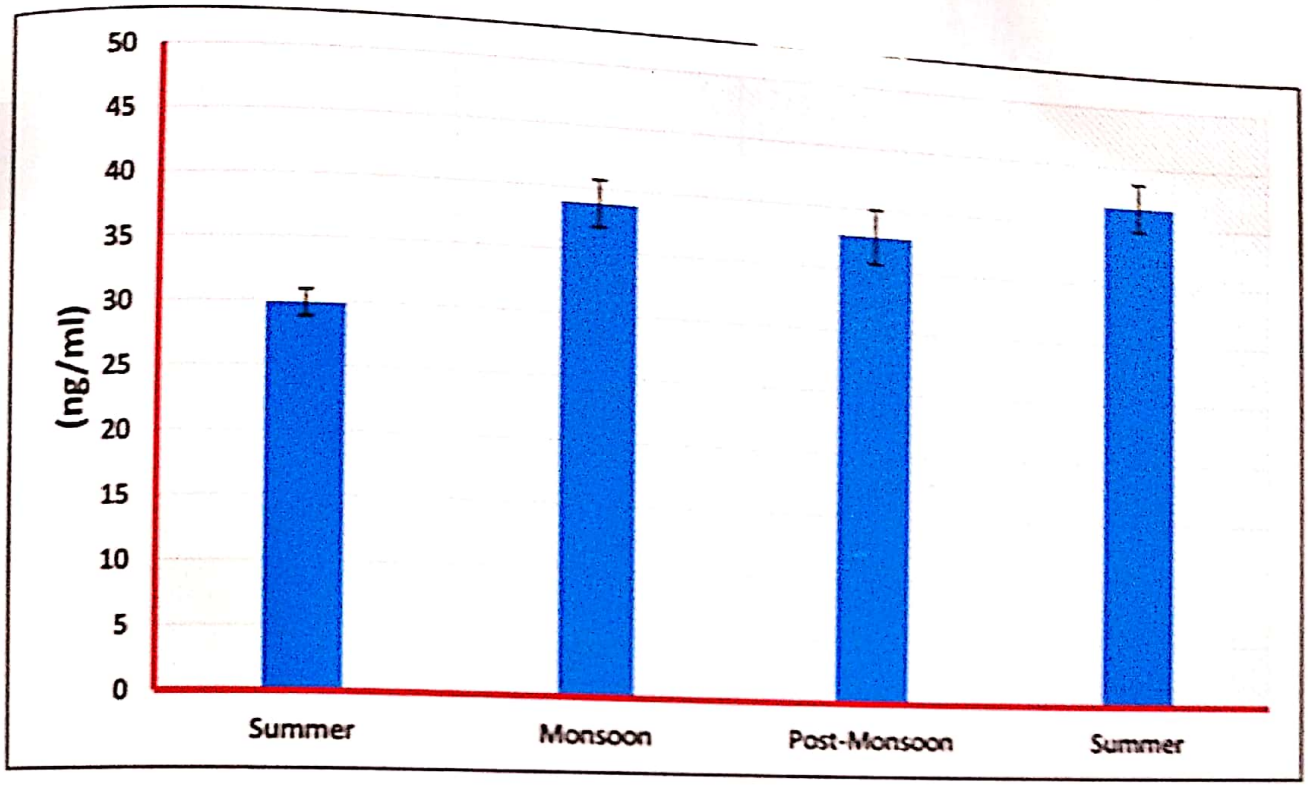


Fig. 9b: Influence of seasons on thyroxine (T4) hormone concentration during experiment

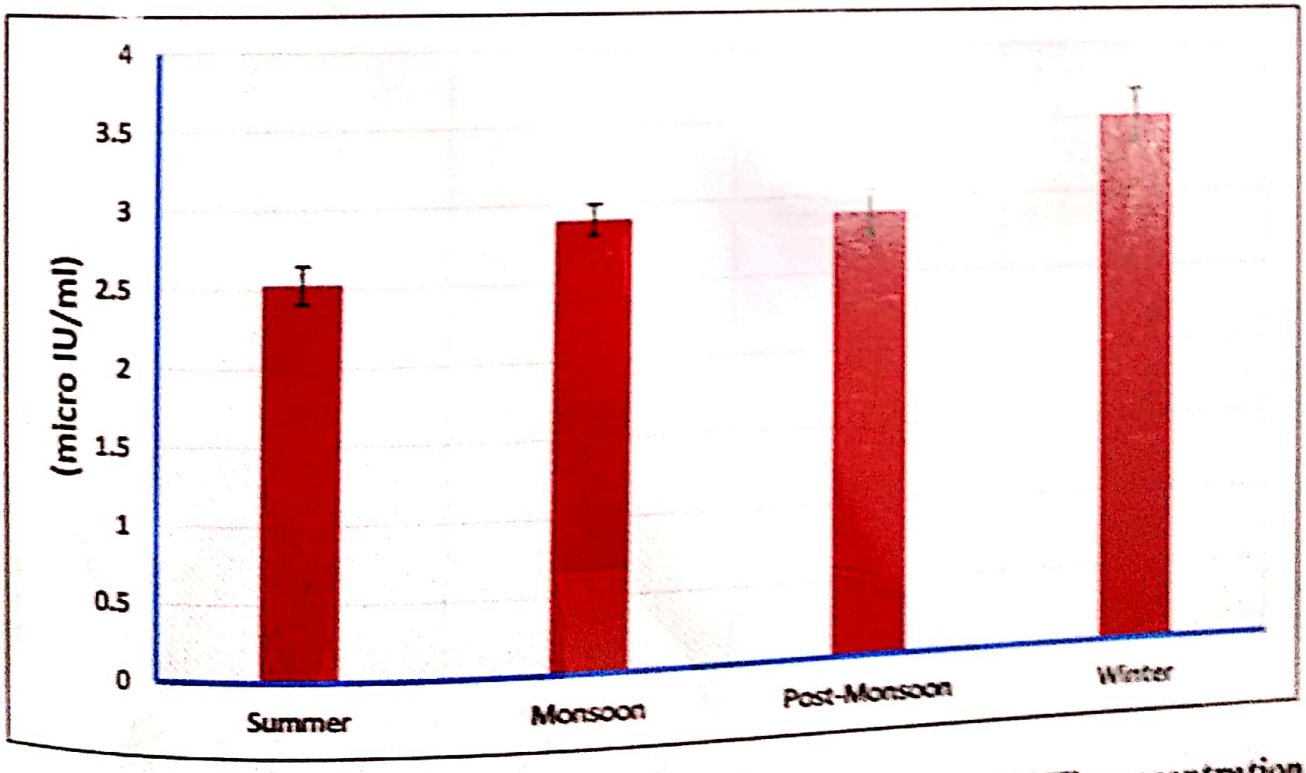


Fig. 9c: Influence of seasons on thyroid stimulating hormone (TSH) concentration during experiment

