EFFECT OF YEA SACC^{1026} SUPPLEMENTATION ON RUMEN PROFILE, PHYSIOLOGICAL AND METABOLIC STATUS OF BUFFALO CALVES IN SUMMER SEASON

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University
In partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY PHYSIOLOGY
(Minor Subject: Veterinary Biochemistry)

By

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CERTIFICATE - I

This is to certify that the thesis entitled, **"Effect of Yea Sacc^{1026} supplementation on rumen profile, physiological and metabolic status of buffalo calves in summer season"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Physiology** (Minor subject: **Veterinary Biochemistry**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Harjap Singh (L-2014-V-102-M)** 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 investigation have been fully acknowledged.

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CERTIFICATE – II

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ABSTRACT

Effect of Yea Sacc^{1026} supplementation on rumen profile, physiological and metabolic status of buffalo calves in summer season was investigated. The experiment was conducted on eight apparently healthy rumen fistulated buffalo calves divided into three groups of four each viz. Pre-summer (group I); Summer control (group II) and Summer treatment (group III). Same animals were used in Pre-summer and Summer treatment groups. Group III animals were supplemented with Yea Sacc^{1026}@ 1 bolus (consisting of 25 billion live yeast cells)/animal per day. The experimental animals were kept on conventional diet consisting of green fodder, wheat straw, concentrate and mineral mixture. Three blood samples were collected from each animal in each group at weekly intervals. Rumen liquor samples were collected before feeding (0 hr) and subsequently at 1, 2, 3, 4, 5 and 6 hr postprandial for 3 consecutive days after the period of microbial adaptation (21 days).The results revealed that there was significant ($P<0.05$) increase in rectal temperature, respiration rate, heart rate, erythrocytic lipid peroxidation levels, plasma glucose, creatinine, urea nitrogen levels and significant ($P<0.05$) decrease in PCV, Hb and plasma total proteins during summer season. However following supplementation of Yea Sacc^{1026} the values of above parameters were restored towards normal except respiration rate and plasma creatnine levels. The plasma cholesterol values did not change in any of the groups. There was no alteration in colour, odour and consistency of rumen liquor. Status of PH, MBRT, SAT, total nitrogen and ammonia nitrogen showed significant increase during summer season. However, level of these parameters declined significantly after oral administration of Yea Sacc^{1026}. In contrast total bacterial count, total protozoal count and TVFA's were significantly decreased in group II and their levels were restored to normal after supplementation of Yea Sacc^{1026}. During summer season there was decrease in entodiniomorphs and increase in holotrichs percentage. But after Yea Sacc^{1026} supplementation there was no significant change in percentage of entodiniomorphs and holotrichs. Molar percentage of acetic acid and butyric acid was significantly higher during summer season whereas molar percentage propionic acid was lower; these values were restored to normal after Yea Sacc^{1026} supplementation. It can be concluded from present investigation that supplementation with Yea Sacc^{1026} can be useful in order to maintain rumen profile, physiological and metabolic status of buffalo calves during summer season.

Keywords: Yea Sacc^{1026}, summer season, blood and rumen liquor parameters, buffalo calves.

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Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

%	:	percent
@	:	at the rate of
°C	:	degree Celsius
am	:	anti meridiem
b. wt.	:	body weight
cm	:	centimeter
dl	:	deciliter
gm	:	gram
H_2SO_4	:	sulphuric acid
hrs	:	hours
kg	:	kilogram
Max.	:	Maximum
MBRT	:	methylene blue reduction time
mEq/L	:	milliequivalent per litre
mg	:	milligram
Min.	:	minimun
ml	:	milllilitre
mm	:	millimeter
N	:	normality
NaOH	:	sodium hydroxide
NH_3-N	:	ammonia nitrogen
no.	:	number
^oF	:	Degree Fahrenheit
pm	:	post meridiem
SAT	:	sedimentation activity time
spp.	:	species
SRL	:	strained rumen liquor
THI	:	temperature humidity index
TVFA	:	total volatile fatty acids
UMMB	:	Urea Molasses Mineral Block
VFA	:	volatile fatty acids
viz.	:	such as
µg	:	microgram

CHAPTER I

INTRODUCTION

The livestock sector globally is highly dynamic. Currently, livestock is one of the fastest growing sector among agriculture in developing countries (Thornton 2010) and it contributes about 33% in agriculture GDP and increasing very rapidly. India is having the largest livestock (ruminant population) in the world which is about 520.6 million, among this cattle and buffalo having largest livestock number (FAOSTAT 2013). Buffaloes are found only in certain regions in the world, principally in Asian countries some countries in eastern Europe and in many countries in Latin America. India has about 102.4 million buffaloes which represents 56.5 percent of the world buffalo population. India is the first country in the world for huge number of buffaloes and milk production (about 55 million tons) and possesses the best milch breeds. (FAO, 2010).

Among these, Indian Murrah is the most important and well-known buffalo breed in the world. Like all other mammals buffaloes are homeotherms i.e. they maintain a constant body temperature by constantly regulating peripheral and internal body temperature with assistance of cutaneous sensors and internal temperature sensors (located in the hypothalamus) along with integration of the endocrine system. When the heat load of an animal is greater than its capacity to lose heat (Wagner 2001), a portion of the metabolizable energy typically used for production must be diverted to assure thermal balance. The primary factors that cause heat stress in dairy animals are high environmental temperature and relative humidity (West 2003). Exposure to high ambient temperature is the major constraint on buffalo productivity in hot climatic areas. An increase in body temperature of around 1.0°C may result in detectable, deleterious effects on metabolism, tissue integrity and a significant depression in production (McDowell *et al* 1976; Shebaita and El-Banna 1982).

Climate change projections for India suggest that temperature is expected to increase between 2.3 and 4.8°C because of doubling of carbon dioxide concentration in the atmosphere (Lonergan 1998) and further this increase in temperature is expected for all the months of the year. Rise in the environmental temperature may impair production through reduced growth, meat, milk and egg, impaired reproductive

performance, imbalanced biochemical and physiological process of metabolism and immune response (Gaughan *et al* 2010).

The rise in environment temperature alters the basic physiology of rumen which negatively affects the nutrient energy balance (Baumgard and Rhoads 2012). Heat stress reduces the dry matter intake, decreases ruminal motility and contraction, changes the fermentation pattern and volatile fatty acid production, affects the digestibility and nutrient utilization, and thus impairs productivity (Madan and Yadav 2013). The biological mechanism by which heat stress impacts production and reproduction is partly explained by reduced feed intake, reduction in rumination and nutrient absorption and increased maintenance requirements (Collier *et al* 2005). Increasing environmental temperature and rising rectal temperature above critical thresholds are related to decrease in the dry matter intake (Albright and Alliston 1972).

The major strategies for lowering the heat stress of the animals during summer is to increase evaporative cooling by providing shade, use of sprinklers, fans, etc (Bucklin *et al* 1991). Another additional/alternative approach to ameliorate the thermal stress experienced by the animals during summer is by use of probiotics. Probiotics are beneficial for animals, affecting their health and production (Shriver-Munsch 2011). Beneficial effects of these probiotics are well reported when these are administrated in adequate amount (FAO/WHO 2002, Senok *et al* 2005, Todorov *et al* 2007). Yeast culture used as a dietary supplement for dairy cattle is thought to improve rumen function, and hence milk production and feed efficiency, by stimulating selective growth of rumen bacteria species (Harrison *et al* 1988). Inclusion of *S. cerevisiae* in ruminant's diets has been shown to alter the molar proportion of ruminal volatile fatty acids (VFAs) (Newblod *et al* 1990*;* Dawson 1993), reduce rumen ammonia concentration, increase the number of ruminal bacteria and protozoa and alter the flow of the nitrogen (N) fraction to the duodenum (Dawson1993, Williams *et al* 1991). Furthermore, a study by (Kumar *et al* 1994) showed that supplementation of yeast culture as a growth promoter for buffalo calves resulted in increased rumen pH, total bacteria and protozoa culture counts, total volatile fatty acids, total nitrogen and microbial protein, with reduced rumen ammonia nitrogen concentration and improved digestion of cellulose and dry matter (DM)

intake. Other researchers have reported that live yeast and yeast culture supplementation may increase feed intake and milk production of dairy cows (Robinson and Garrett 1999; Dann *et al* 2000).

Yeast culture is considered as a non-hormonal growth promoter which increase milk yield as well as improve composition of milk in dairy animals (Sune, 1998; Garg *et al* 2000). Perhaps the oldest hypothesis is that the yeast are able to grow, at least for a short period of time in the rumen, thereby directly enhancing fiber digestion and/or producing nutrients that stimulate growth of rumen bacteria which do the bulk of the fiber digestion. It has also been suggested that yeasts utilize nutrients, such as lactic acid which, if allowed to accumulate in the rumen could suppress bacterial growth and/or suppress DM intake by driving rumen pH down (Robinson 2010).

The ability of specific yeast culture preparations to stimulate the growth of ruminal bacteria and to increase the concentrations of specific groups of beneficial bacteria in the rumen has been well documented (Garcia *et al* 2000, Abd El- Ghani 2004, Kamel *et al* 2004 and Moharrery and Asad 2009). Yeast culture improve the cellulolytic activities of rumen microorganisms in such a way that they increase their total numbers, improve fiber digestion, reduce lactate accumulation and improve utilization of starch supplied in the feeding ration. Besides that, Yeast culture improves ruminal fermentation by scavenging excess oxygen, thus creating a more optimal environment for rumen anaerobic bacteria (Chaucheyras-Durand *et al* 2008). *Sacccharomyces cerevisiae* naturally boosts the rumen functions by improving the beneficial biomass. *Saccharomyces cerevisiae* reduces acidosis and stabilises the ruminal pH by supplementing malic acid, the growth stimulating factor for lactate utilizing bacteria. It also chelates several important trace minerals like zinc, copper and manganese and increases their availability to the animals as well as it is a natural source of vitamins including the B-complex vitamins. Yea-Sacc^{1026} is the naturally produced live yeast culture (LYC) of *Saccharomy cescerevisiae* that has been specifically chosen due to its superior ability to stimulate rumen microflora and to optimize rumen function.

In ruminants probotics supplementation improves digestibility, weight gain and FCR (Chiofalo *et al* 2004, Antunovic *et al* 2006, Whitley *et al* 2009). It also

increases blood total protein (El-Shaer 2003), glucose (Sharma *et al* 1998), decreases cholesterol (Fayed *et al* 2005). Yeast Culture also helps in maintaining body homeostasis by maintaining the physical parameters like respiration rate (Huber *et al* 1994) and rectal temperature (Bruno *et al* 2009) within normal physiological limits, not only this it also have positive effect on hematological parameters (PVC and Hb).

This response to Yea-Sacc^{1026}supplementation is varied, it may be due to variation in diet composition, forage to concentrate ratio, type of forage fed, yeast dose and feeding strategies and stage of lactation (Robinson and Garret 1999, and Dann *et al* 2000). Therefore keeping in view the importance of yeast in improving rumen microbial population, optimizing rumen fermentation and enhancing digestion the present study was carried out to evaluate the effect of Yea Sacc^{1026} supplementation on buffalo calves during summer season with the following objectives:

- To study the effect of Yea Sacc^{1026}on rumen profile during summer season in buffalo calves.
- To study the effect of Yea Sacc^{1026}on physiological and metabolic parameters during summer season in buffalo calves.

CHAPTER II

REVIEW OF LITERATURE

2.1 PHYSIOLOGICAL PARAMETERS

2.1.1 Respiration Rate, Heart Rate and Rectal Temperature

Radadida *et al* (1980) observed a positive correlation between ambient temperature, respiration and pulse rate in buffaloes. The seemingly contradictory finding that heart responds to heat exposure either by a rise or by a fall may be largely explained by the fact that heart rate is positively correlated with metabolic rate.

Montsma *et al* (1985) studied the effect of high environmental temperature on rectal temperature and respiration rate in goats and concluded that the rectal temperature increased from 39.0ºC to 39.9 ºC and respiration rate from 30 to 60 times/min with a rise in environmental temperature from 20 ºC to 35 ºC.

Verma and Husain (1986) observed a significant increase in the rectal temperature in buffaloes during the hotter part of the year where environmental temperature exceeded than the critical limits. The increase in rectal temperature observed in the heat stressed animals was an indicator of disturbance in the homoeothermic status of the animals which was not being effectively countered by the enhanced heat loss by the physical and physiological processes of thermoregulation.

Joshi and Tripathy (1991) reported an increase in rectal temperature from 102.0 ºF to 103.8 ºF and respiration rate from 29 to 59 beats/minute when buffalo calves were exposed to 40.5ºC for eight hours daily for three months. The increase in respiratory frequency was two and half times in heat stressed animals than control animals.

Higginbotham *et al* (1993) Supplementation of Aspergillus oryzae to cows during heat stress resulted in improvement in digestibility feed conversion efficiency and lowered rectal temperature and respiration rate.

Sethi *et al* (1994) recorded an increase of 2.6 ºC in rectal temperature of buffaloes when exposed to direct sun rays in the months of june and july.

Huber *et al* (1994) suggested that fungal cultures such as A. oryzae, when fed to lactating dairy cows exposed to high ambient temperatures, might improve signs of

heat stress such as rectal temperature and respiration rate but the authors indicated that the mechanism was not clear.

Das *et al* (1999) observed an increase in respiration rate from 14 to 70 beats / minute in the month of june in murrah buffalo calves when exposed to direct sunlight for six hours.

Singh *et al* (2003) reported that there is significant increase in physiological responses in lactating Murrah buffaloes when there is increase in environmental temperature and level of humidity. Rectal temperature shows positive relationship with ambient temperature and humidity.

Koga *et al* (2004) observed that rectal temperature is recognized as an important measure of physiological status as well as the ideal indicator for assessment of stress in animals. Rectal temperature and skin temperature have been reported to fluctuate much more in buffaloes than in tropical cattle under increased ambient temperature.

Sabuncuoglu (2004) observed that the repiration rate is a good indicator of heat stress. In cattle respiration is the most sensitive physiological parameter which changes with change in climate and physical environment.

Al-Haidary (2006) reported the negative impact of heat with increased body temperature, higher respiration and heart rate followed by a drop in feed intake, redistribution in blood flow and alteration in endocrine function in sheep.