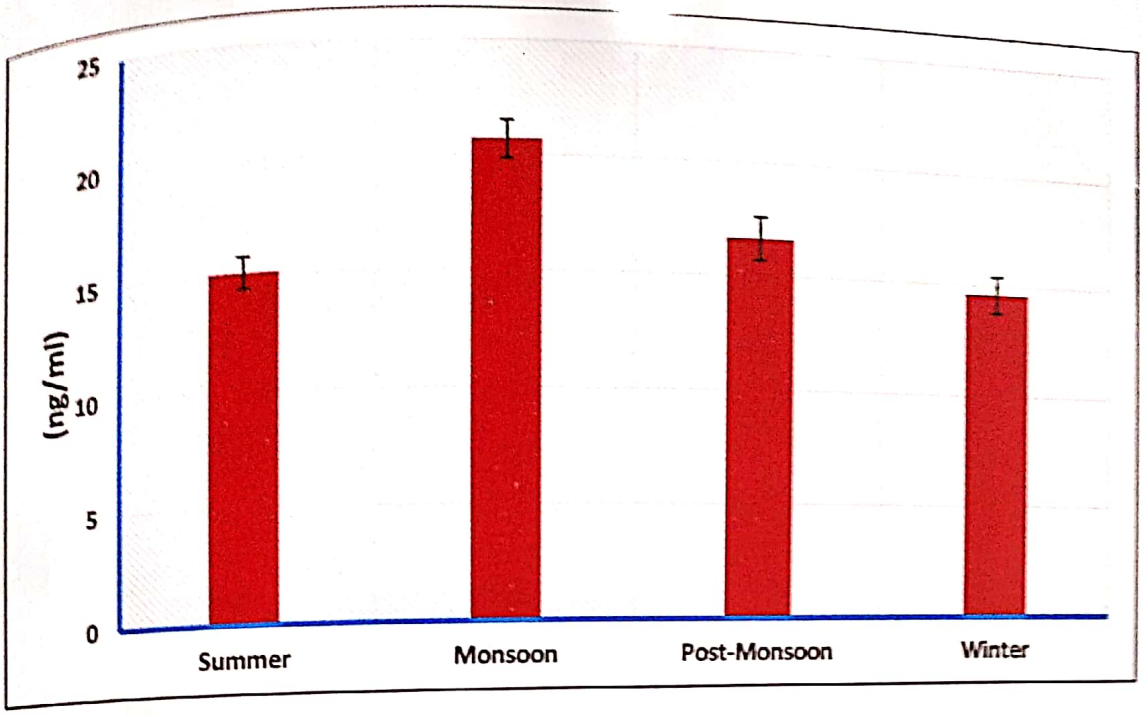


Fig. 9d: Influence of seasons on cortisol concentration during experiment

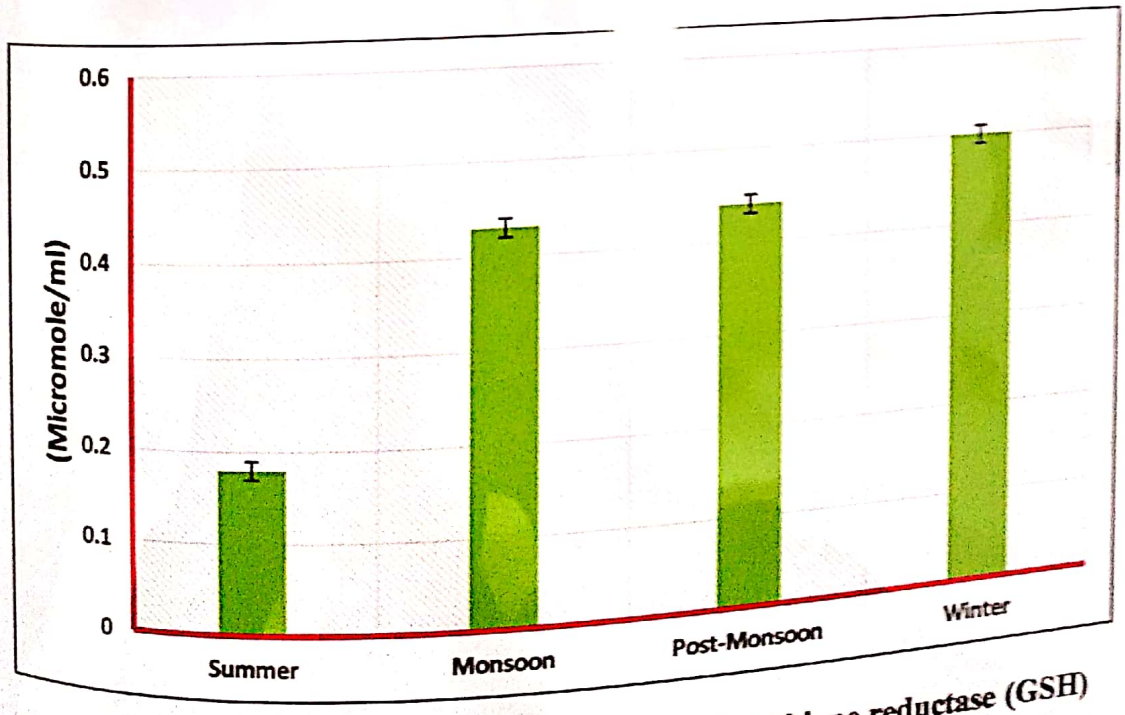


Fig. 10a: Influence of seasons on serum reduced glutathione reductase (GSH) concentration during experiment

The statistical analysis (ANOVA) shows that there were significant ($p<0.05$) influences of the climatic season on the concentrations of T_3, T_4, TSH and cortisol.

During the winter the concentration of T_3, T_4 and TSH was observed significantly ($p<0.05$) higher than other three seasons. The lowest concentration of these three thyroid hormones was observed during the summer season.

The stress hormone i.e. cortisol concentration was highest during the summer season when the environmental temperature was highest, and during winter season when the environmental temperature was lowest the serum cortisol concentration was also significantly lower than the summer season.

4.6 Effect of seasonal variation on oxidative stress related parameter in serum and erythrocyte

4.6.1 Effect of seasonal variation on oxidative stress related parameter in serum

The concentrations of Reduced glutathione (GSH) and Malondialdehyde (MDA) in serum in different seasons is tabulated in Table 10 and Fig.10a and 10b. Both were observed significant ($p<0.05$) during different seasons. Significant ($p<0.05$) effect were also observed in activities of Superoxide dismutase (SOD, EC 1.15.1.1) and Catalase (CAT, EC 1.11.1.6) during different seasons (Table 10 and Fig. 10c and 10d).

Data pertaining to MDA and GSH concentration, SOD and CAT activities were maximum during summer and minimum during winter and moderate during monsoon and post- monsoon.

The mean ± SE values of Ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazine (DPPH) activities were observed to be maximum during summer and minimum during winter and moderate during monsoon and post-monsoon. (Table.10 and Fig. 10e,10f).

4.6.2 Effect of seasonal variation on oxidative stress related parameter in erythrocyte

GSH and Malondialdehyde concentration in erythrocyte are tabulated in Table 11 and Fig.11a, 11b.Both were significant differ ($p<0.05$) during different seasons. The activities of Superoxide dismutase (EC 1.15.1.1) and Catalase (EC 1.11.1.6) during different seasons (Table 11 and Fig. 11c and 11d) were also significantly ($p<0.05$) different.

Table: 10 Influence of seasons on serum antioxidant during experiment. Values are Mean ± SE

Season	GSH (micromole/ml)	LPO (nmol/ml)	SOD (U/ml)	CATALASE (U/ml)	FRAP (mmol/ml)	DPPH (%)
Summer	$0.50^{c} \pm 0.01$	$4.50^{c} \pm 0.12$	$91.39^{c} \pm 1.20$	$184.73^{c} \pm 7.27$	$181.67^{b} \pm 4.58$	$22.81^{c} \pm 1.30$
Monsoon	$0.43^{b} \pm 0.01$	$3.20^{b} \pm 0.09$	$74.81^{b} \pm 0.96$	$155.65^{b} \pm 8.03$	$171.28^{ab} \pm 5.33$	$20.36^{bc} \pm 0.84$
Post-Monsoon	$0.44^{b} \pm 0.01$	$2.93^{ab} \pm 0.09$	$73.06^{b} \pm 1.54$	$144.69^{b} \pm 8.34$	$171.89^{ab} \pm 5.73$	$19.67^{b} \pm 0.71$
Winter	$0.18^{a} \pm 0.01$	$2.72^{a} \pm 0.08$	$58.26^{a} \pm 0.98$	$94.09^{a} \pm 4.09$	$162.48^{a} \pm 5.25$	$15.99^{a} \pm 0.60$

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

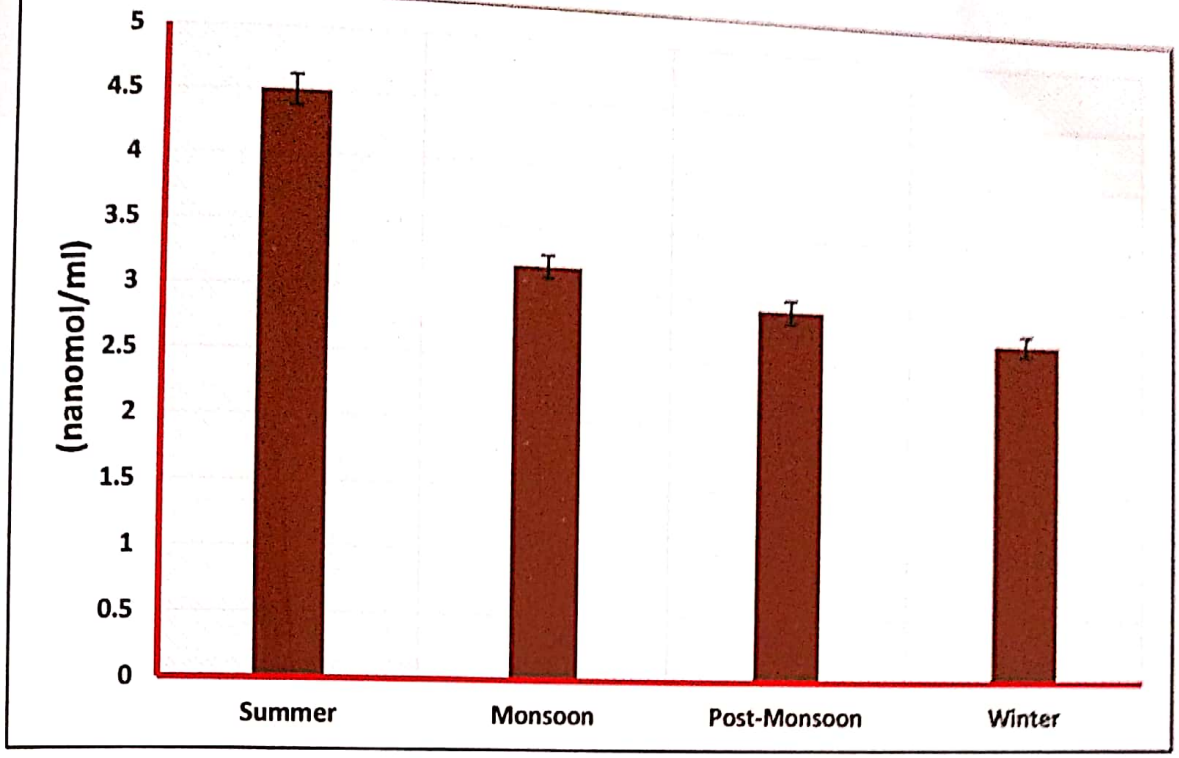


Fig. 10b: Influence of seasons on serum Malondialdehyde (MDA) production during experiment