Gudev *et al* (2007) reported that the rectal temperature and respiration rate of buffaloes were significantly higher during direct sun exposure than the values obtained when the animals were kept under shade in barn. Therefore, when kept in the barn the buffaloes maintained their rectal temperature within the thermo neutral zone at the expense of higher respiration rate.

Maurya *et al* (2007) observed that the rise in temperature affected physiological responses of Malpura sheep and reported that animal kept in asbestos shed showed significantly lower rectal temperature, respiration rate and pulse rate as compared to animals kept in hot chamber.

Perriera *et al* (2008) reported that during thermally stressful period, the respiratory frequencies and rectal temperature in Alentejana, Frisian, Limousine and

Metrolenga cattle breeds increased significantly by 2.7, 2.8, 2.5, 2.9% and 1.1, 2.0, 1.8 and 0.2%, respectively.

Bruno *et al* (2009) found slight reduction in rectal temperature in cows fed yeast culture after 72 days postpartum which might suggest that feeding a culture of Saccharomyces cerevisiae positively impacted the rectal temperatures of cows at the time when dry matter intake is usually highest.

Shwartz *et al* (2009) claimed that cows fed yeast culture had lower rectal temperatures at 1200 and 1800 hrs (40.29 vs. 40.02°C and 40.35 vs. 40.12 °C, respectively) compared with control (housed in climatic chambers).

Haque *et al* (2012) observed physiological parameters like rectal temperature (RT) and respiratory rate (RR) to assess magnitude of stress in the animals due to thermal exposure. A significant increase ($P<0.001$) in rectal temperature and respiration rate in both young and adult buffaloes was observed after exposure to 40°, 42° and 45°C for 4 hrs as compared to thermo neutral temperature (22°C).

Wanker *et al* (2014) reported that rectal temperature and respiration rate differed with the increasing exposure to temperature and was higher ($P<0.001$) at 35ºC and 40ºC as compared to lower temperature (25ºC and 30ºC) in adult buffaloes, In contrast, the pulse rate decreased significantly ($P<0.001$) during heat exposition and highest values were recorded at 25ºC and 30ºC, respectively.

Lakhani *et al* (2015) reported that values of rectal temperature, pulse rate and respiration rate were higher ($P<0.05$) in summer season as compared to those in pre summer indicating increased heat load on buffaloes.

Yadav *et al* (2016) reported that the mean of rectal temperature, respiration rate and pulse rate after thermal exposure at 25°C, 30°C, 35°C and 40°C indicated that animals were in stress at 35°C exposure however intensity of stress was quit higher at 40°C temperature exposure.

2.2 Hematological parameters

2.2.1 Packed cell volume (PCV) and Hemoglobin (Hb)

Silankove *et al* (1980) reported lower values of erythrocytic count, PCV and Hb concentration during summer in Nubian goats, which could be attributed partly to a low level of nutrition.

Shaffer *et al* (1981) reported that blood hematocrit and hemoglobin of cows decreased under hot environment and the depression was related to the reduction in cellular oxygen requirements to compensate for elevated environmental heat load.

Shebaita and El-Banna (1982) reported that there is decrease in hemoglobin concentration during heat stress due to decrease in the rate of hematopoiesis and hemodilution.

Loch *et al* (1984) studied that the addition of 545 mg of iron sulfate plus 113.5 gms of live yeast culture to the ration caused a slight over-all increase in Hb, however, the difference was significant ($P<.05$) only in the 6th week of the experiment.

Habeeb *et al* (1992) suggested that decline in PCV and Hb in heat stressed animals could be attributed to haemolysis, haemodilution and/or to reduction in cellular O_2 requirements to minimize metabolic heat production. The decrease in hematological indices of empty cows during summer could also partly be associated with a decline in feed intake.

Marai *et al* (1995) reported that haemoglobin concentration and PCV decreased during heat stress in animals due to red cell destruction and/or to hemodilution.

Srikandakumar *et al* (2003) reported that a reduction of hemoglobin and PCV level could be due to either increased attack of free radicals on the RBCs membrane, which is rich in lipid content and ultimate lysis of RBC or inadequate nutrient availability for hemoglobin synthesis as the animal consumes less feed or decreases voluntary intake under heat stress.

Al-Haidary (2006) studied the physiological responses of Naimey sheep to heat stress challenge under semi-arid environment and observed a significant increase in PCV level.

Singh *et al* (2008) studied the effect of housing system on blood constituents of Marwari and Patanwadi ewe in subtropical climate to determine changes in haematological and biochemical parameters and reported that PCV level decreased significantly ($P<0.05$) when animals were exposed to sun during summer season as compared to animals kept in shed . It was shown that mean haemoglobin did not differ between treatments.

Bhan *et al* (2012) conducted an experiment to observe the effect of temperature variability on hematology during hot humid, winter, spring and summer season in growing and adult sahiwal cattle. Hematological parameters viz. Packed cell volume and hemoglobin levels were higher during winter season than other seasons. Higher levels of cortisol were found during summer (8.91ng/ml) as compared to spring (1.92ng/ml). The plasma enzymes increased ($P<0.05$) during summer over spring season. Hematological parameters showed negative correlation ($P<0.05$) with Temperature Humidity Index (THI).

Jabbar *et al* (2012) and Gangwar *et al* (1984) found that an increasing trend of PCV was seen during winter, whereas, these decreased during hot days. In contrast Haque *et al* (2013) reported that acute heat stress evokes a series of drastic changes in the animal's hematological functions. Packed cell volume and haemoglobin increased significantly during acute heat stress in Murrah buffaloes.

Hussein (2014) Probiotics supplementation have significant effect on the hematological parameters like Hb and PCV in second and third groups (fed with 5g and 10g/kg probiotics diet with a concentrate feed mixture respectively) when compared with control group (fed ration composed of 60% concentrate feed mixture). The results of present study are consistent with Sarwar *et al* (2011) who found that Hb and PCV were higher ($P<0.05$) in growing Kajli lambs fed diets containing probiotics than those without it.

Lakhani *et al* (2016) found significant decrease in PCV and Hb in summer stressed buffaloes (Group II), when compared with pre summer (Group I).

2.3 Summer stress and biochemical parameter

2.3.1 Plasma glucose, Plasma total protein and cholesterol

Canfield *et al* (1984) evaluated different biochemical parameters in normal mature swamp buffaloes and reported mean plasma values of glucose, total protein and creatinine to be 61.2±48.6 mg/dl, 6.89±0.46g/dl and 1.00±0.3mg/dl, respectively.

Huntington and Eisemann (1988) reported that the glucose increase in yeast-treated animals may be attributed to increased gluconeogenesis, which raises blood glucose levels in ruminants.

El-Masry and Habeeb (1989) reported that significant decline in serum proteins with rising temperature seems to be due to dilution of plasma proteins as a result of the increase in body water content and decrease protein synthesis as a result of the depression of the anabolic hormonal secretion. The decrease in serum protein may also be due to decrease in fed nitrogen and mineral intake, which occurs under heat stress condition.

Ahmed (1990) found that the total protein levels decreased from 7.7 in winter to 6.4 g/dl in summer in Friesian cattle under Egyptian environmental conditions.

Yousef (1990) reported that Egyptian buffalo calves had slightly higher serum total protein concentrations of 7.4 and 9.5 g/dL during summer and winter seasons, respectively.

Joshi and Tripathi (1991) opined that a concurrent increase in total protein might be due to increase protein catabolism to meet out the need of energy for maintaining homeothermy.

El-Masery and Marai (1991) studied comparison between friesians and water buffaloes in blood constituents, during winter and summer conditions and observed that in summer, cholesterol level in the plasma were higher ($P < 0{\cdot}01$), and total proteins were lower ($P< 0{\cdot}01$) in buffalo than in friesian calves.

Marai *et al* (1992) reported that blood glucose level was found to be significantly higher during summer than in winter in mature ossimi ewes.

Verma *et al* (2000) found that blood cholesterol lowered during summer than winter seasons in lactating murrah buffaloes. This may be due to dilution as a result of increase in total body water or decrease in acetate concentration which is the primary precursor for the synthesis of cholesterol. Marked increase in glucocorticoid hormone level in heat stressed animals may be another factor causing decline in blood cholesterol.

Sahin *et al* (2003) revealed that higher plasma glucose concentration in summer stressed buffaloes was probably due to greater catabolic effect of corticosterone, and increased gluconeogenesis yielding more of glucose.

Rasooli *et al* (2004) found an increase in total protein concentration in buffaloes exposed to high temperature stress. This increase in serum protein could be

a physiological attempt to maintain extended plasma volume. In cattle, dehydration during heat stress caused sharp increase in ADH levels associated with a significant decrease in urine output and a significant increase in plasma protein (El-Nouty *etal*1980).

Habeeb *et al* (2007) reported that heat stress conditions significantly decrease total protein concentration and significantly increase in heat shock protein concentration in their molecular nature in buffalo claves. Adaptability to the heat stress conditions can be detected with the least deviation of heat shock protein from their normal level.

Gudev *et al* (2007) reported that the lactating bufaloes exhibited lower level of plasma cholesterol and total protein during heat stress while plasma urea was higher when cows were exposed to direct solar radiation. The observed trend of cholesterol decline in buffaloes under heat stress could be related to decrease feed intake in hot environment. Consequently the decreased feed intake is related to reduce intake of dietary cholesterol. The observed trend of plasma total protein decline in buffalo could be due heat exposure, with initial hemoconcentration followed by hemodilution.

Guedes *et al* (2008) reported that the elevation in the levels of serum total protein in probiotic treated animals may be attributed to the fact that yeast supplementation stimulates the rumen microbial protein synthesis, so it elevates the populations and the activity of cellulolytic bacteria in rumen, consequently enhancing the fiber digestion, lactate utilization in the rumen and increase flow of microbial protein from the rumen to duodenum.

Bruno *et al* (2009) reported that concentrations of glucose in plasma of dairy cows during heat stress were not influenced ($P>0.10$) by feeding yeast culture.

Chakiroglu *et al* (2010) reported that the diet supplemented with Saccharomyces cerevisiae (Yea Sacc^{1026}/alltech) implied an extension at peak lactation period and a statistically significant increase of serum cholesterol level ($P < 0{\cdot}05$) in early lactation of jersey cow.

Singh *et al* (2012) reported that glucose and total cholesterol concentration decreased ($P<0.05$) significantly whereas total protein increased in animals which

were exposed to psychrometric chamber at 40°C. The levels of these parameters tended to be normal in animals fed with yeast powder during heat stress.

Mousa *et al* (2012) while working on Rahmani sheep where control group was fed the basal ration without any supplementation and reported that feeding diets treated with yeast culture resulted in significantly increased ($P<0.05$) glucose concentrations during late pregnancy and suckling period and also found that the average blood cholesterol concentration decreased ($P<0.05$) only in response to the low levels of dry yeast.

Abu El-Ella & Kommonna (2013) divided Damascus goat into three groups and all groups were fed 60% concentrate feed mixture (CFM) plus 40 % clover and rice straw. First group was considered as control group while group two and group three were supplemented with 2.5g and 5.0g of YC/head/day. The results revealed that the total protein concentration was highest in group two followed by group three as compared to control group.

Hussein (2014) reported that plasma glucose concentration in Najdi Ram lambs when fed on diets supplemented with probiotics increased ($P<0.05$) significantly from control group (fed ration composed of 60% concentrate feed mixture plus 40 % alfalfa hay) till the end of experimental period, respectively.

Bakr *et al* (2015) investigated the effects of Saccharomyces cerevisiae feeding on rumen, blood and milk parameters together in high producing dairy cattle during the transition and early lactation period and found that serum glucose levels were significantly higher ($P<0.01$) in yeast supplemented animals than controls at 30, 45 and 60 DMI. Furthermore, a significant reduction was found for serum cholesterol concentrations in the yeast-fed group compared to the control group at 30 ($P<0.01$), 45 ($P<0.01$) and 60 ($P<0.05$) DMI.

Yadav *et al* (2016) observed an increase in total protein concentration which indicated a change in protein metabolism shifting towards catabolic side, which was evident with decrease in body weight of the animals, perhaps the need of energy for maintaining homeothermy was met by increase in tissue protein catabolism which resulted in increased serum protein, urea and creatinine concentration.

Lakhani *et al* (2017) studies revealed that mean plasma glucose concentration, plasma total protein concentration was found to be higher in summer stressed

buffaloes (Group II) as compared to pre summer group (Group I), and lowered level of cholesterol in Group II to as compared Group I.

2.3.2 Creatnine and Blood Urea

Segura *et al* (1979) and Shaffer *et al* (1981) observed that heat stressed Friesian calves showed lowered blood urea-nitrogen levels.

El-Masry and Habeeb (1989) and Mc-Veign and Tarrent (1982) reported that creatnine concentrations were higher under heat stress than in mild conditions in Friesian cows and bulls.

Ahmed (1990) reported blood urea-nitrogen in Friesian cattle during winter and summers to be 18.4 and 15.7 mg/dl respectively.

El-Ashry *et al* (2003) and Shakweer (2003) reported that sheep fed diets treated with yeast or fungi exhibited lower blood urea concentration than control.

Khattab *et al* (2003) in sheep; Salem *et al* (2002) in lactating buffaloes and El-Kholi *et al* (2005) in buffalo calves reported no significant effect or a decrease of creatinine concentration with the addition of yeast culture.

Rasooli *et al* (2004) and Gudev *et al* (2007) reported that cows and lactating buffaloes exhibited higher levels of plasma urea during heat stress condition.

Habeeb *et al* (2007) reported a significant increase in creatinine concentration under heat stress conditions in Egyptian buffalo calves.

Gudev *et al* (2007) reported that plasma urea level tend to be higher when the heat load was the highest. The enhanced urea level could be due to the negative effect of the elevated core temperature on utilization of rumen ammonia for microbial protein synthesis.

Petr Dolezal *et al* (2011) found lower concentration in serum urea-nitrogen of cows in response to yeast culture supplementation which suggested as an indicator of better nitrogen metabolism and utilization of protein inside rumen.

Abdel-Rahman *et al* (2012) and Mousa *et al* (2012) conducted an experiment on sheep, who found that feeding diets treated with yeast culture resulted in an increase of creatinine concentration.

Das *et al* (2013) reported that there were no significant differences between urea and creatnine in Nilli-Ravi buffaloes during hot dry and hot humid seasons.

Lakhani *et al* (2017) reported that there was significant ($p<0.05$) increase in plasma creatinine and urea concentrations during summer as compared to pre summer season.

2.3.3 Lipid peroxidation

Donkoh (1989) reported that high ambient temperature causes impaired antioxidant status which is characterized by elevated lipid peroxidation in serum.

Altan *et al* (2003) reported that heat stress increased lipid peroxidation which was associated with associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids.

Yarovan *et al* (2008) observed that free radicals oxidation is activated in animals under various types of stresses and lipid peroxidation products accumulate in various organs.

Lakhani *et al* (2016) found significant rise in the erythrocytic lipid peroxidation level in summer stressed buffaloes (Group II), when compared with Group I (pre summer).

2.4 Physical characteristics of rumen liquor

Garry (2002) reported that the physical nature of rumen fluid is affected by the type of diet ingested by the animal. Even the healthy animals maintained on normal diet show considerable variations in physical characteristics of rumen fluid. Normal rumen fluid is greenish brown in colour, aromatic in odour with slightly viscous in consistency.

Singh *et al* (2016b) found that rumen liquor exhibited putrid odour, dark brown colour and watery consistency during rumen dysfunction which after treatment with HB strong, returned to normal with ammonical smell, yellowish green colour and viscous consistency.

Singh (2005) studied the effect of feed additives on rumen profile of buffaloes and found that supplementation of yeast culture did not alter the physical characteristic of rumen liquor.

Similarly, Rao (2013) did not find any change in physical characteristics upon supplementing UMMB to the buffalo calves.

2.5 Ruminal pH

Ruminal pH is a physiochemical measure for the fermentation in the rumen. It fluctuates within a broad range of normal values according to the type of feed and the time interval between last feeding and taking a sample for pH determination Radostitis *et al* (2000). The normal range of ruminal pH, however, is between 5 to 7 (Hungate 1966).

Mutsvangwa *et al* (1992) studied the effect of dietary inclusion of yeast culture on patterns of rumen fermentation, food intake and growth of intensively fed bulls and observed significantly lower pH of rumen liquor after dietary inclusion of yeast culture.

Corona *et al* (1999) studied the evaluation of two yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed a corn stover diet and found that the ruminal pH was lower with supplementation of yeast culture. Similarly, Mruthunjaya *et al* (2010) reported that rumen pH was significantly lower in Holstein Friesian crossbred cows supplemented with yeast culture when compared to exclusively roughages fed animals. However, Hucko *et al* (2009) studied the effect of yeast culture supplements in pre-weaning calves on rumen fermentation and Lopuszanska and Krzystof (2011) evaluated the effect of live yeast on dairy cows rumen fermentation and observed no significant effect on pH after supplementation of Yea-Sacc^{1026} when compare with control diets.

Incontrast, Dolezal *et al* (2011) found significant higher pH in dairy cows fed total mixed ration and yeast culture than animals fed only mixed ration. Similar findings were reported by Mwenya *et al* (2004) in sheep and Sawsan *et al* (2012) in lamb.

Yadav *et al (*2013) reported that high environmental temperature increased rumen liquor pH from 5.73 to 5.82 on average, whereas (Hall and M B 2009) reported an increase in ruminal pH form 5.82 to 6.03 during heat stress in lactating dairy cattle.

Singh and Singh (2015) observed that mean value of the ruminal pH was significantly lower ($P<0.05$) in animals supplemented with Yea Sacc^{1026} along with wheat straw, than the animals of control group (fed with conventional diet consisting of green fodder and wheat straw). Feeding of yeast culture to buffalo calves revealed a significant ($P<0.05$) decrease in the level of ruminal pH post-prandial which could

be attributed to increase in microbial fermentation which significantly ($P<0.05$) increased the TVFA production. The pH of strained rumen liquor was reported to be negatively correlated with concentration of TVFA which tended to lower the ruminal pH (Mruthunjaya *et al* 2010).

2.6 Qualitative examination for microbial activity

2.6.1 Sedimentation activity test (SAT)

Unfavorable changes in the rumen liquor could be detected by observing the time taken for floatation of sediment (Nicholas and Penn 1958). Settling of particulate materials rapidly and prolongation of time required for floatation indicates abnormality of rumen function (Chakrabarti 2001).

Dirksen (1979) found that sedimentation activity time in normal healthy cattle varies from 4 to 8 minutes depending upon ration and time after feeding.whereas change in diet from good to poor quality roughages resulted in a significant increase in SAT with an average value of 39.9 minutes (Misra *et al* 1972a).

Similarly, Gnanaprakasam *et al* (1986) observed retardation to complete absence of floatation of sediment occurred in starvation, in appetence and following feeding of nutritionally poor diets.

Misra *et al* (1972b) reported sedimentation activity time of 12.8 minutes in healthy zebu cattle.

Singh (2005) studied the effect of feed additives on rumen profile in buffaloes and observed that SAT was significantly decreased in animals supplemented with Yea Sacc^{1026} than animals fed conventional diet only.

Singh and Bhatia (2012) examined the effect of herbally formulated drug AV/DAC on healthy buffalo calves and concluded that the SAT was highest before feeding and there was a sharp decrease at 2hr after feeding in both control as well as treatment group.

Singh and Singh (2015) reported significant ($P<0.05$) decrease in sedimentation activity time in yeast treated animals when compared to control group which might be due to an increase in microbial activity because of supplementation of Yea Sacc^{1026} which increases the microbial population significantly ($P<0.05$).

2.6.2 Methylene blue reduction test (MBRT)

Methylene blue reduction test reflects the anaerobic fermentative metabolism of the bacterial population (Dirksen 1979).

Garry (2002) found that a rapid discolouration of methylene blue dye indicates a very active microflora, whereas delayed reaction indicates less microbial activity. MBRT of a normal ruminal fluid ranged from 3 to 6 minutes but with inactive microflora, it may be prolonged to 15 minutes or more.

Singh (2005) studied the effect of feed additives on rumen profile in buffaloes and recorded significantly lower value of MBRT in animals supplemented with Yea Sacc^{1026} when compared to control group fed with conventional diet.

Singh and Singh (2015) observed the lowest values of MBRT at 3hr post feeding and highest values at 0 hr before feeding. The highest values before feeding at 0 hr could be attributed to low activity of microflora because of non-availability of nutrients before feeding. The overall mean value of MBRT was significantly lower ($P<0.05$) in yeast treated group followed by animals kept on normal diet as compared to experimental animals kept on wheat straw.

2.7 Rumen microbiology.

2.7.1 Total bacterial count

Hungate (1966) observed that an abnormal increase or decrease in the pH of rumen liquor considerably suppresses the microbial activity.

Kamra and Pathak (1996) reported that normally total number of rumen bacteria may vary from 10^7 to 10^{12} per ml of rumen fluid. However, the population changes both qualitatively and quantitatively in response to change in diet.

Kumar *et al* (1994) reported that the number of total bacteria and protozoa were increased proportionately by 0.554 ($P<0.02$) and 0·079 ($P<0·05$),respectively when treated with yeast culture (5g/animal/day for 6 weeks) supplementation as compared to control group (diet consisting of, a dry-matter basis, wheat straw, berseem and concentrate).

Another reason could be that S. cerevisiae might have provided growth factors, pro-vitamins and micronutrients that stimulated the growth of bacteria in the rumen (Chaucheyras-Durand *et al* 1995).

Kaur *et al* (2003) and Singh *et al* (2008) studied the effect of Yea Sacc^{1026} on ruminal fermentation in buffalo calves and found increase in number of total bacteria, total viable bacteria and cellulolytic bacteria.

Lascano *et al* (2009) and Patra (2012) observed that yeast additives exert positive effects on digestibility especially fiber components, probably by stimulating cellullolytic activities of rumen microorganisms.

Romero-Pérez*et al* (2011) reported that the bacterial population diversity in rumen liquor samples appeared to decrease concurrently with the ambient temperature.

Kumar *et al* (2013) observed the mean total bacterial count (x10^9/ml of SRL) in the yeast supplemented group (14.46±0.44) was higher (P<0.01) than the control (8.67±0.27). The increase in the total bacterial count in the yeast supplemented group may be attributed to the positive effect of the yeast culture. Time of sampling had a significant (P<0.01) effect on the total bacterial count. The total bacterial count concentration was the highest (14.78±1.11) at 4 h post-feeding. It increased (P<0.01) from 0 hr to 4 hr post-feeding, and then declined The probable reason for increased rumen microbial numbers after yeast culture supplementation could be assigned to the capacity of yeast to remove oxygen from the rumen.

Singh and Singh (2014) reported that total bacterial count was significantly higher (P<0.05) in animals supplemented with Yea Sacc^{1026} as compared to animals which were exclusively fed wheat straw. Increased bacterial number in the rumen could be attributed to the capacity of yeast to remove oxygen from the rumen. Yeast may scavenge available oxygen on the surfaces of freshly ingested feeds which creates better conditions for the growth of strict anaerobic cellulolytic bacteria thereby stimulating their attachment to forage particles and increasing the initial rate of cellulolysis (Chaucheyras-Durand *et al* 2008).

2.7.2 Total and differential protozoal count

Hungate (1966) reported that rumen protozoa are anaerobic, can ferment plant materials for energy and can grow symbiotically with rumen bacteria. Two chief groups of protozoa, holotrichs and entodiniomorphs, occur in the rumen. Total and differential protozoal count in rumen liquor differs depending upon the diet of animal.

Purser and Moir (1966) studied the dietary effects upon the concentrations of protozoa in the rumen and noticed that greater protozoal concentrations were associated with high concentration of ammonia in the rumen liquor.

Naga and El-Shazly (1969) recorded total protozoal count ranging from 2.6 to 3.6 x 10^5per ml of SRL from healthy buffalo calves whereas, Misra *et al* (1972a) obtained an average protozoal count of 3.58 x 10^5 per ml of SRL in healthy cattle.

Misra *et al* (1972b) revealed an increase in diplodinia percentage with decrease in entodinia population. They also reported decrease in total protozoal count from base value of 3.58 x 10^5 to 2.24 x 10^5 per ml of SRL when feeding pattern was shifted from good to poor quality roughages.

Mwenya *et al* (2005) while working on the effect of yeast culture and galacto oligosaccharides on ruminal fermentation in Holstein cows, observed that the total protozoal count did not differ significantly amongst supplements. However, all supplemented cows had numerically higher protozoal counts than cows fed control diets.

Dolezal *et al* (2011) reported that the supplementation of yeast culture significantly increased the number of protozoa in the rumen fluid of dairy cows fed total mixed ration and yeast culture than animals fed only mixed ration. Similar findings were observed in lambs (Chaucheyras-Durand and Fonty 2002), steers (Plata *et al* 1994), sheep (Garcia *et al* 2000), heifers (Miranda *et al* 1996) and buffaloes (Singh 2005).

Kumar *et al* (2013) observed that the mean total protozoal count (x10^4 / ml of SRL) in the yeast culture supplemented group (21.50±1.48) was higher ($P<0.05$) than in the control (15.42±1.36). Time of sampling had significant ($P<0.01$) effect on the total protozoal count. The total protozoal count was the highest (25.83±1.44) at 4 hr post-feeding. It increased ($P<0.01$) from 0 hr to 4 hr post-feeding, and then declined. Feed offer was associated with an abrupt increase in the total protozoal population within the first 4 hr of post-feeding, which could be due to migration of protozoa from the reticulo-ruminal wall where they sequester from the rumen medium in response to chemical stimuli originating from the diet. The migration of protozoa into rumen liquor was due to chemotactic movement towards the feed entering the rumen. After the feed was utilized, the protozoa gradually migrated back to the reticulo-ruminal

wall after 6 hr post-feeding resulting in the observed drop in their numbers in the rumen liquor in both groups.

Singh and Singh (2014) reported that protozoal count was significantly higher ($P<0.05$) in animals supplemented with Yea Sacc^{1026} as compared to animals which were fed wheat straw and green fodder alone. This increase in the total protozoal count in animals treated with Yea Sacc^{1026} might be due to anaerobic conditions created by oxygen-scavenging activity of the yeast which favoured the growth and multiplication of protozoa of rumen (Chaucheyras-Durand and Fonty 2002).

2.8 Rumen metabolites

2.8.1 Total volatile fatty acids (TVFA)

Microbial fermentation of feed ingredients in the rumen produces volatile fatty acids which are the main source of energy for ruminants. The total concentrations of VFA in the rumen and amount of individual volatile fatty acids present are dependent on the composition of ration and feeding regime (Annison and Lindsay 1962).

Singh and Singh (2015)reported that there was progressive increase in the levels of TVFAs, which attained peak at 3 hr. post feeding and subsequent fall up to 6 hr post feeding in group I (kept on convential diet consisting of wheat straw and green fodder), group II (wheat straw alone) and group III (wheat straw plus yeast supplementation). An initial increase in the concentration of TVFAs in all the group might be attributed to increase in fermentation rate due to increased availability of nutrients (Wanapat *et al* 2013). Peak concentration of TVFAs at 3 hrs post feeding could be ascribed to maximum microbial fermentation of carbohydrates and catabolism of amino acids leading to formation of these organic acids. However, decline in TVFAs at 6 hr post-prandial might be due to absorption of these acids through rumen wall into blood stream and decrease in availability of carbohydrates and proteins for microbial fermentation (Sharma *et al* 2007). This increase in the levels of TVFAs could be attributed to stimulatory effect of Yea Sacc^{1026} supplementation on viable or total bacterial population specifically cellulolytic bacteria, which in turn, enhanced the fermentation in the rumen and resulted inincreased production of TVFAs (Mruthunjaya *et al* 2010).

Adans and Ishiar (2006) reported that volatile fatty acids production in the rumen decreases in animals exposed to heat stress, Similarlly Beed and Collier (1986)

reported that heat stress decreases volatile fatty acids produced in the rumen probably due to the decrease in feed intake.

According to Annison and Armstrong (1970) the vast majority of metabolizable energy available to ruminant animals is volatile fatty acids (VFA) from ruminal fermentation.