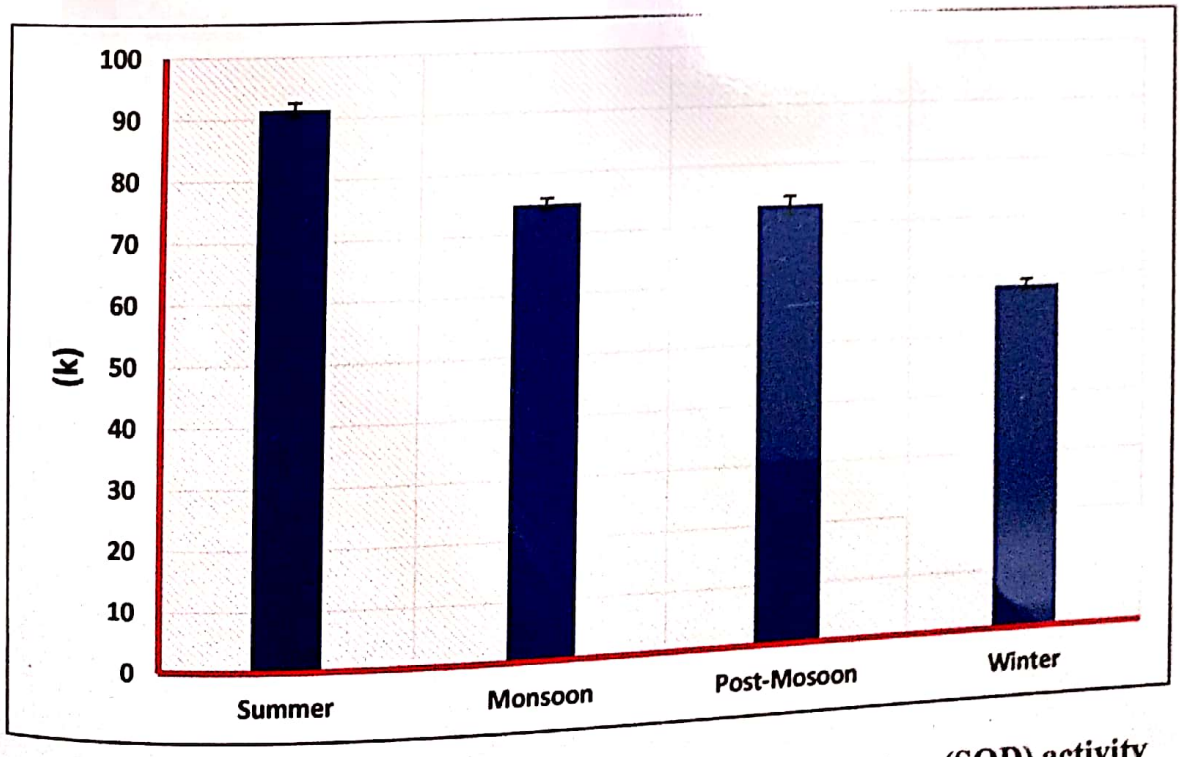


Fig. 10c: Influence of seasons on serum superoxide dismutase (SOD) activity during experiment

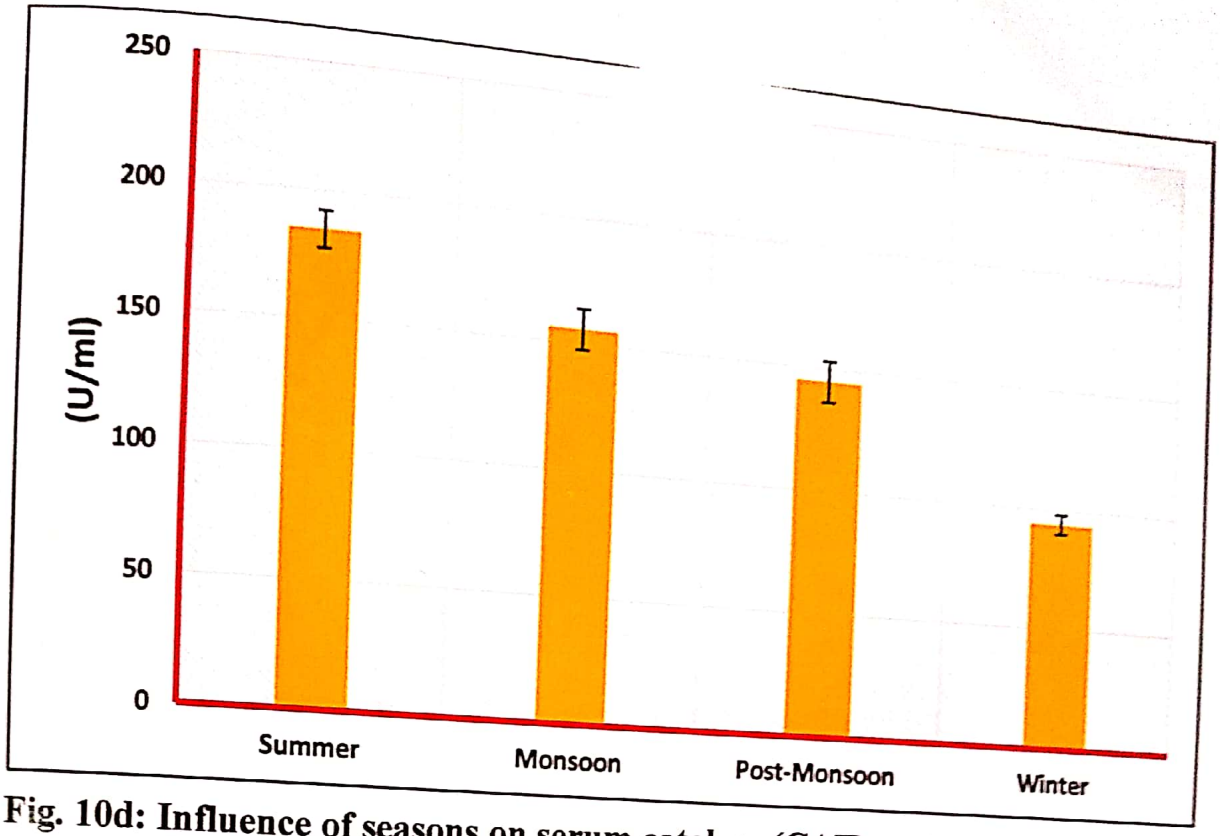


Fig. 10d: Influence of seasons on serum catalase (CAT) activity during experiment

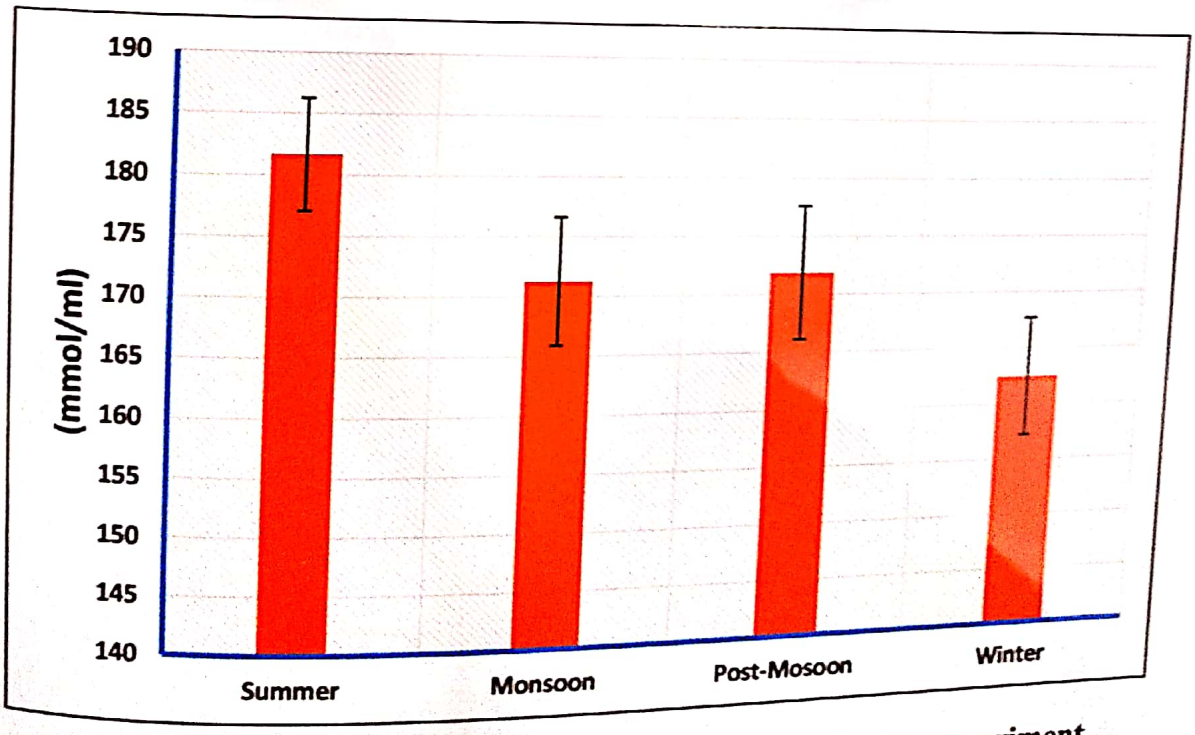


Fig. 10e: Influence of seasons on serum FRAP activity during experiment

Tabular data reveals that LPO and GSH concentration, SOD and CAT activities in erythrocyte was maximum during summer and minimum during winter and moderate during monsoon and post-monsoon.

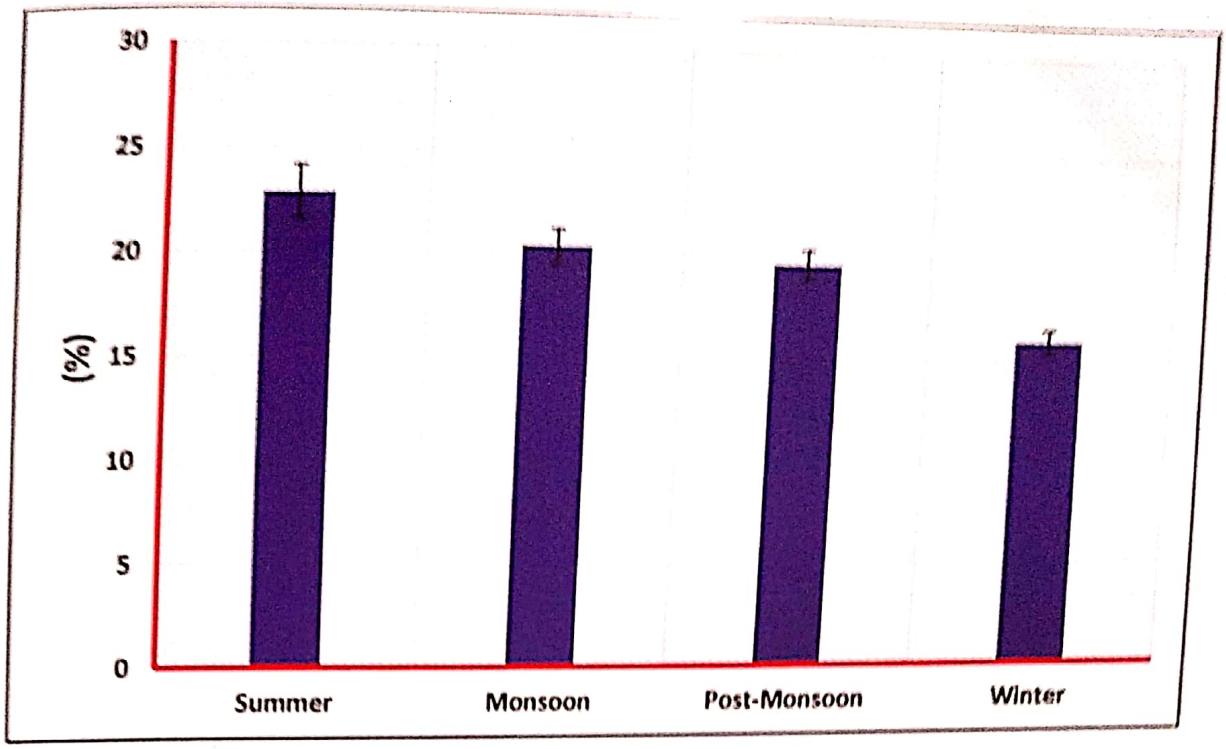


Fig. 10f: Influence of seasons on serum DPPH activity during experiment

Table:11 Influence of seasons on erythroc xidant during experiment.
Values are Mean ± SE

Season	GSH (μ mole/gm Hb)	LPO (nmol/gm Hb)	SOD (U/gm Hb)	CATALASE (U/gm Hb)
Summer	$1.82^{c} \pm 0.02$	$9.17^{c} \pm 0.24$	$332.34^{c} \pm 5.15$	$650.32^{c} \pm 55.87$
Monsoon	$1.31^{b} \pm 0.06$	$6.41^{b} \pm 0.19$	$250.87^{b} \pm 5.60$	$549.55^{cb} \pm 40.73$
Post-Monsoon	$1.22^{b} \pm 0.04$	$5.86^{ac} \pm 0.19$	$238.85^{b} \pm 8.26$	$522.00^{b} \pm 40.28$
Winter	$0.80^{a} \pm 0.04$	$5.45^{a} \pm 0.14$	$167.27^{a} \pm 3.73$	$330.60^{a} \pm 21.05$

Mean bearing different superscripts (a, b, c) in a column differ significantly ($P<0.05$) between seasons.

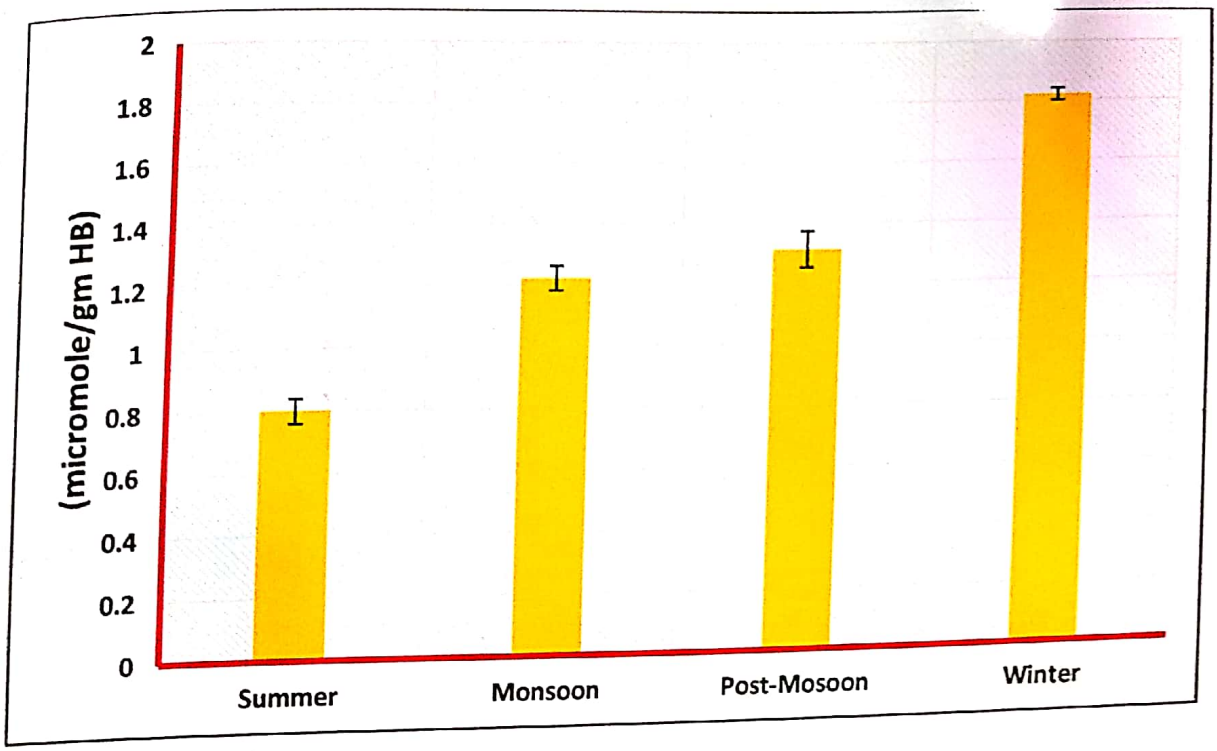


Fig. 11a: Influence of seasons on erythrocyte reduced glutathione (GSH) c ncentration during experiment

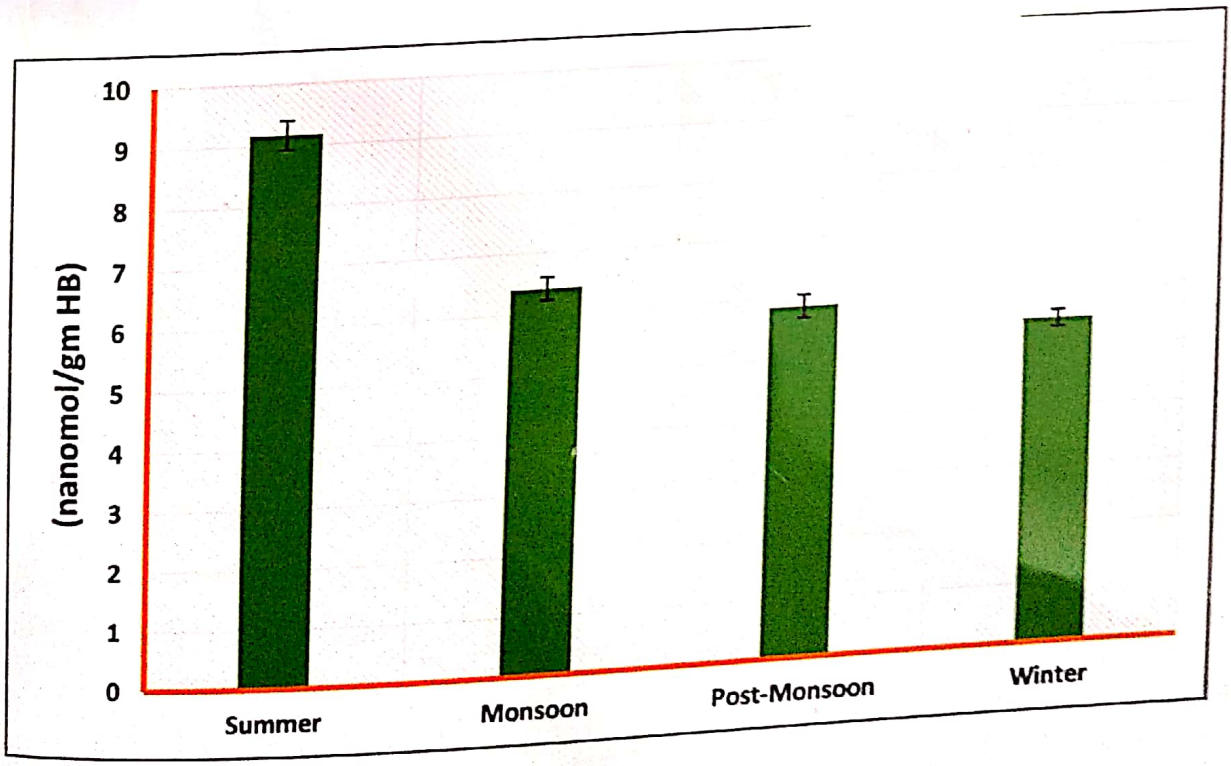


Fig. 11b: Influence of seasons on erythrocyte Malondialdehyde (MDA) production during experiment