Thermal stress reduces quantity of VFA apparently produced in the rumen. Lower ruminal concentrations of VFA likely are related to reduced feed consumption (Gengler *et al* 1970).

Pfander and Phillipson (1953) reported that the concentration of TVFA in rumen at any particular time interval after feeding does not necessarily represent daily production but indicates a balance between the VFA production and its removal through absorption and other metabolic processes.

Devi (1987) reported that feeding of concentrate and roughage diet to buffalo calves resulted in TVFA concentration of 104.5 mEq/L of rumen liquor with peak levels at 4 hrs post feeding.

Harrison *et al* (1988) stated that supplemented yeast did not affect total VFA in lactating cows. Similarly, no effect of Saccharomyces cerevisiae supplementation on total VFAs in vivo and vitro in sheep was recorded (Singh 2005). While, Kumar *et al* (1994) recorded significantly higher concentration of total volatile fatty acids in rumen fluid of buffalo calves supplemented with Saccharomyces cerevisiae up to 12 hours and peak obtained at 4 hours post feeding.

Singh *et al* (2008) studied the effect of Saccharomyces cerevisiae on rumen profile in buffaloes and observed that total VFA were significantly increased in animals fed Yea Sacc^{1026} and conventional diet than animals fed conventional diet only.

Significant different levels ($P < .01$) of volatile fatty acids was obtained when temperatures of 18.2 and 37.7 ^{o}C were compared (Kelley *et al* 1967). The mean level for volatile fatty acids was 153.1, 147.9 and 66.3 mEq/liter of rumen fluid for 1.6, 18.2, and 37.7 ^{o}C, respectively.

Kumar *et al* (1994) observed that the concentrations of total volatile fatty acids, particularly at 4 h post feeding ($P < 0{\cdot}01$) was higher in the yeast culture

(5g/animal/day for 6 weeks) compared with the control group (diet consisting of, a dry-matter basis, wheat straw, berseem and concentrate).

Mruthunjaya *et al* (2010) observed significant increase in total VFA concentration in Holstein Friesian crossbred cows supplemented with yeast culture when compared with cows kept on roughages only.

Lopuszanska and Krzysztof (2011) also observed that the supplementation of diets with yeast preparation increased total VFA content and individual fatty acid content in ruminal fluid.

Decrease in molar concentration of volatile fatty acid during heat stress was mainly attributed to decrease in roughage intake (Kelly *et al* 1967) and variation in fermentation pattern due to changes in microbial population (Uyeno *et al* 2010).

Patra (2012) also stated the high level of total VFA content as a beneficial response of use of live yeast as a microbial feed additive in ruminant nutrition.

Stella *et al* (2007) found that yeast product supplementation plays an important role in digestibility of nutrient by altering the volatile fatty acids production in the rumen, decrease the production of ruminal ammonia and increase in ruminal microbial population.

2.8.2 Individual volatile fatty acids (IVFA)

Nonaka *et al* (2008) reported that (during high environmental temperature) at 33°C, acetic acid concentration decreased (67.8 mol%, $P<0.05$). Propionic acid and valeric acid did not differ among the thermal treatments Butyric acid at 33 °C (11.49 mol%) tended to be lower ($P<0.08$) than that at 20 °C or 28 °C (9.39 mol% or 9.37 mol%, respectively). The ratio of acetic acid to propionic acid at 33 °C (3.86 mol%) was significantly ($P<0.05$) lower than that at 20 °C or 28 °C (4.40 mol% or 4.33 mol%, respectively). Animals under heat stress have reduced acetate production whereas propionate and butyrate production increased as rumen function altered. As a response animal consumed less roughages, changes rumen microbial population and pH from 5.82 to 6.03 (Hall 2009).

Kumar *et al* (1994) observed that acetate ($P < 0{\cdot}01$) and propionate and the acetate to propionate ratio were also higher in yeast treated group as compared with control group (diet consisting of, a dry-matter basis, wheat straw, berseem and concentrate).

Tajima *et al* (2007) reported that total concentration of SCFA in the rumen dropped when the animals were kept at 33ºC. Only the proportion of propionic acid among other SCFAs was stable throughout the temperature shifts, while two other SCFAs, acetic and butyric acids, demonstrated the reverse tendencies, with the former decreasing and the latter increasing with the environmental temperatures rising. Accordingly, the ratio of acetic acid to propionic acid was decreased with the increased temperature.

Significant different levels (P <.01) of acetic acid and propionic acid were obtained when temperatures of 18.2 and 37.7 °C were compared (Kelley *et al* 1967). The mean levels for acetic acid and propionic acid were 94.4, 37.6; 94.7, 33.3; and 47.2, 10.6 mEq/liter of rumen fluid for 1.6, 18.2, and 37.7 °C, respectively.

2.8.3 Ammonia nitrogen (NH_3-N)

Kumar *et al* (1994) reported that on yeast culture inclusion the concentration of ammonia nitrogen was decreased in experimental group when compared with control group.

Warner (1956) reported that hydrolysis of proteinaceous and non-protein nitrogenous moieties of feed followed by deamination of peptides and amino acids by rumen microbial population produces NH3-N in rumen liquor.

Chaucheyras-Durand and Fonty (2001) reported that, in gnotobiotically-reared lambs harbouring a very simplified rumen microflora, ammonia concentration decreased in the presence of an active dry yeast , similar phenomenon were also observed in the rumen of newborn lambs (Chaucheyras- Durand and Fonty, 2002). In a study with adult ruminants, a similar effect on ammonia concentration occurred with daily yeast feeding (Kumar *et al* 1994). Combined, these data suggest some changes in the nitrogen metabolism of rumen microorganisms in the presence of yeasts.

Enjalbert *et al* (1999) reported that supplementation of YC (0.5% DM) significantly decreased rumen ammonia from 148.5 to 103.1 mg/dl, 3 hr post-feeding.

Alshaikh *et al* (2002) reported that rumen ammonia nitrogen concentration decreased significantly after yeast culture supplementation.

Putnam *et al* (1997) and Corona *et al* (1999) evaluated the effect of yeast on dairy cows and sheep, respectively, and found that the yeast culture had no effect on ruminal ammonia nitrogen.

Lopuszanska and Krzysztof (2011) also observed that there was no effect on ruminal ammonia nitrogen with the oral supplementation of yeast fed to cows.

Singh *et al* (2008) studied the effect of Saccharomyces cerevisiae on rumen profile in buffaloes and found significant increase in ruminal ammonia nitrogen concentration in buffaloes receiving yeast supplementation.

Similarly, Galip (2006) observed significant increase in ruminal ammonia nitrogen concentrations at 4 hours ($P < 0.05$) with yeast supplementation irrespective of the ratio diet. Similar results were found in cows (Miranda *et al* 1996, Roa *et al* 1997), sheep (Garcia *et al* 2000, Kamel *et al* 2004), calves (Pinos-Rodriguez *et al* 2008) and goats (Ozsoy *et al* 2013).

In contrast, lower concentration of ruminal ammonia nitrogen with yeast supplementation has been reported by Harrison *et al* (1988), Kumar *et al* (1994) and Sawsan *et al* (2012).

Salles *et al* (2010) reported that the heat-stressed animals presented higher levels of ammonical nitrogen in the ruminal liquid.

According to Nocek & Russell (1988), the interaction between the carbohydrates and the protein in the rumen metabolism is particularly intense, and if there is any kind of deficiency or inefficiency of the feed protein use in the rumen, the carbohydrate digestibility may decrease. If the diet carbohydrate is insufficient to support the microbial growth, the nitrogen will be lost as rumen ammonia and its concentration will increase in the rumen. As there was a decrease on feed ingestion by the animals under heat stress, carbohydrate in the rumen decreased to support microbial growth causing increase in ruminal ammonia nitrogen.

Another explanation would be a possible increase in the retention time of protein fraction in the rumen leading to a higher soluble protein degradation therefore increasing NH_3-N concentration in these animals. Because of the decrease on ruminal concentrations in animals exposed to high environmental temperatures (Yousef, 1985), there is an excessive concentration of ammonia in the rumen when the protein degradation exceeds the amino acid and ammonia assimilation rates to the microbial protein synthesis (NRC, 2001).

2.8.4 Total nitrogen

Hendrix and Martin (1963) reported that concentration of various nitrogen fractions in the rumen is influenced by a variety of factors viz. quality and quantity of proteins in diet, proportion of non-protein to protein nitrogen in feed as well as the physical form, solubility and chemical makeup of the protein in diet.

El-Waziry *et al* (2000) studied the effect of baker's yeast (Saccharomyces cerevisiae) on protein digestion in sheep and found that nitrogen balance was slightly increased by addition of yeast.

Sawsan *et al* (2012) also concluded that the addition of yeast to sheep rations containing different levels of roughages improve the nitrogen balance.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental animals

Eight apparently healthy male buffalo calves of 10-12 months were procured from Private dairy farm in and around Ludhiana and housed separately under proper hygienic conditions at the animal shed of department of Veterinary Physiology and Biochemistry. The experimental animals were dewormed before the start of the experiment. The animals of all the groups were fed conventional diet comprising of wheat straw, concentrate, mineral mixture and green fodder. The animals of all the groups were fed twice daily on their respective diets at 9.00 A.M and 4.00 P.M. individually for a period of 21 days for microbial adaptation. Fresh and clean drinking water was provided *ad libitum* immediately after feeding. The study was conducted during two seasons, i.e. Pre-summer (March-April) and summer (June to August).

3.2 Grouping and feeding of animals

The experimental animals were divided into three groups having four animals in each group.

a) **Group I (Pre-Summer): (**n=4). No supplementation was given to these animals.

b) **Group II (Summer Control): (**n=4). No supplementation was given to these animals.

c) **Group III (Summer Treatment): (**n=4). The animals of this group were supplemented with Yea Sacc^{1026} @ one bolus (consisting of 25 billion live yeast cells) / animal / day for 21 days after acclimatization with summer season for one month.

Same animals were used in pre summer and summer control groups.

3.3 Fistulation of animals

The animals of all the groups were surgically operated for rumen fistulation on left flank. Permanent rumen fistula was fitted to each animal following the technique adopted by Roychoudhury (1981). Animals were operated four weeks prior to the commencement of experiment, so that they could adopt themselves to fistulae. Post operative care was taken throughout the study to avoid infection and maggot infestation.

3.4 Collection of rumen liquor

Rumen liquor samples were collected through rumen fistula from various positions and depths to obtain representative sample with the help of suction pump. Each animal was sampled for three consecutive days after the period of microbial adaptation. First sample was taken before feeding i.e. at 0 hr and subsequent samples were obtained at 1, 2, 3, 4, 5, and 6 hrs interval post prandial. Collected samples were strained through double layer of muslin cloth to remove solid particles as suggested by Lengemann and Allen (1955) and designated as strained rumen liquor (SRL).

3.5 Preservation of samples

Sufficient quantity of rumen liquor samples were preserved by adding few drops of saturated mercuric chloride solution for the analysis of various rumen metabolites viz. total volatile fatty acids, ammonia nitrogen and total nitrogen. For microbial count, rumen liquor samples were preserved in equal volume of 10% formalin solution. The samples were stored at -20°C until analyzed for different parameters. Rest of the parameters viz. physio chemical characteristics like PH, Colour, odour and consistency, sedimentation activity test (SAT), methylene blue reduction test (MBRT) were analyzed in the fresh rumen liquor**.**

3.6 Parameters studied

3.6.1Physiochemical characteristics of SRL

i) pH

ii) Colour, odour and consistency

iii) Sedimentation activity test (SAT)

iv) Methylene blue reduction test (MBRT)

3.6.2 Rumen Microbiology examination

i) Total bacterial count

ii) Total protozoal count

iii) Differential protozoal count

(a) Percent holotrichs

(b) Percent entodiniomorphs

3.6.3Rumen metabolites

i) Total volatile fatty acids (TVFA)

ii) Ammonia nitrogen (NH_3 –N)

iii) Total nitrogen

iv) Individual volatile fatty acids (IVFA)

3.7 Analytical procedures

3.7.1 Physical characteristics of rumen liquor

Rumen liquor samples were physically examined for its colour, odour and consistency immediately after its collection as described by Garry (2002).

3.7.2 pH of rumen liquor

pH of rumen liquor was determined immediately after collection of sample by digital pH meter (Systronics digital pH meter 802).

3.7.3 Sedimentation activity test (SAT)

Sedimentation activity test was noticed according to the method adopted by Dirksen (1979). Freshly collected rumen contents were observed in glass test tubes kept in water bath at 39^0 C. Normally most of the fine food particles begin to settle at once, while the larger and more fibrous constituents are carried upward by gas bubbles resulting from fermentation forming a broad, foamy upper layer. The time required for sedimentation and floatation was noted and was referred as sedimentation activity time.

3.7.4 Methylene blue reduction test (MBRT)

Methylene blue reduction test was recorded as per the method described by Dirksen (1979). This test measures the redox potential of rumen liquor by measuring the time required by rumen fluid to discolour methylene blue dye. 1 ml of 0.03% methylene blue solution and 20 ml of freshly collected rumen liquor were mixed in a test tube and incubated in a water bath at 39°C. Time required for discolouration of sample was noted using a plain rumen fluid as a basis for comparison.

3.7.5 Total bacterial count

Total bacterial count was determined according to the method of Gall *et al* (1949) using nigrosine slide technique. The preserved sample of SRL was thawed and shaken vigorously in order to separate microbes from feed particles and to break microbial clumps. The thawed rumen liquor sample was centrifuged @ 3000 rpm for 5 min and supernatant was taken. After that, the supernatant was serially diluted in

1:10,000 ratio with distilled water. The diluted bacterial suspension was mixed well and 0.01 ml of diluted suspension was taken onto a clean grease free glass slide. A loopful of saturated nigrosine (water soluble) stain was added to 0.01 ml of diluted suspension. The sample was mixed thoroughly and uniformly spread over 2x2 cm area of glass slide with the help of platinum loop. The smear was dried immediately over a preheated (about 60°C) hot plate. Bacterial counting was done in total 30 microscopic fields from 2x2cm area of stained smear under oil immersion objective of microscope. The total bacterial count per ml of rumen liquor was calculated by the formula given below:

Total bacterial count per ml of rumen liquor	=	Average no. of bacterial per filed	×	Microscopic factor (1000)	×	Dilution factor (10^6)

3.7.6 Protozoal count

Rumen protozoal count was done as per the method described by Naga and El-Shazly (1969). 5 ml of formalinized sample was taken through wide bore (3.5 mm) pipette into a test tube. Then 15 ml of normal saline solution (0.85%) was transferred and thereafter 5 ml lugol's iodine was added. The solution was mixed gently and 0.1 ml of sample was transferred swiftly to a dry clean slide and spread under a glass cover of known area (24x60 mm). 30 fields were counted per slide both for ease and accuracy and total protozoal count per ml of RL was calculated by the formula given below:

Total protozoal count per ml of rumen liquor	=	Average no. of protozoa per filed	×	Microscopic factor (1000)	×	Dilution factor (100)

Differential counting of holotrichs and entodiniomorphs was carried out using a lowpower objective (10 X). Observations were made on the basis of morphological features compiled by Hungate (1966).