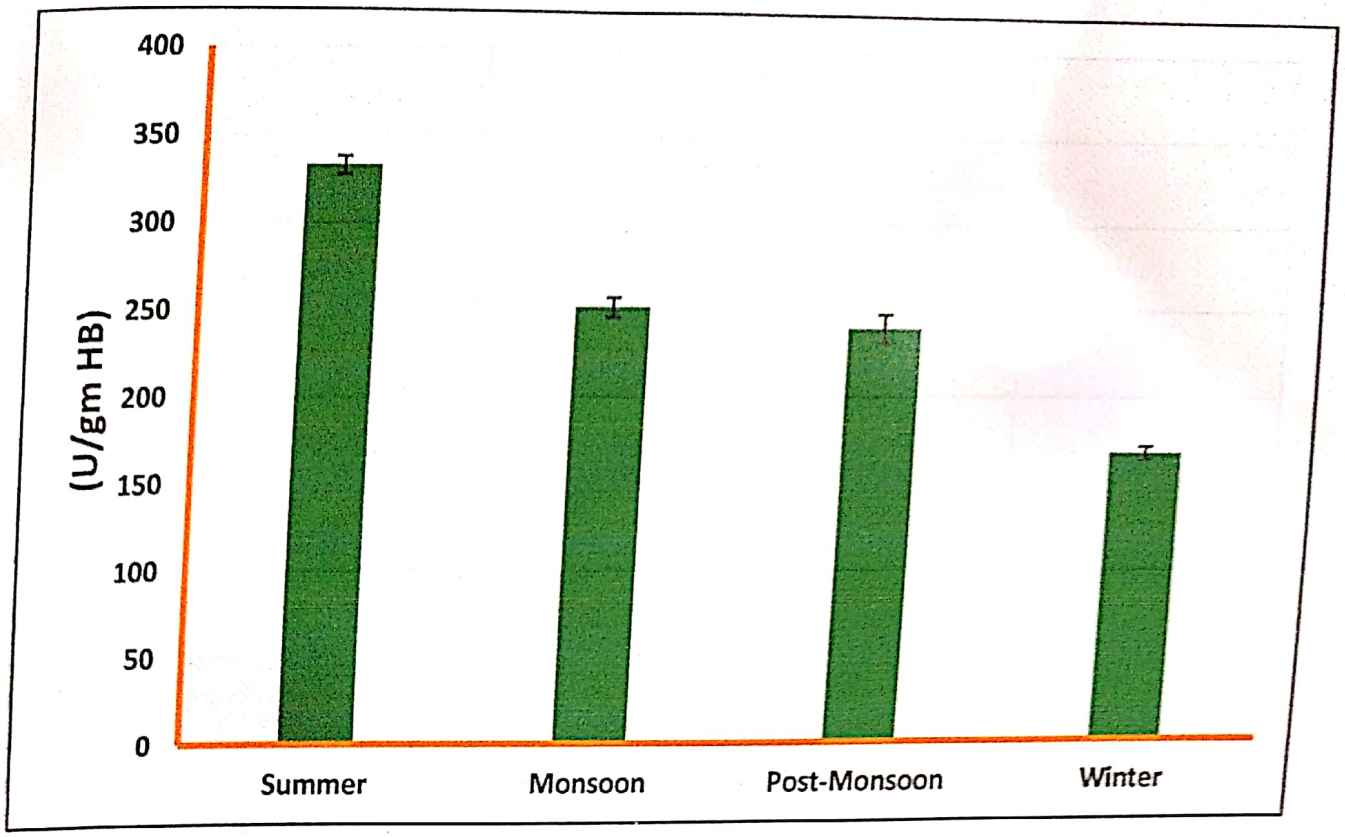


Fig. 11c: Influence of seasons on erythrocyte superoxide dismutase (SOD) activity during experiment

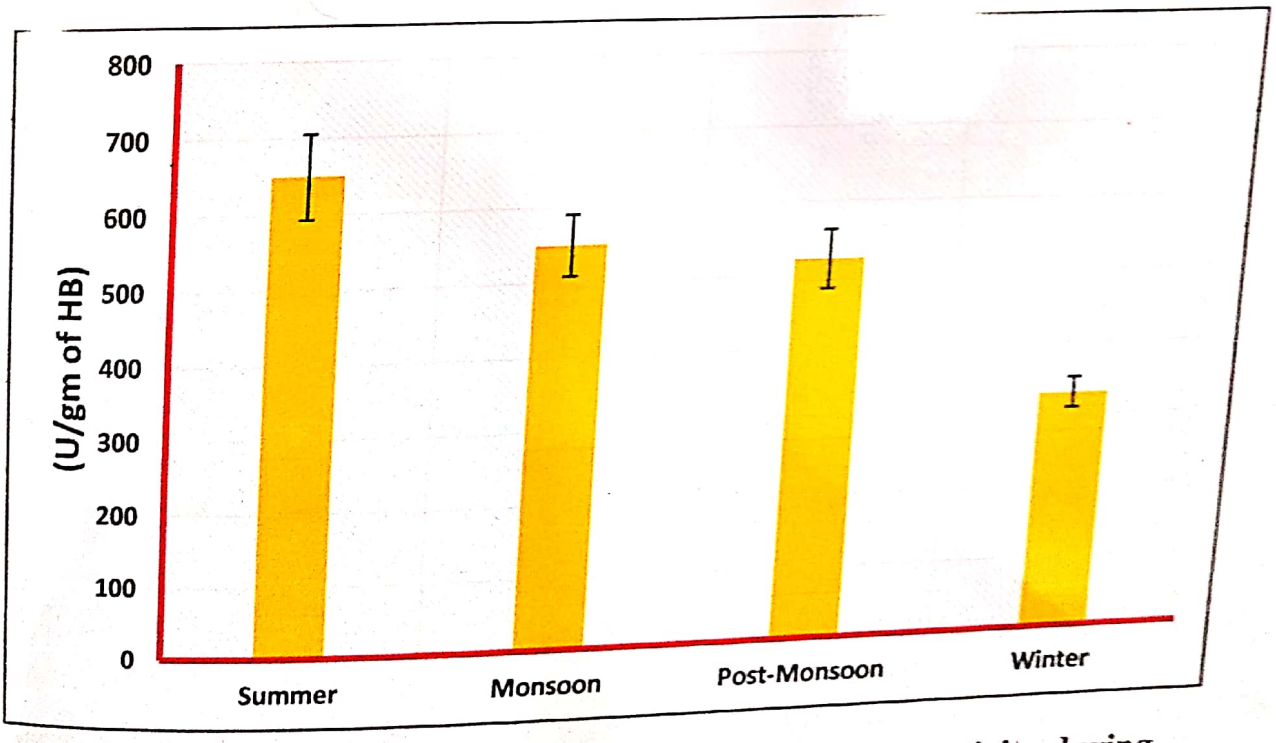
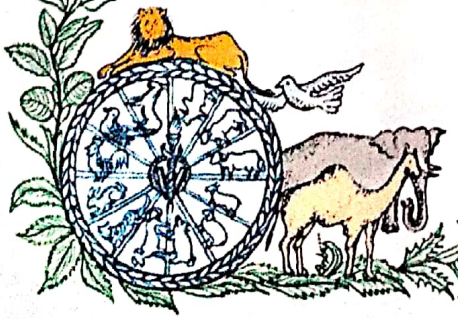


Fig. 11d: Influence of seasons on erythrocyte catalase (CAT) activity during experiment

Discussion



Abiotic factors such as temperature, relative humidity, and rainfall play a major role in the animal breed adaptability to a certain agro-climatic condition. Indigenous breeds are better adapted to the local environments where they have evolved adaptation than the exotic and crossbred animals (Pragna, *et al*. 2018a). Therefore, the seasonal influence on physiological, hemato-biochemical, hormonal parameters antioxidant status in serum and erythrocyte of indigenous Black Bengal goat breeds raised in tropical condition were studied.