3.7.7 Total volatile fatty acids (TVFA)

Total volatile fatty acids concentration in rumen fluid was estimated by the method of Barnett and Reid (1957). 1 ml of SRL was transferred into the Markham's micro-kjeldahl distillation apparatus and 1ml of scaribrick buffer (10% potassium oxalate and 5% oxalic acid in equal volumes) was added to it. The cup was made air tight with stop cork and by adding some water in it. Steam distillation was carried and

approximately 75 ml distillate was collected. To the distillate, few drops of phenolphthalein indicator were added and titrated against standard 0.01 N NaOH solution.

TVFA (mEq/L) = Amount of standard base used x 10

3.7.8 Individual volatile fatty acids (IVFA)

Individual volatile fatty acids were estimated using Netchrom 9100 gas chromatograph (Netal, New Delhi, India) equipped with flame ionozation detector. The gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 was used for the estimation of TVFA. The gas flow for nitrogen hydrogen and zero air were 30, 30 and 320 ml/min, respectively.

Temperature for injector oven, column even and detector were 270°C, 172°C respectively. Samples were prepared by adding 0.2ml of 25% metaphophoric acid per ml of rumen liquor/ content of in vitro syringes, allowing it to stand for 2 hrs followed by centrifugation at 4000 rpm for 7 min, supernatant was used for estimation of VFA. Standard VFA mixture was prepared by mixing stock solution (each of 25mg/ml concentration of standard VFAs and distilled water in the following amounts: acetic acid 1.68ml, propionic acid 0.48 ml, butyric acid 0.24 ml, distilled acid 7.24 ml to obtain final concentration of acetic acid 7.0, propionic acid 1.62, valeric 0.68, mm/100ml. The standard was stored in deep freezer until further use.

3.7.9 Ammonia nitrogen (NH_3-N)

Conway micro diffusion technique of Conway (1957) was used to estimate NH3-N in SRL. In the inner chamber of Conway cell, 1 ml of 2% boric acid solution containing mixed indicator was taken. I ml of clear SRL was pipetted into the outer compartment. 1 ml of 50% potassium carbonate solution was then slowly added into the outer compartment opposite to SRL. After covering the micro-diffusion cell, it was gently rotated clockwise and anticlockwise at a horizontal plain to mix contents of outer chamber followed by incubation for 1 hr at 38°C. Thereafter, contents of the inner chamber were titrated against standard 0.01N H_2SO_4 solution. Simultaneously, a blank of 1 ml distilled water was also titrated against standard acid.

$$\text{Ammonia nitrogen (mg/dl)} = \frac{\text{ml of standard acid used} \times 0.14}{\text{Volume of sample taken}} \times 100$$

3.7.10 Total nitrogen

The total nitrogen in the SRL was determined by the method of McKenzie and Wallace (1954). 1 ml of SRL was pipetted into a digestion flask and 2 ml of concentrated H_2SO_4 was added to it, followed by about 200 mg digestion mixture (copper sulphate 1 part and potassium sulphate 10 parts). The contents of digestion flask were heated till the solution becomes clear. After cooling, required amount of distilled water was added to it to make final volume approximately 50 ml. The contents of flask were transferred to the steam distillation apparatus. Thereafter, 10 ml of 40% NaOH solution was added to it. The distillate was collected into 10 ml of 2% boric acid solution containing mixed indicator till the final volume becomes approximately 50 ml. This distillate was titrated against standard 0.01 N H_2S0_4. Duplicate samples were run. A distillate blank of distilled water was also titrated simultaneously.

$$\text{Total nitrogen (mg/dl)} = \frac{\text{ml of standard acid used} \times 0.14}{\text{Volume of sample taken}} \times 100$$

3.8 BLOOD SAMPLING

Three Blood samples (6-8 ml each) were collected aseptically by jugular vein-puncture from each animal in each group at weekly intervals. Sodium fluoride vials were used to collect 1-2 ml blood for estimation of blood glucose and heparinized vials were used to collect 7-8 ml of blood for analysis of other biochemical parameters. The blood samples were kept in ice bucket and transported quickly to the laboratory for further processing.

3.8.1 Sampling schedule

Three blood samples were collected from each animal at weekly interval in pre-summer group, summer group and summer treatment group. In summer treatment group animals the first sampling was done after one week of start of supplementation.

3.8.2 Processing of samples

The blood samples were analyzed shortly after collection for hematological parameters viz. hemoglobin and packed cell volume and rest of samples were

processed for the separation of plasma and preparation of hemolysate. The upper meniscus of the blood sample in each collection vial was marked and samples were immediately centrifuged at 2500-3000 rpm for 30 minutes. Plasma was separated and stored in small aliquots at -20°C for analysis of various parameters. Buffy coat was removed and sediment (erythrocyte pellet) was washed thrice with normal saline solution. Distilled water was added up to the marked level and the resulting hemolysate was stored at -20°C till analyzed for erythrocytic lipid peroxidation (LPO) as per Placer *et al* (1966) and Nishikimi *et al* (1972), respectively.

3.9 Observations to be Recorded

i) Physiological

a. Rectal temperature
b. Respiration rate
c. Heart rate

ii) Biochemical

a. Blood Glucose
b. Total Protein
c. Plasma cholesterol
d. Urea
e. Creatinine
f. Erythrocytic Lipid Peroxidation

iii) Hematological

a. Hemoglobin
b. Packed cell Volume

iv) Environmental

a. Environmental temperature
b. Relative humidity

3.9.1 Rectal Temperature

Rectal temperature (˚F) of all the animals were recorded with the help of clinical thermometer by carefully inserting it into the rectum of each animal and keeping the same in contact with the rectal mucous membrane of for a minimum of 2

minutes. The thermometer was cleaned between animals using cotton wool soaked in methylated spirit and lubricated with liquid paraffin.

3.9.2 Respiration Rate

The respiration rate (per minute) was recorded by observing and counting the movement of the thoracic cage of the experimental animals. Care was taken not to disturb the animals.

3.9.3 Heart Rate

Heart rate was recorded with the help of stethoscope on left side at a point behind the elbow.

3.9.4 Biochemical Parameters

Blood glucose, total protein, plasma cholesterol, urea and creatinine, all these parameters were estimated with automatic BPC biosed chemistry analyser within one week of collection using BPC biosed kits.

3.9.5 Lipidperoxidation (Placer *et al* 1966)

The method is based on the principle that the reaction of malondialdehyde (MDA), an end product of lipid peroxidation, with thiobarbituric acid (TBA) yields a pink coloured trimethine complex which is measured at 548 nm.

Reagents

i) 0.2M tris - 0.16M KCl buffer (pH 7.4)
ii) 7% perchloric acid
iii) 1N NaOH
iv) TBA reagent: 0.8% TBA solution was prepared by dissolving TBA in a small amount of 1N NaOH and then neutralized with 7% perchloric acid or pyridine.
v) Pyridine / n-butanol reagent (3/1, v/v)

Procedure

Test and control were prepared as follows:

- 1.4 ml of buffer was added to 0.1 ml of hemolysate.
- All test solutions were incubated at 37^{o} C for 30 min whereas control solution was not incubated.
- Added 1.5 ml of TBA reagent to all the tubes.
- Heated the test solutions in boiling water bath for 10 min using marbles as condenser.

- Cooled the test solutions
- Control solutions were not heated/cooled.
- Added 3 ml of pyridine / n-butanol (3/3, v/v) and 1.0 ml of 1N NaOH to all the solutions and mixed thoroughly.
- Read absorbance of test and control against a water blank at 548nm.

$$\text{Lipid peroxidation} = \frac{(A_T\text{-}A_c) \times 46 \times 1000 \text{ (nmol MDA produced/ g Hb}}{X}$$

Where X is Hb in mg /0.1 ml

3.9.6 Haemoglobin (Dacie and Lewis 1975)

The method is based on the principle that haemoglobin (Hb) is converted into cyanomethaemoglobin by addition of KCN or NaCN and Ferricyanide. The colour of cyanomet haemoglobin is recorded at 540 nm against a standard haemoglobin solution. Since cyanide has the maximum affinity for Hb, the method estimates the total Hb.

Reagents

Drabkin's solution: Dissolved 0.05g of KCN or NaCN, 0.20g of $K_2Fe\ (CN)_6$ and 1.0 g of $NaHCO_3$ in 1 litre of distilled water.

Procedure

20 µl of blood was transferred with the help of a haemoglobin (Hb) pipette into a test tube containing 5 ml of Drabkin's solution and mixed thoroughly. The colour was read at 540 nm against reagent blank (Drabkin's diluent). The standard curve was plotted using the standard cyanomethaemoglobin solution.

3.9.7 Packed cell volume (Benjamin 1985)

Packed cell volume (PCV) was estimated in freshly collected heparinised blood samples by micro capillary method

3.9.8 Environmental temperature and Relative humidity

The weekly records of meteorological data viz. temperature and relative humidity in the buffalo shed were recorded at the time of blood samplings using thermo-hygrometer. The temperature humidity index (THI) was calculated using the following formula:

$$THI = (0.81 \times Ta) + \{(RH \div 100) \times (Ta - 14.4)\} + 46.6$$

Where,

Ta = Average ambient temperature in ºC and RH = Average relative humidity in %

The meteorological parameters and THI are given in Annexure – I

The study was conducted during two seasons, i.e. Pre-summer (March-April; Mean THI=68.5) and summer (June to August; Mean THI= 83.5).

Statistical analysis

The effect of supplementation of Yea Sacc^{1026} during summer season on various physiological, blood and ruminal parameters of buffalo calves was compared by applying ANOVA at 5% level of significance as per the method described by Snedecor and Cochran (1968) using computer software SAS.

CHAPTER IV

RESULTS AND DISCUSSION

The results of present study have been discussed as below:

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 Respiration Rate

The overall mean respiration rate (breaths/min) in present study was 15.33±0.5, 19.83±1.06 and 18.03±0.89 in Group I (pre-summer), Group II (summer control) and Group III (summer treatment) respectively (Table 1).

The respiration rate is a good indicator of heat stress. In cattle respiration is the most sensitive physiological parameter which changes with change in climate and physical environment (Sabuncuoglu, 2004). This study represents that respiration rate was significantly higher in Group II as compared to Group I, which clearly represents the effect of summer stress in buffalo calves. Similar, increase ($P<0.05$) in respiration rate in Murrah buffalo during summer has been reported by Lakhani *et al* (2015) and Lallawmkimi (2009). Singh *et al* (2003) in another study observed significantly higher respiration rate in buffaloes with increase in ambient temperature and relative humidity. (Haque *et al* 2012) also recorded significant higher rectal temperature and respiration rate in young and adult buffalo after exposure to environment temperature of 40°,42°,45°C for 4 hrs as compared to thermoneutral zone temperature at 22◦C.

Present study shows that respiration rate was slightly lower in Group III (18.03±0.89) but it was not significantly lowered after supplementation of Yea Sacc[1026]. Similarly, Huber *et al* (1994) observed slight improvement in respiration rate after following supplementation of *Aspergillus oryzae* to dairy cows during summer but the author indicated that mechanism was not clear.

4.1.2 Heart Rate

In the present study overall mean heart rate was found to be 48.5±0.7, 60.08±0.45 and 48.33±1.54 in Group I, Group II and Group III respectively (Table 2).