Temperature humidity index (THI) allows an objective comparison of environmental conditions (El-Tarabany 2016). During experimental period the values of THI were more than 72 in summer and monsoon seasons, indicating that goats were under environmental stress, while during post-monsoon winter season the THI value was closer to or below 72. In earlier reports THI of 70 or less is considered comfortable, 75 –78 is stressful, while greater than 78 shows that animals are under stress and are unable to maintain homeothermy (Silanikove 2000; Dikmen and Hansen, 2009; Banerjee *et al*. 2015; Ocheja *et al*. 2017; Ocheja *et al*. 2020).

5.1. Effect of seasonal variation on physiological response

Physiological responses like rectal temperature (RT), respiration rate (RR) and pulse rate (PR) showed a significant variation between different seasons and found maximum during summer and minimum during winter in this study. In several studies goats RT was elevated as rise in environmental temperature and they cope up with this by increasing the evaporative cooling mechanism and enhancing RR which ultimately reduce the extra heat load in them (Singh *et al*. 2016; Kumar *et al*. 2017). According to Devendra (1987) change in metabolism and muscle activity of goats also changes pulsation and respiration rates. Increase in heart rate (HR) and PR is attributed to two causes. One is the increase in muscular activity controlling the rate of respiration, at the same time with elevated RR. The second is the reduction in resistance of peripheral vascular beds Banerjee *et al*. (2015). Similar kind of result was also reported by Banerjee *et al*. (2015) and Rathwa*et al.* (2017) in goat and by Bhan *et al*. (2013),

Kumar *et al*. (2017), Grewal and Aggarwal (2018) and Sailo *et al.* (2017) during different seasons.

5.2. Effect of seasonal variation on blood profile and erythrocyte indices

Blood cells could experience sequence of adaptive transformation on exposure to different seasons environment and sensitive to temperature variation and acts as key indicator of physiological responses to stressors (Casella *et al*. 2013). The results of hematological parameters in this study were within the reported ranges in goat Banerjee *et al*. (2015), Habibu *et al*. (2017b). Seasonal variations were observed in most of the haematological parameters. The levels of Hb, PCV, TEC and TLC were observed to be higher during winter and lower during summer. Our result in this study is in agreement with the findings of Banerjee *et al*. (2015) and Habibu *et al*. (2017b) in goat. In hot and humid environment decrease in hematological parameter is associated with enhanced water intake due to heat stress Habibu *et al.* (2017b). Decrease in feed intake occurs in animals under long-term heat stress, reduces the number of erythrocytes and hemoglobin level, resulting in a decrease of erythrocyte concentration in the bloodstream Swenson and Reece (2006), Ribeiro *et al.* (2018b). Decline in erythrocyte count in during summer is associated with decrease of thyroid hormones secretion which is related to declining the process of erythropoiesis Bhatta *et al*. (2014).

The levels of different leukocyte (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were within the reported ranges in goat Azab and Abdel-Maksoud (1999), Phulia *et al*. (2007), Banerjee *et al.* (2015). Leucocyte profiles are particularly useful in the field of adaptation physiology because they are altered by stress and can be directly related to stress hormone levels Minka and Ayo (2007), Banerjee *et al.* (2015). Higher value of neutrophils and monocytes during summer season due to an increased cortisol level leads to an increase in numbers of neutrophils and decreases in lymphocyte numbers as reported by Banerjee *et al.* (2015) and Habibu *et al.* (2017b).

There is no significant difference was observed in Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin concentration (MCHC), while Mean Corpuscular Volume (MCV) was significantly lower during summer season as compared to monsoon, post-monsoon and summer. The decreased MCV value obtained

could be related to the negative correlation among size and number of erythrocyte Holman and Dew (1966), Habibu *et al.* (2017b). Our finding is in agreement with the findings of Bhatta et al. (2014), Banerjee *et al.* (2015) and Habibu *et al*. (2017b).

5.3. Effect of seasonal variation on serum metabolites, electrolytes and minerals concentration and activity of serum enzymes

Blood glucose level is an adaptation mechanism that can be affected by high ambient temperatures and its level shows greater variations during heat stress condition than in the comfort zone. Some researchers reported that heat stress decrease blood glucose level in goats (Pandey *et al*. 2012; Hooda and Upadhyay 2014; El-Tarabany *et al*. 2017). Decrease in glucose and cholesterol was also observed in hot dry and hot humid season as compared to winter season by Chaudhary *et al*. (2015). Report of above researcher support our finding which shows shown a gradual increasing trend of blood glucose concentration from summer to winter season with recording maximum during winter and minimum during summer.

The season had no significant effect on the concentrations of serum total protein, albumin and globulin. Concentrations of serum total proteins and albumin have been used as indices for nutritional status and a direct relationship between nutritional status or protein intake and serum protein and albumin level has been established in ruminants (Caldeira *et al.* 2007; Attia 2016) and no significant changes in serum protein, albumin and globulin during summer may be due to good nutritional status of the animal. Previous studies revealed no significant change in total protein level in goats during different seasons (Abdelatif *et al*. 2009; Rathwa *et al*. 2017). Cozzi et al., 2011 reported that albumin concentration in serum was unaffected by season in cattle. The globulin level in blood plasma was also insignificantly affected by season (Sejian *et al*. 2013; Attia 2016).

Blood urea nitrogen (BUN) concentration was significantly higher during summer as compared to post-monsoon and winter seasons. Creatinine concentration was higher during summer season than winter season. The increased level of BUN and creatinine during summer season may be due to reduced blood flow toward kidney due to heat stress. The other reasons may be due to protein mobilization from muscle tissue

due to heat stress (Ghosh*et al*. 2013; Indu *et al*. 2014; Rathwa *et al*. 2017; Abdelnour *et al*. 2019). Further, this increase in plasma urea nitrogen can be due to inefficient rumen ammonia incorporation into microbial protein, or hepatic deamination of amino acids mobilized from skeletal muscle (Wheelock *et al*. 2010).

Activities of enzyme alanine amino transferase (ALT) and lactate dehydrogenase (LDH) are significantly influenced by seasons, while activity of aspartate aminotransferase (AST) was not influenced by seasons. Serum level of aspartate transaminase (AST) and alanine transaminase (ALT) is helpful in assessing the animal welfare during stress condition. The serum level of these enzymes increases during summer seasons due to their higher adaptive capability (Banerjee *et al*. 2015; Rathwa *et al.* 2017). On another hand, no significant changes were observed in AST level of heat-stressed goats (Sharma and Kataria 2011). The higher AST activity in summer in goats was also reported by Sharma and Kataria (2011), Banerjee *et al*. (2015); Rathwa *et al.* (2017). Increase in LDH enzyme activity during the summer season may be due to an increased muscular activity involved in cellular respiration in which pyruvate from glucose was converted into usable energy as lactate in the cells catalyzed by LDH (Bhan *et al*. 2013; Chetia *et al*. 2017).

Electrolytes are a crucial for cellular metabolism, muscle contraction, nerve transmission, and enzymatic reactions (Piccione *et al*. 2007). Concertation of sodium, potassium and chloride is varying with season due to variation in forages and in soil mineral content. Spring–summer seasons are richer in minerals than other seasons of the year (Xin *et al*. 2010). Seasonal changes in sodium, potassium and chloride of goats has been reported by Piccione *et al*. (2012) and Rathwa *et al.* (2017). Increase in sodium, potassium and chloride concentration during summer season due to dehydration (Chaudhary*et al*. 2015; Rathwa *et al.* 2017).

Calcium is required for the normal functioning of a wide variety of tissues and physiologic processes (Horst *et al*. 1994). Dietary content of phosphorus can influence calcium absorption and utilization (Huber *et al*. 2002). The lower concentrations of Ca and P observed during summer season may be due to decreased feed intake (Ramana *et*

al. 2013). Low Ca and P during winter season as compare to other season was also reported by Piccione *et al*. (2012);Inbaraj *et al*. (2017) and Rathwa *et al*. (2017).

Magnesium supports the conversion of blood sugar to energy and is also considered as an anti-stress mineral, regulating HR and blood clots (Ribeiro *et al*. 2018a). In our study The magnesium concentration was gradually decreased from summer to winter season. The present finding is in accordance with report of Kaneko *et al*. (2009) and Ribeiro *et al*. 2018a.

There was no significant difference observed in serum copper concentration. Erdogan *et al*. (2004) and Noaman, (2014) find that serum Cu levels were not affected by season. While Yokus and Cakir, (2006) found elevated serum Cu during summer and lower values during winter.

Serum zinc concentration was significantly lower during summer season as compare to other seasons. This finding corroborates the observation of Noaman *et al*. (2012). However, Yokus and Cakir (2006) found season has no effects on serum Zn concentration in crossbred cows. Actually, Zn is a vital constituent of many enzymes including those involved in the synthesis of DNA and RNA. It may affect immunity by means of its significant role in cell replication and proliferation (Weiss and Spears, 2006).

5.4. Hormonal parameters

In the present study plasma T_3, T_4 and TSH hormone concentration was lower in the summer season and higher in winter season. As per the values of THI animals were in stress during summer, monsoon and post-monsoon. So it is clear from the result that during stressful condition animal reduce their metabolic hormone (T_3, T_4 and TSH) as a physiological response and this could be attributed to the direct effect of heat stress on hypothalamic-pituitary-thyroid axis in an effort to produce less thyroid hormones to prevent more metabolic heat production to cope with elevated ambient temperature (Aleena et al. 2017). Several researchers reported decreased T3 and T4 production in animals during high environmental temperature (Todini *et al*. 2007; Ribeiro *et al*. 2016; Pragna *et al*. 2018b).