These results indicated significant increase in heart rate during summer as compared to pre-summer. (Maurya *et al* 2007) also reported that the rise in temperature affects physiological responses of Malpura sheep and found that animals kept in shed showed significantly lower rectal temperature, respiration rate and pulse

Table 1: Effect of yeast culture supplementation on respiration rate (Mean ± S.E.) in buffalo calves during summer season

Sampling	Respiration rate (breath/min)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	17.25±0.75	17.5±1.89	19±1.47
2	14.25±0.48	19±1.47	15.5±0.71
3	14.5±0.29	23±1.08	19.6±1.5
Overall Mean ± SE	**15.33±0.5^{B}**	**19.83±1.06^{A}**	**18.03±0.89^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

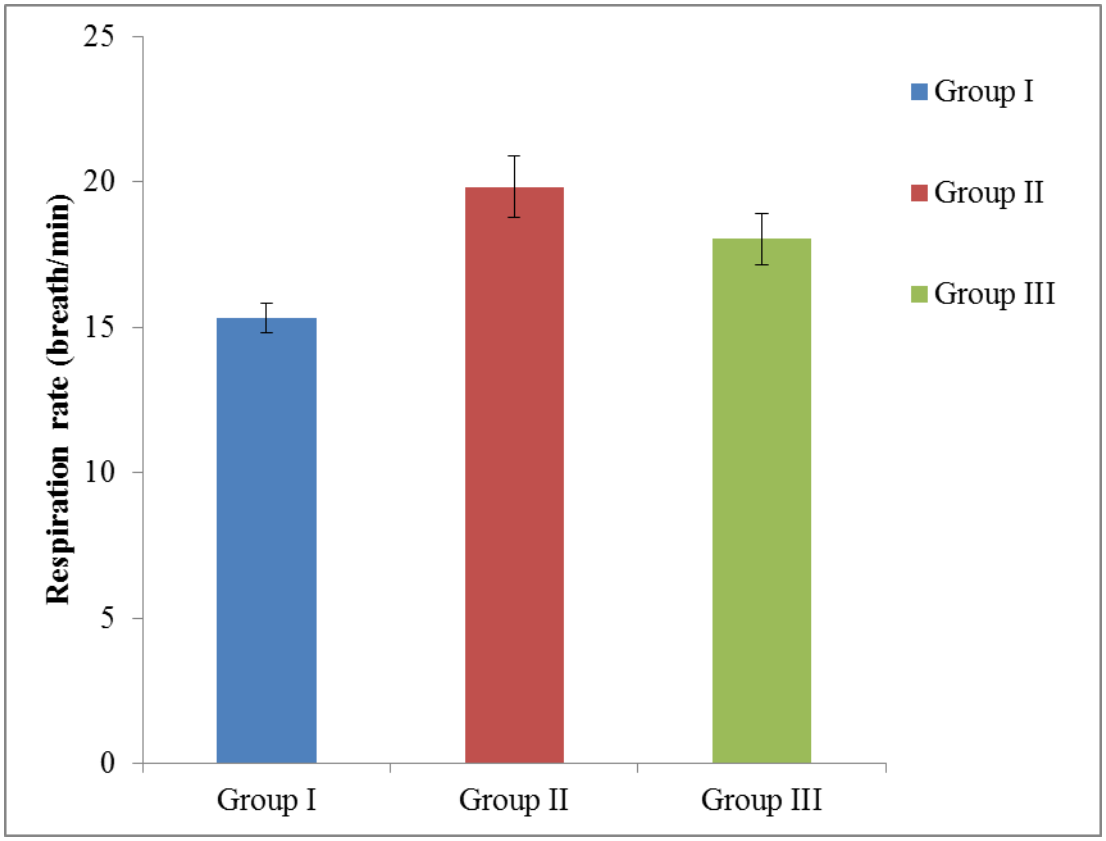

Fig. 1: Effect of yeast culture supplementation on respiration rate (Mean ± S.E.) in buffalo calves during summer season

Table 2: Effect of yeast culture supplementation on Heart rate (Mean ± S.E.) in buffalo calves during summer season

Sampling	Heart rate (beats/min)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	49.5±1.85	59.75±0.85	49.25±2.75
2	48±1.22	60.5±1.19	45.25±1.18
3	48±0.29	60±0.10	50.5±3.50
Overall Mean ± SE	**48.5 ± 0.7^{B}**	**60.08 ± 0.45^{A}**	**48.33 ± 1.54^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

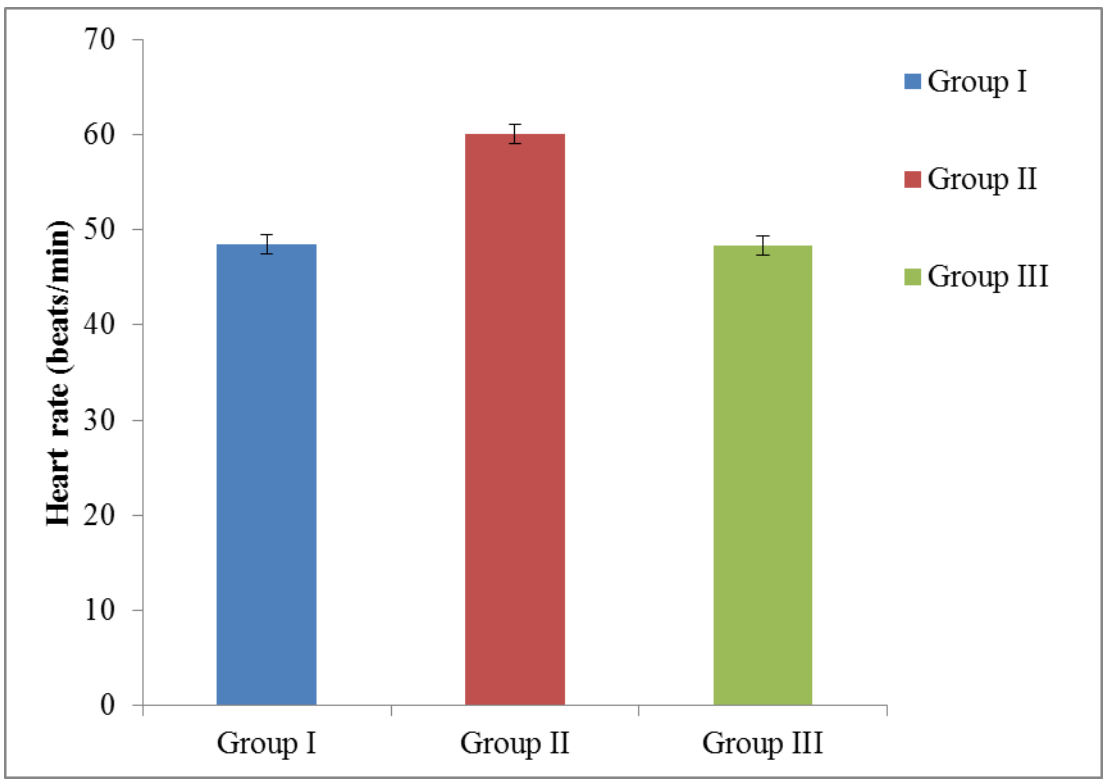

Fig. 2: Effect of yeast culture supplementation on Heart rate (Mean ± S.E.) in buffalo calves during summer season

rate as compared to animals kept in hot chamber. Yadav *et al* (2016) also observed significant increase in heart rate in buffaloes at temperature 35°C and 40ºC compared to 25°C. Similarly, Lakhani *et al* (2015) reported that values of pulse rate in murrah buffaloes were higher ($P<0.05$) in summer season as compared to those in pre summer. This increase in heart rate could be due to heat loss mechanism due to peripheral vasodilation Singh and Bhattacharya (1991). Vasodilation stimulates the motor center to flatten the hair cover to allow better heat dissipation through conduction, convection and radiation (Yousef and Johson 1966).

The Heart rate was restored to normal Pre-summer values following supplementation of Yea Sacc^{1026} in group III.

4.1.3 Rectal temperature

Table 3 depicts overall mean rectal temperature which was 101.27±0.08, 102.29±0.1 and 101.17±0.09 in Group I (pre summer), Group II (summer control) and Group III (summer treatment) respectively.

Results revealed that rectal temperature was significantly higher in Group II as compared to Group I and Group III. This rise in rectal temperature may be due to increase load of heat on buffaloes and their inability to dissipate the excess heat Shivakumar *et al* (2010). Singh *et al* (2003) observed a positive correlation between rectal temperature and ambient environment temperature & humidity in lactating Murrah buffaloes. Joshi and Tripathy (1991) reported an increase in rectal temperature from 102.0 ºF to 103.8 ºF when buffalo calves were exposed to 40.5ºC for eight hours daily for three months. The high rectal temperature observed for the heat stressed animals, was an indicator of disturbance in the homoeothermic status of the animals which was not being effectively countered by the enhanced heat loss by physical and physiological processes of thermolysis. Similar findings were reported by Lakhani *et al* (2015) and Lallawmkimi (2009) in buffaloes. Banerjee and Ashutosh (2011) also observed significantly higher rectal temperature in Tharparkar and Kran Fries during summer season when compared to Pre summer values.

In the treatment group oral supplementation of Yea Sacc^{1026} resulted in significant decrease in rectal temperature in buffalo calves during summer season. Our results are in accordance with findings of Higginbotham *et al* (1993) who observed significant reduction in rectal temperature, improves digestibility and feed

Table 3: Effect of yeast culture supplementation on Rectal Temperature (Mean ± S.E.) in buffalo calves during summer season

Sampling	Rectal Temperature °F		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	101.13±0.13	102.13±0.13	101.13±0.13
2	101.4±0.14	102.13±0.13	101±0.14
3	101.28±0.16	102.63±0.13	101.38±0.24
Overall Mean ± SE	**101.27±0.08^{B}**	**102.29±0.1^{A}**	**101.17±0.09^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

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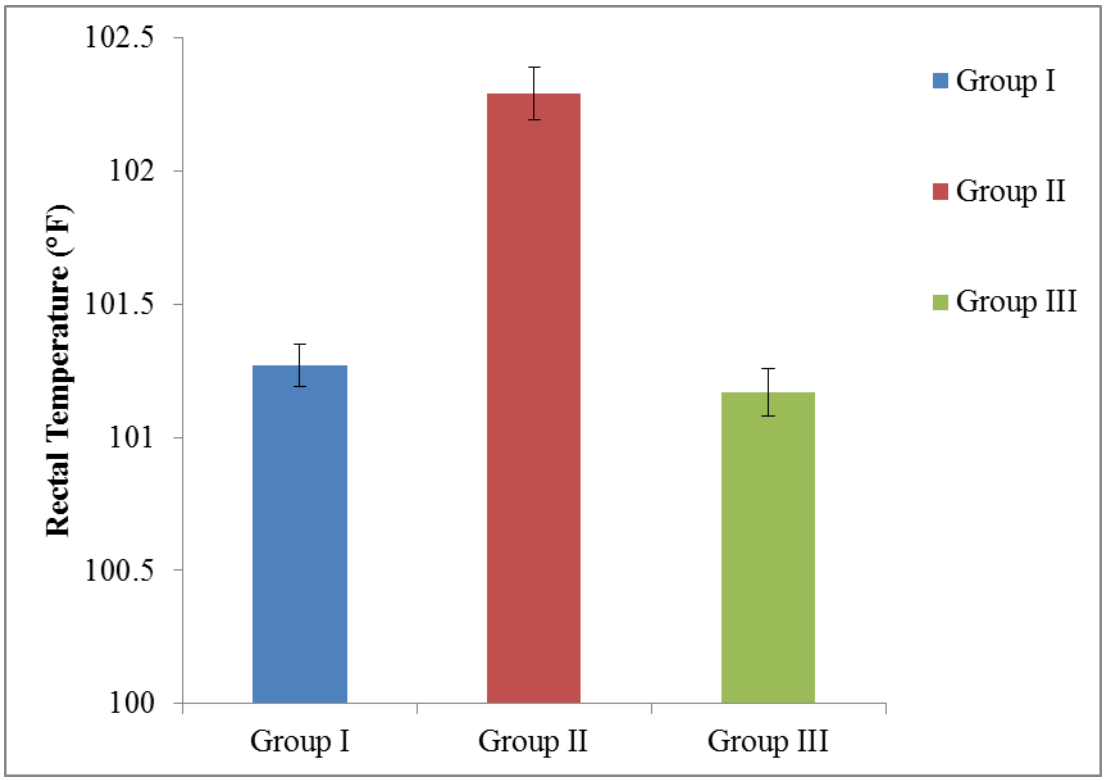

Fig. 3: Effect of yeast culture supplementation on Rectal Temperature (Mean ± S.E.) in buffalo calves during summer season

conversion efficiency in cows supplementation with Aspergillus oryzae. Similarly (Bruno *et al* 2009) also observed that there was reduction in rectal temperature in summer stressed cows after supplementation with yeast culture of Saccharomyces. cerevisiae. Huber *et al* (1994) suggested that fungal cultures such as Aspergillus. oryzae, when fed to lactating dairy cows exposed to high ambient temperatures, might improved signs of heat stress such as rectal temperature and respiration rate.

4.2 HEMATOLOGICAL PARAMETERS

4.2.1 Packed Cell Volume and Hemoglobin

In this experiment average packed cell volume (PCV) was 35.67±0.5, 30±0.78 and 34.83±0.77 % and hemoglobin concentration in (g%) was 12.38±0.08, 10.07±0.31 and 11.63±0.25 in Group I, Group II and Group III respectively (Table 4 and 5). This study shows that PCV and Hb is invariably less in Group II (summer control) as compared to Group I (Pre-summer). Lakhani *et al* (2016) also found significant decrease in PCV and Hb during summer season in buffaloes, when compared pre summer control group.

The decline in PCV and Hb in heat stressed animals might be due to haemolysis, or reduction in cellular oxygen requirements to minimize metabolic heat production (Habeeb *et al* 1992). Similarly, Bhan *et al* (2012) observed the effect of temperature variability on hematological parameters during winters, spring and summer seasons in Sahiwal cow. These parameters (PCV and Hb) were on higher side during winter as compared to other seasons. These hematological parameters show negative correlation ($P<0.05$) with Temperature Humidity Index (THI). Marai *et al* (1995), Silankove *et al* (1980), Shaffer *et al* (1981) and Shebaita and El-Banna (1982) concluded that PCV and Hb decreased in heat stressed animals during summer seasons.

This present study revealed that there was significant increase in PCV and Hb in Group III as compared to Group II. Similar results were reported by Hussein (2014) who observed that supplementation of probiotic have significant effect on hematological parameters like PCV and Hb in animals fed with 5 g and 10 g/kg probiotics diet with a concentrate feed mixture. Study done by Sarwar *et al* (2011) revealed that PCV and Hb were higher ($P<0.05$) in growing *Kajli* lambs fed diets

Table 4: Effect of yeast culture supplementation on Packed Cell Volume (Mean ± S.E.) in buffalo calves during summer season

Sampling	Packed Cell Volume (%)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	35.75±0.48	29.75±1.03	33.75±0.63
2	36.25±1.03	31.25±1.65	36.75±1.75
3	35±1.08	29±1.41	34±1.08
Overall Mean ± SE	**35.67±0.5^{A}**	**30±0.78^{B}**	**34.83±0.77^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

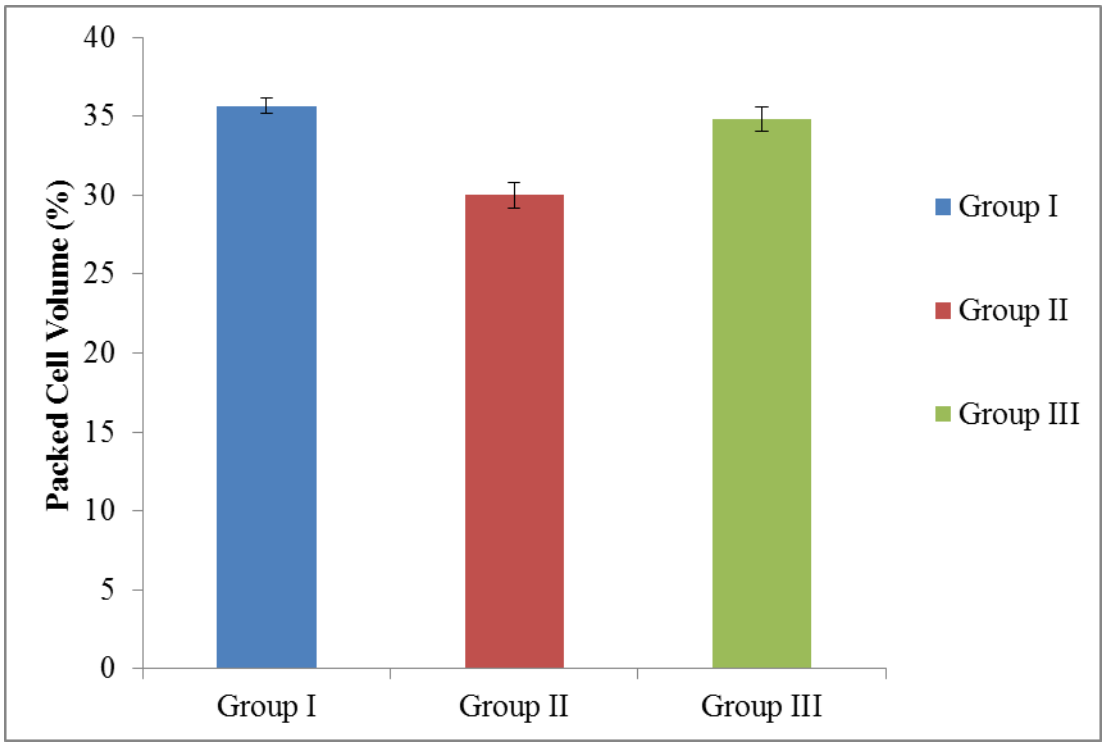

Fig. 4: Effect of yeast culture supplementation on Packed Cell Volume (Mean ± S.E.) in buffalo calves during summer season

Table 5: Effect of yeast culture supplementation on Hemoglobin Concentration (Mean ± S.E.) in buffalo calves during summer season

Sampling	Hemoglobin Concentration (g %)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	12.38±0.14	9.87±0.39	11.21±0.18
2	12.2±0.14	10.62±0.65	12.26±0.59
3	12.55±0.10	9.72±0.55	11.41±0.35
Overall Mean ± SE	**12.38±0.08^{A}**	**10.07±0.31^{C}**	**11.63±0.25^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

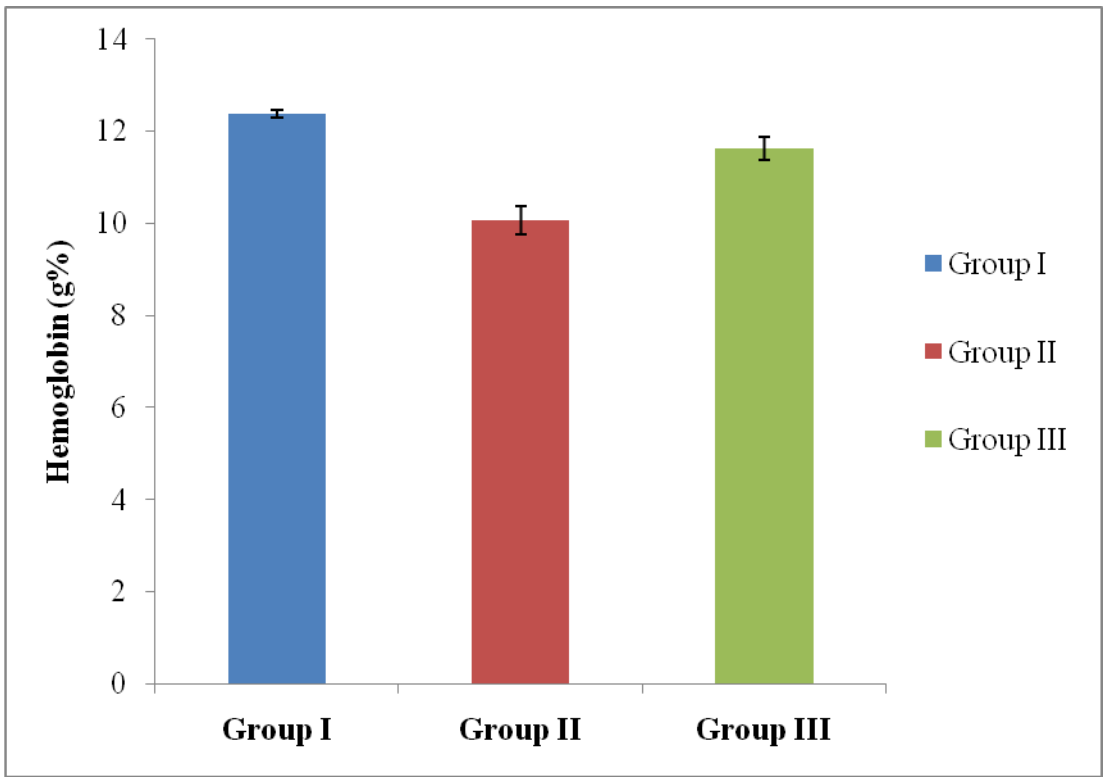

Fig. 5: Effect of yeast culture supplementation on Hemoglobin Concentration (Mean ± S.E.) in buffalo calves during summer season

containing probiotic than those without it. This rise in PCV and Hb was attributed to better iron absorption from small intestine and vitamin B synthesis following supplementation of probiotic (Kander 2004).

4.3 BIOCHEMICAL PROFILE

4.3.1 Plasma Glucose

The results of plasma glucose concentration in buffalo calves of group I, group II, and group III are 62.39±1.47, 69.26±1.01 and 63.86±1.7 mg/dl, respectively (Table 6).

Results revealed that the overall mean plasma glucose concentration was significantly decreased during summer season and was restored to normal following feeding of Yea Sacc ^{1026}. These results are supported by Sahin *et al* (2003), who observed that glucose concentration in summer may be higher due to greater catabolic effect of corticosteron and also due to increased gluconeogenesis yielding more glucose. Similarly, Singh *et al* (2008) and Chaiyabuter *et al* (1987) that during summer stress glucose level increases in buffaloes. Marai *et al* (1992) reported that blood glucose level was significantly ($p<0.005$) higher in ossimi ewes during summer than in winters.

Lakhani *et al* (2017) also reported that mean plasma glucose concentration was found to be higher in summer stressed buffaloes as compared to pre summer group.

Present study revealed that plasma glucose concentration in treatment group (group III) was restored to normal (lower) Pre-summer values as compared to group II. The fall in blood glucose concentration following Yea Sacc^{1026} supplementation during summer may be due to stabiliation of rumen fermentation and reducing the overall stress in the animals. In contrast Singh and Bhatia (2012) reported that glucose concentration decreased ($P<0.05$) significantly in animals which were exposed to psychometric chamber at 40°C. The levels of plasma glucose tended to be normal in animals fed with yeast powder during heat stress.

4.3.2 Plasma Total Protein

Mean plasma total protein concentration (g/dl) in buffalo calves of group I (pre-summer), group II (summer control) and group III (summer treatment) were 7.11±0.17, 6.558±0.1 and 6.97±0.1 g%, respectively (Table 7).

Table 6: Effect of yeast culture supplementation on Plasma Glucose Concentration (Mean ± S.E.) in buffalo calves during summer season

Sampling	Plasma Glucose Concentration (mg/dl)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	60.81±3.4	71.94±1.3	65.81±3.47
2	63.08±2.28	66.66±1.81	61.08±1.9
3	63.29±2.42	69.17±1.28	64.7±3.45
Overall Mean ± SE	**62.39±1.47^{B}**	**69.26±1.01^{A}**	**63.86±1.7^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

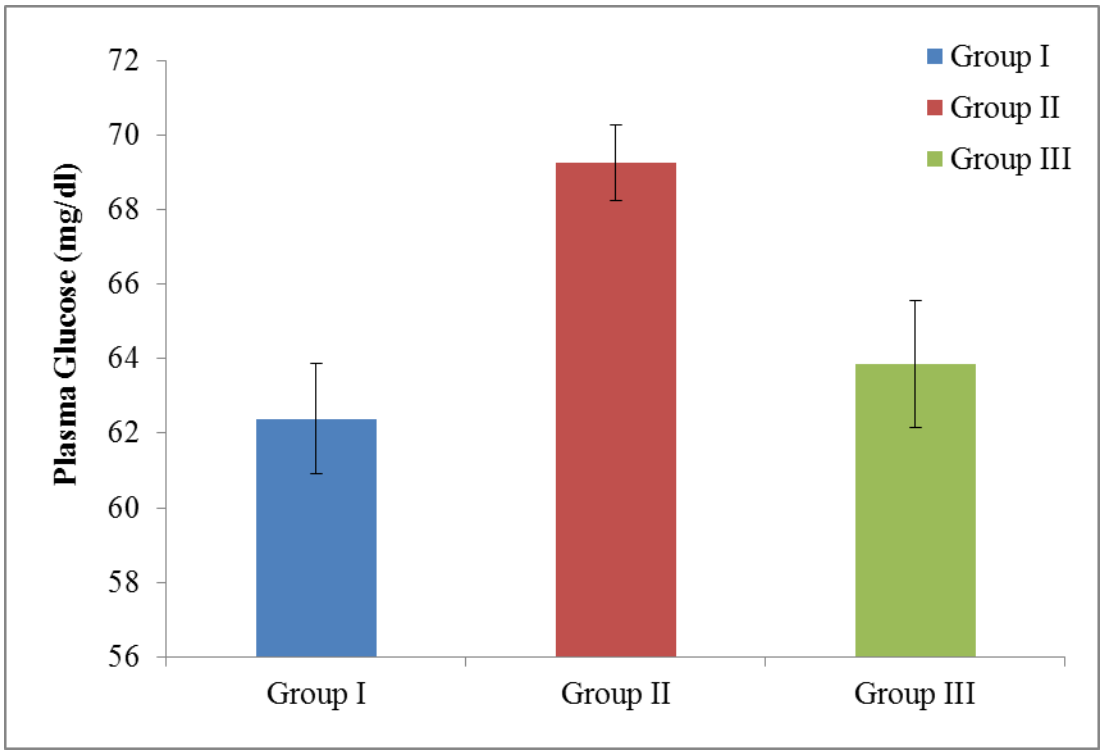

Fig. 6: Effect of yeast culture supplementation on Plasma Glucose Concentration (Mean ± S.E.) in buffalo calves during summer season

Table 7: Effect of yeast culture supplementation on Plasma Total Protein (Mean ± S.E.) in buffalo calves during summer season

Sampling	Plasma Total Protein (g%)		
	GROUP I (Pre-summer)	GROUP II (Summer control)	GROUP III (Summer treatment*)
1	7.06±0.26	6.44±0.15	6.76±0.19
2	7.12±0.38	6.48±0.09	7.15±0.18
3	7.16±0.34	6.82±0.23	7.01±0.15
Overall Mean ± SE	**7.11±0.17^{A}**	**6.58±0.1^{B}**	**6.97±0.1^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

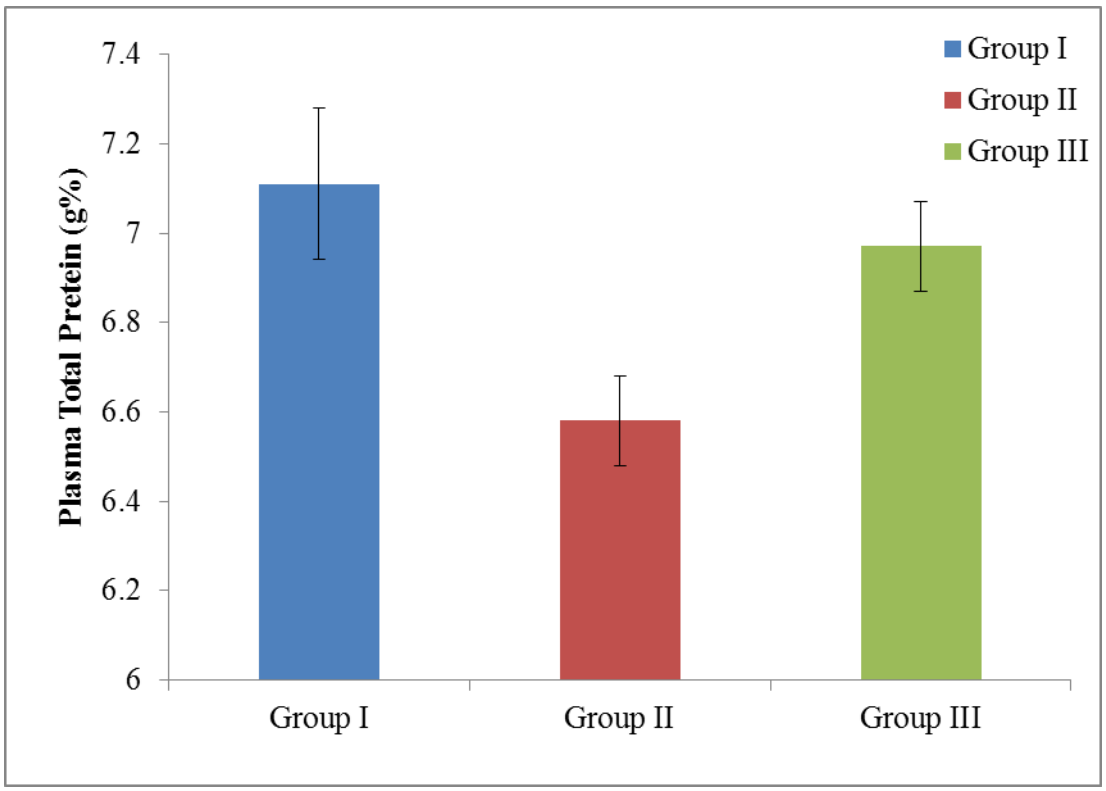

Fig. 7: Effect of yeast culture supplementation on Plasma Total Protein (Mean ± S.E.) in buffalo calves during summer season

Present study revealed that mean plasma total protein concentration was lower in group II (summer control) as compared to group I (pre-summer) which were restored to normal values following supplementation of Yea Sacc^{1026}. The significant decline in protein with rising temperature may be due to dilution of plasma protein that result in increase in water content and decease in protein synthesis, due to depression in anabolic hormonal secretion El-Masry and Habeeb (1989). Similarly Gudev *et al* (2007), reported that decline in plasma total protein in buffalo after heat exposure may be due to hemoconcentration followed by hemodilution. Verma *et al* (2000) in Murrah buffaloes and Ahmed (1990) in Friesian cattle, reported that protein level decreases in summer as compared to winters.