In the study cortisol level were higher during summer and lower during winter. This indicated that heat stress experienced by the animals stimulated the hypothalamo–pituitary–adrenal (HPA axis) to secrete cortisol in order to relieve the stress condition and to supply energy for normal physiological processes (Ali and Hayder 2008; Sejian *et al*. 2008b; Sejian and Srivastava 2010). The association between heat stress and increased secretion of cortisol in small ruminants is well documented (Shilja *et al*. 2016; Tajik *et al*. 2016; Chergui *et al*. 2017; Sejian *et al*. 2018). Further, cortisol is the principal stress reliever in ruminant species and hence its higher level is beneficial to cope up with heat stress condition (Shaji *et al*. 2017).

5.5. Effect of seasonal variation on oxidative stress related parameter in serum

Elevated environmental temperatures increase the production of reactive oxygen species (ROS), with subsequent induction of oxidative damage to lipids (Maibam *et al*. 2018; Abdelnour *et al*. 2019). The unsaturated lipids are more prone to free radicals damage and hence, lipid peroxidation is considered as the biomarker of free radical load (Wagner *et al*. 1994). Malonaldehyde (MDA) is a low molecular weight end product which is generated as the free radical damages the lipids (Wong-Ekkabut *et al*. 2007). In our study also MDA concentration was maximum in summer during which environmental temperature and minimum in winter when environmental temperature is low.

Elevated Superoxide dismutase (SOD) and catalase (CAT) activities found during summer as compare to other season indicating that more ROS is produce during summer (Maibam *et al*. 2018; Abdelnour *et al.* 2019). SOD in conjugation with catalase and GPx scavenges both intracellular and extracellular superoxide radicals and prevents lipid peroxidation (Agarwal and Prabhakaran, 2005). Similar finding was also observed by Ghosh *et al*. 2013 and Gupta *et al*. 2013 in goat and in cattle and buffalo by Lakhani *et al*. 2018; Lakhani *et al*. 2020; Kalmath G. and Swamy, 2020.

Higher concentration of GSH was found during summer season and it may be due to decrease in activity of enzyme glutathione-S-transferase (John *et al*. 2001; Bernabucci *et al.* 2002). GSH is an essential cofactor for glutathione-S-transferase (Dhanasree et al., 2020).

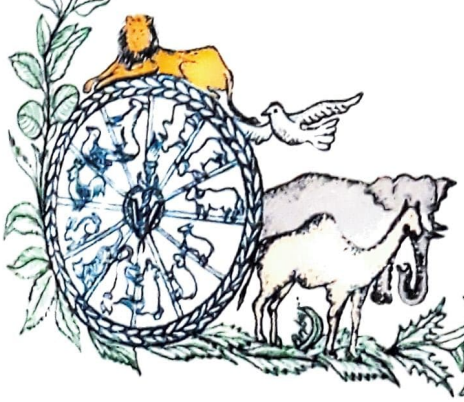
Ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazine (DPPH) activities were found maximum during summer and minimum during winter. Higher FRAP and DPPH activities during summer may be due to increase antioxidant activity of serum to neutralize more ROS produced due to heat stress. Chaudhary*et al*. 2015 reported that elevated serum total antioxidant capacity (TAS) during summer and monsoon seasons in surti buffalo. Kumar *et al*. 2019 also reported increase in FRAP and DPPH activity due to high altitude stress in goat.

5.5. Effect of seasonal variation on oxidative stress related parameter in erythrocyte

Erythrocytes are carrier of oxygen and they are more prone to oxidative stress caused by ROS (Mohanty *et al*. 2014). Presence of hemoglobin in erythrocytes also promotes oxidative processes in erythrocyte (Radwan et al., 2013). Activities of SOD and CAT in erythrocyte were higher during summer season in cattle (Chetia *et al*. 2017; Verma *et al.* 2020; Kalmath and Swamy, 2020). In our study also activities of SOD and CAT in erythrocyte was higher during summer season as compare to other seasons. SOD along with CAT neutralizes ROS produce due to stress (Agarwal and Prabhakaran, 2005) and elevated temperature increases ROS production (Maibam *et al*. 2018; Abdelnour *et al.* 2019).

Polyunsaturated fatty acids are abundant in erythrocyte and most susceptible substrate of lipid peroxidation (Bolchoz *et al*. 2002; Fazio *et al.* 2016). MDA is an important product of lipid peroxidation and acts as good indicator of oxidative damage of erythrocyte (Banerjee *et al*. 2020). Increase in MDA concentration during summer season may be due to higher ROD mediated lipid peroxidation. GSH is keystone to the cellular antioxidant defenses as it acts as an essential cofactor for antioxidant enzymes such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase (Dhanasree *et al*. 2020). The elevated GSH concentration during summer season as compare to other season indicating that erythrocyte in under oxidative stress and this increase may be due to the decrease in activity of enzyme glutathione-S-transferase in erythrocytes. (John *et al*. 2001; Bernabucci *et al.* 2002).

Summary and Conclusions



6. SUMMARY AND CONCLUSION

Goats (Capra hircus) experience a diversity of environmental challenges resembling extensive variation in temperature, humidity and pathogenic invasion (Haldar 2012). The tropical climate of Indian sub-continent provides several environmental indications to different animal species to develop adaptive strategies to cope up with environmental stress. Blood being the most important specialized tissue of the body creates an open channel system to provide the equal amount of nutrients, hormones and other important factors to different organs. Thus, blood biochemistry is not only the vital marker for the general health and basic metabolic pattern of an animal but also for adaptive modifications to different geographical distributions/conditions (Ghosh *et al*. 2013).

In the present scenario of global climate changes, the physiological response of goat to elevated temperature is a major focus to maintain the goat rearing a sustainable venture. Goat undergo various kinds of stressors, i.e. physical, nutritional, chemical, psychological and heat/thermal stress. Among all, heat stress is the most concerning issue nowadays in the ever-changing climatic scenario. Stress causes production of reactive oxygen species (ROS). Under normal physiological conditions, ROS are counterbalanced by the antioxidant defense system present in animal body (Omidi *et al*. 2017). Any inequity between production of ROS and antioxidant defense system induces oxidative stress. Oxidative stress and antioxidant defence status depends upon disease seasonal variations (Maibam *et al*. 2017). The levels of thyroid hormone may fluctuate according to the physiological stage, growth, breed, age, environmental temperature, and sex. The thyroid gland is prone to the variation of environmental temperature since its hormones are related to thermogenesis (Ribeiro *et al*. 2018b). The another hormone cortisol synthesized by the adrenal cortex stimulates the degradation and release of glucose, amino acid, and fat in the liver, muscle and adipose tissue and its concentrations changes according to several factors, such as circadian rhythm, season, photoperiod and diet composition (Sejian *et al*. 2010).

The susceptibility of red blood cells (RBCs) to oxidative damage is due to the presence of PUFA, heam iron and oxygen which may produce oxidative changes in

RBC (Kumar *et al.* 2011). Fully mature RBC, lacking nucleus, cannot produce new proteins in response to stress. They have to rely on proteins synthesized earlier in development to protect themselves from damage by ROS and thus ensure their own survival. The activities of antioxidant enzymes and lipid peroxidation are significantly increased during oxidative stress. So, they can be used as markers of oxidative stress (Agarwal and Sengupta, 2020).

Keeping in view the lack of available knowledge about the adaptability of Black Bengal goat in the climatic condition of Bihar, the study was conducted with objectives to study the impact of environmental condition (four seasons) on physio-biochemical profile along with the stress biomarkers in the blood of Black Bengal goats.

This experiment was carried out for four different seasons which includes summer (April, May, June), monsoon (July, August, September), post monsoon (October, November) and winter (December, February) on ten Black Bengal female goat. The data on meteorological variables like temperature and relative humidity was obtained from meteorological station Patna. Rectal temperature (°C), respiration rate per minute and Pulse rate per minute of each animal was recorded.

Blood sample was collected two times in month at 15 days' interval in each season from jugular vein in tube containing anticoagulant EDTA and tube without anticoagulant. Blood with anticoagulant was used for hematological study viz. Haemoglobin (Hb), total leucocyte count (TLC), total erythrocyte count (TEC), differential count (DLC) and packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated by using standard formula. Erythrocytic hmolysate was also prepared from blood having anticoagulant and hemolysate was used for estimation of Reduced glutathione (GSH) and Malondialdehyde (MDA) concentration, Superoxide dismutase (SOD) and Catalase (CAT) activity. Serum was serrated from clotted blood was used for estimation of some serum metabolites such as total protein, albumin, globulin albumin: globulin ratio, glucose, blood urea nitrogen (BUN), creatinine and serum enzyme like Alanine Transaminase (ALT), Aspartate

Aminotransferase (AST), Lactate Dehydrogenase (LDH). Electrolyte and mineral like Na, K, Cl, Ca, P, Cu, Zn Mg was also estimated in serum. Serum was also used for estimation of hormone like T3, T4, TSH, cortisol and also for estimation of GSH, MDA concentration and SOD, CAT, DPPH and FRAP activity in serum.

Temperature humidity index (THI) allows an objective comparison of environmental conditions (El-Tarabany 2016). During experimental period the values of THI were more than 72 summer and monsoon season, indicating that goats are under environmental stress. Physiological responses like rectal temperature (RT), respiration rate (RR) and pulse rate (PR) showed a significant variation between different seasons and found maximum during summer and minimum during winter in this study. Increased in physiological response may be due to stress caused by environment.

The levels of Hb, PCV, TEC and TLC were observed to be higher during winter and lower during summer. In hot and humid environment decrease in hematological parameter is associated with enhanced water intake due to heat stress Habibu *et al*. (2017b). Decrease in feed intake occurs in animals under long-term heat stress, reduces the number of erythrocytes and hemoglobin level, resulting in a decrease of erythrocyte concentration in the bloodstream Swenson and Reece (2006); Ribeiro *et al.* (2018b). Higher value of neutrophils and monocytes during summer season was found due to an increased cortisol level leads to an increases in numbers of neutrophils and decreases in lymphocyte numbers as reported by Banerjee *et al*. (2015) and Habibu *et al*. 2017b.There is no significant difference was observed in MCH and MCHC, while MCV was significantly lower during summer season.

Blood glucose level is an adaptation mechanism that can be affected by high ambient temperatures and its level shows greater variations during heat stress condition than in the comfort zone. The season had no significant effect on the concentrations of serum total protein, albumin and globulin. Concentrations of serum total proteins and albumin have been used as indices for nutritional status and a direct relationship between nutritional status or protein intake and serum protein and albumin level has been

established in ruminants (Caldeira *et al*. 2007; Attia, 2016). The increased level of BUN and creatinine during summer season may be due to reduced blood flow toward kidney due to heat stress.

Serum level of AST and ALT is helpful in assessing the animal welfare during stress condition. The serum level of these enzymes increases during summer seasons due to their higher adaptive capability (Banerjee *et al*. 2015; Rathwa *et al*. 2017). Increase in LDH enzyme activity during the summer season due to an increase muscular activity involve in cellular respiration in which pyruvate from glucose was converted into usable energy as lactate in the cells catalyzed by LDH (Bhan *et al*. 2013; Chetia *et al*. 2017).

Concertation of sodium, potassium and chloride is varying with season due to variation in forages and in soil mineral content. Spring–summer seasons are richer in minerals than other seasons of the year (Xin *et al*. 2010). The lower concentrations of Ca and P observed during summer season may be due to decreased feed intake (Ramana *et al*. 2013). Magnesium supports the conversion of blood sugar to energy and is also considered as an anti-stress mineral, regulating HR and blood clots (Ribeiro *et al*. 2018a). In our study the magnesium concentration was gradually decrease from summer to winter season. There was no significant difference was observed in serum copper concentration. Serum zinc concentration was significantly lower during summer season as compare to other seasons.

During stressful condition animal reduce their metabolic hormone (T3, T4 and TSH) as a physiological response and this could be attributed to the direct effect of heat stress on hypothalamic-pituitary-thyroid axis in an effort to produce less thyroid hormones to prevent more metabolic heat production to cope with elevated ambient temperature (Aleena *et al*. 2017). In the study cortisol level were higher during summer and lower during winter. This indicated that heat stress experienced by the animals stimulated the hypothalamo–pituitary–adrenal (HPA axis) to secrete cortisol in order to relieve the stress condition and to supply energy for normal physiological processes (Ali and Hayder, 2008; Sejian *et al*. 2008; Sejian and Srivastava 2010).

Elevated Superoxide dismutase (SOD) and catalase (CAT) activities found during summer as compare to other season indicating that more ROS is produce during summer (Maibam *et al*. 2018; Abdelnour *et al*. 2019). Higher concentration of GSH was found during summer season and it may be due to decrease in activity of enzyme glutathione-S-transferase (John *et al*. 2001; Bernabucci *et al*. 2002). Ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazine (DPPH) activities were found maximum during summer and minimum during winter. Higher FRAP and DPPH activities during summer may be due to increase antioxidant activity of serum to neutralize more ROS produced due to heat stress.

In the present study the goats were in environmental stress during summer and monsoon season, which might be a reason to the observed variations in different physio-biochemical parameters. Further it was concluded that the Black Bengal goats varies its biological response to the different environmental condition, showing its adaptability to the climatic condition of Bihar.

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Appendix

Annexure I

1. 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)- SISCO Research Laboratory, Mumbai, India.
2. 5, 5' Dithiobis (2- Nitrobenzoic acid) (DTNB)- Himedia Laboratories Pvt. Ltd. India.
3. Acetonitrile- Thermo Fisher Scientific, USA.
4. Ascorbic acid- Himedia Laboratories Pvt. Ltd. India.
5. Dimethyl sulphoxide (DMSO)- Merk Ltd. Mumbai, India.
6. Disodium Hydrogen Phosphate (anhydrous) purified- Sigma-Aldrich Chemicals Pvt Ltd.USA
7. Drabkin^{s} solution - Coral clinical system, India
8. Ethylene diamine tetra acetic acid (EDTA) Disodium salt- British Drug House Pvt. Ltd., India.
9. Ferric chloride- Himedia Laboratories Pvt. Ltd. India.
10. Glacial acetic acid- Thermo Fisher Scientific, A.
11. Hydrogen peroxide- Thermo Fisher Scientific, USA.
12. Hydrochloric acid- Himedia Laboratories Pvt. Ltd. India.
13. Methanol- Thermo Fisher Scientific, USA.
14. Potassium chloride- Himedia Laboratories Pvt. Ltd. India.
15. Potassium dihydrogen orthophosphate- Sigma-Aldrich Chemicals Pvt Ltd.USA.
16. Pyragallol- Thermo Fisher Scientific, USA.
17. Sodium acetate trihydrate- Sigma-Aldrich Chemicals Pvt Ltd.USA.
18. Sodium chloride- Merk Ltd. Mumbai, India.
19. Sodium dihydrogen orthophosphate- Merk Ltd. Mumbai, India.
20. Sodium hydroxide- British Drug House Pvt. Ltd., India.
21. Sodium tungstate hydrated- British Drug House Pvt. Ltd., India.
22. Sulphuric acid- Himedia Laboratories Pvt. Ltd. India.
23. Thiazolyl blue (MTT)- Himedia Laboratories Pvt. Ltd. India.

24. Thiobarbituric acid- Himedia Laboratories Pvt. Ltd. India.
25. Trichloro acetic acid- Himedia Laboratories Pvt. Ltd. India.
26. Tripyridyltriazine (TPTZ)- SISCO Research Laboratory, Mumbai, India.
27. Tris (hydroxyl methyl) amino methane- SISCO Research Laboratory, Mumbai, India.

Composition of reagent

1. Reagents for estimation of catalase activity

a. Phosphate buffer solution (50 mM) pH 7.4

Disodium Hydrogen Phosphate	50 mM
Potassium dihydrogen phosphate	50 mM

Add the former to the latter in the ratio of 61: 39

b. PBS- hydrogen peroxide solution

Hydrogen peroxide	0.34 ml
50 mM PBS to make	100 ml

2. Reagents for estimation of SOD activity

a. 1.25 mM MTT solution

MTT	2.58 mg
Distilled water to make	5 ml

b. 100 mM pyragallol solution

Pyragallol	6.3 mg
Distilled water to make	100 ml

3. Reagents for lipid peroxidation

a. Trichloroacetic Acid (TCA) 30 % solution

Trichloroacetic Acid	30 g
Distilled water to make	100 ml

b. 1% Thiobarbituric acid (TBA) solution

Thiobarbituric acid	1 g
0.05 N NaOH to make	100 ml

c. EDTA solution (0.01M)

EDTA	3.722 g

Distilled water to make	100 ml

4. Reagents for glutathione estimation

a. H_2SO_4- 0.08 N in distilled water

b. Tungstate solution

Sodium tungstate	0.3 M
EDTA	0.1 M

c. Tris buffer (1M) pH 8

tris-hydroxymethyl aminomethane	1 M

d. DTNB reagent

Sodium chloride	0.14 M
Disodium Hydrogen Phosphate	0.009 M
Sodium dihydrogen phosphate	0.000013 M
DTNB	40 mg
Distilled water to make	100 ml

5. Reagents for DPPH assay

a. DPPH radical

DPPH reagent	39.4 mg
Methanol	10 ml

b. Acetonitrile 0.009 M

6. Reagents for FRAP assay

a. Acetate buffer 3mM

Sodium acetate trihydrate	4.08 gm
Distilled water to make	100 ml

Glacial acetic acid to set pH 3.6

b. Ascorbic acid 100 µM

Ascorbic acid	17.6mg

	Distilled water to make	100 ml
c.	Ferric chloride solution	2 Mm
	Ferric chloride	3.24 gm
	HCl	40 mM
d.	Tripyridyl triazine	10 mM
	Tripyridyl triazine	20.25 mg
	HCl (40 Mm)	6.5 ml

VITAE

Name of the student	:	Dr. Komal
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EDUCATIONAL QUALIFICATION

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Any specific information:

- Got best poster award in the 4th National seminar on 'current Research in Veterinary Biochemistry and Biotechnology in the improvement of Animal Health and Production' of SVBBI AT College of Veterinary Science, SVVU,TIRUPATI

- Got third position in 'International poster competition on Different versatile Pharmacophore used for Covid-19 organised by Association of Professionals in biotechnology and Pharmaceutical research.

- Attended 21 days National Training Course organised by NADCL, Baramulla (J & K)