This study shows that plasma total protein concentration was significantly higher in treatment group (Group III) than in control (Group II) which may be due to fact that yeast supplementation stimulates the rumen microbial protein synthesis due to which population of cellulolytic bacteria increases in rumen, which leads to increase in fiber digestion and more lactate utilization in rumen and leads to increase flow of microbial protein from rumen to duodenum (Guedes *et al* 2008). These results are also supported by Abu El-Ella and Kommonna (2013) in Damascus goat.

No significant difference in mean levels of plasma total protein concentration was observed between group I (pre-summer) and III (summer treatment) which justifies that yeast supplementation brings protein level back to normal and help in releiving stress.

4.3.3 Plasma cholesterol

The mean cholesterol concentration of group I, group II and group III were 103±2.66, 94.28±2.04 and 101.45±4.24 mg/dl respectively (Table 8). The results revealed that there was decrease in plasma cholesterol concentration during summer as compared to pre summer control and values were improved following supplementation of yeast culture. However the change in values of plasma cholesterol concentration were not statistically significant. Similarly Lakhani *et al* (2016) recorded decrease in plasma cholesterol concentration during summer and increased with supplementation of amla powder.

Table 8: Effect of yeast culture supplementation on Plasma Cholesterol Concentration (Mean ± S.E.) in buffalo calves during summer season

Sampling	Plasma Cholesterol Concentration (mg/dl)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	100.3±3.9	90.38±2.17	102.38±7.31
2	106.8±6.79	99.6±4.03	103.68±8.43
3	103.15±3.09	92.85±3.08	98.3±8.23
Overall Mean ± SE	**103.42±2.66^{A}**	**94.28±2.04^{A}**	**101.45±4.24^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

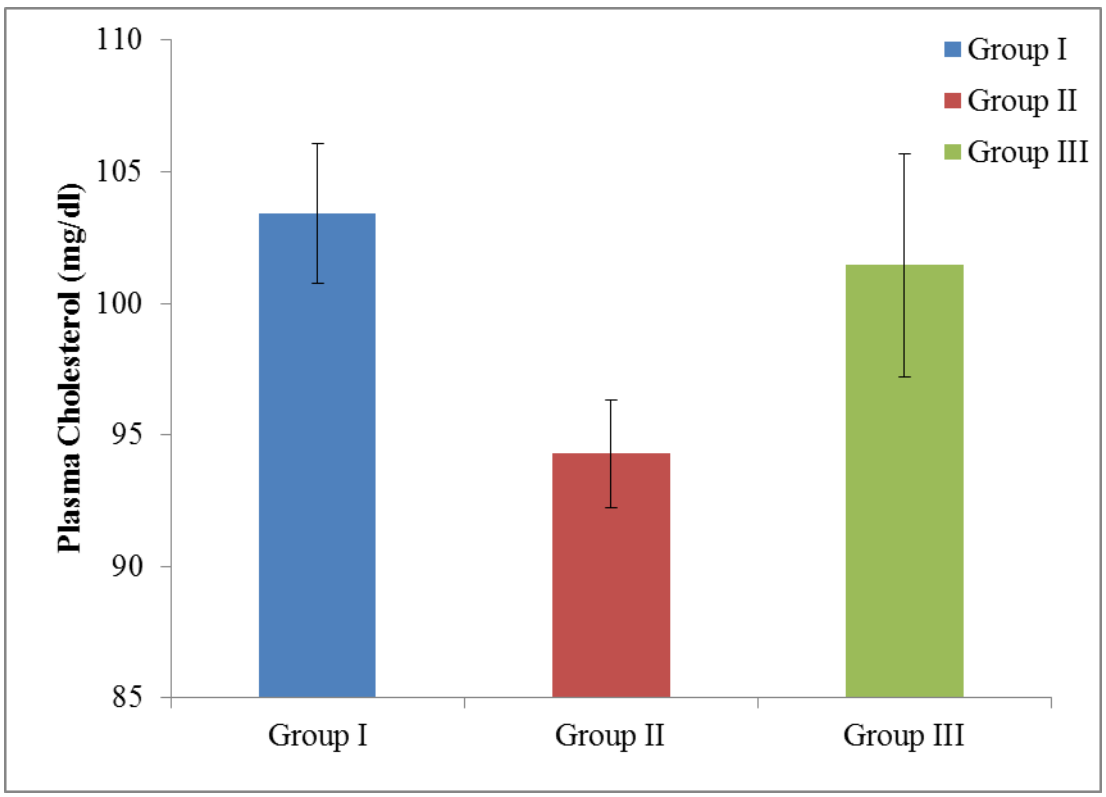

Fig. 8: Effect of yeast culture supplementation on Plasma Cholesterol Concentration (Mean ± S.E.) in buffalo calves during summer season

4.3.4 Creatinine and Urea

Overall mean plasma creatnine concentration (Table 9) is Group I (pre summer), Group II (summer control) and Group III (summer treatment) were 1.19 ±0.09, 1.58±0.08 and 1.57±0.12 mg/dl, respectively and mean plasma urea concentration 33.41±1.92, 39.95±1.94 and 34.28±1.9 mg/dl respectively (Table 10).

Table 9 and 10 depicts significant increase in plasma creatnine and urea concentration during summer control (group II) as compared to pre summer (group I). Under heat stress conditions, there is decrease utilization of NH_3-N for microbial protein synthesis which is absorbed through rumen wall and converted to urea in liver which could lead to increase in blood urea nitrogen (BUN) associated with heat stress. Gudev *et al* (2007) also reported that plasma urea level begun to be higher during heat stress. This rise in urea level could be due to negatively effect of high temperature on rumen micro flora activity.

Habeeb *et al* (2007) found increase in level of creatinine concentration during hot environment conditions in Egyptian buffalo calves. But However, Das *et al* (2013) reported no significant difference in levels of urea and creatnine in nilli-ravi buffalo during heat stress.

In the present study revealed that there was significantly decrease in plasma urea level in group III as compared to group II which could be due to better utilization of ammonia for microbial protein synthesis (Petr Dolezal *et al* 2011) which leads to lower concentration of blood urea-N in cows when treated with yeast culture supplementation. El-Ashry *et al* (2003) and Shakweer (2003) also reported similar results. However no significant change in plasma level of creatnine was observed.

4.3.5 Erythrocytic Lipid Peroxidation

The results of Yea Sacc^{1026} supplementation on erythrocytic LPO in buffalo calves is presented in Table 11. Mean values of LPO were 216±6.33, 329.99±8.6 and 240.98±6.07 nmol MDA produced/g Hb in Group I, II and III, respectively.

Present study revealed that Erythrocytic LPO level was higher in summer stressed buffalo claves (Group II) as compared to pre summer (Group I). This increase in lipid peroxidation during summer might be associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated

Table 9: Effect of yeast culture supplementation on Creatinine (Mean ± S.E.) in buffalo calves during summer season

Sampling	Creatinine (mg/dl)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	1.25±0.15	1.52±0.05	1.63±0.21
2	1.1±0.2	1.42±0.16	1.81±0.24
3	1.23±0.15	1.79±0.11	1.27±0.07
Overall Mean ± SE	**1.19 ± 0.09^{B}**	**1.58 ± 0.08^{A}**	**1.57 ± 0.12^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

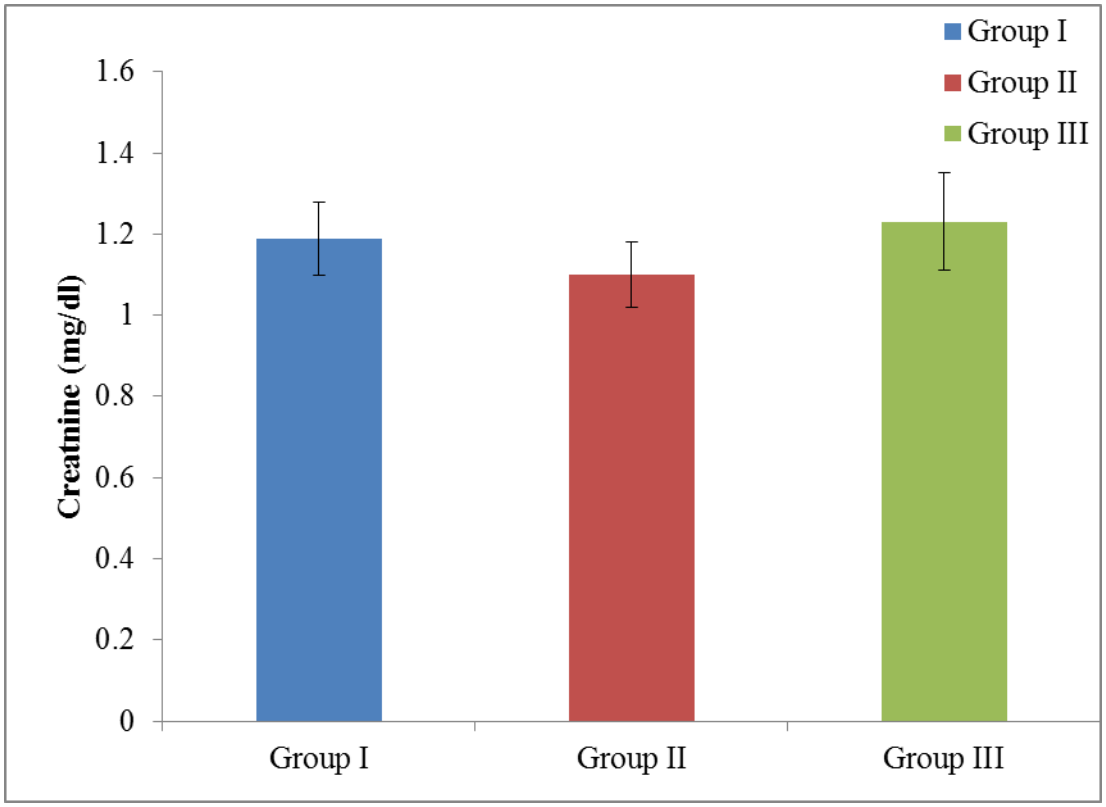

Fig. 9: Effect of yeast culture supplementation on Creatinine (Mean ± S.E.) in buffalo calves during summer season

Table 10: Effect of yeast culture supplementation on Urea (Mean ± S.E) in buffalo calves during summer season

Sampling	Urea (mg/dl)		
	GROUP I (Pre-summer)	**GROUP II (Summer control)**	**GROUP III (Summer treatment*)**
1	33.78±2.67	33.25±2.16	31.38±0.84
2	38.98±2.2	45.7±1.83	32.7±4
3	27.48±2.54	40.9±2.65	38.75±3.57
Overall Mean ± SE	**33.41±1.92^{B}**	**39.95±1.94^{A}**	**34.28±1.9^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

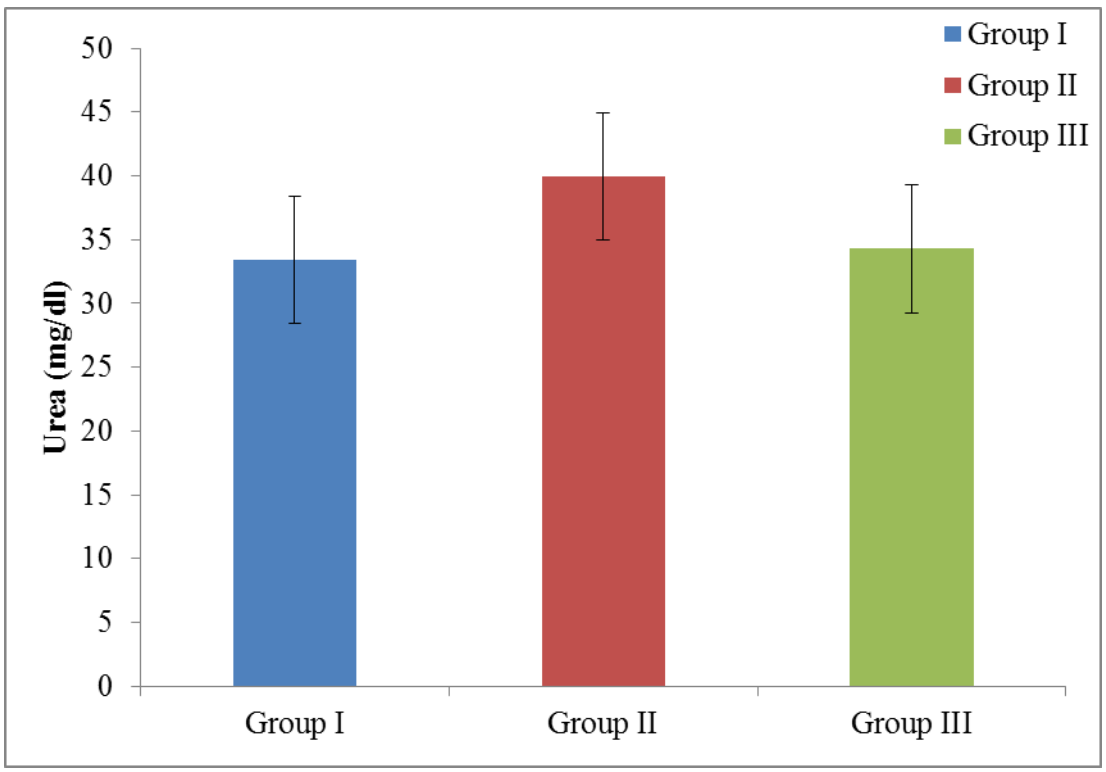

Fig. 10: Effect of yeast culture supplementation on Urea (Mean ± S.E) in buffalo calves during summer season

Table 11: Effect of yeast culture supplementation on Erythrocytic Lipid Peroxidation (Mean ± S.E.) in buffalo calves during summer season

Sampling	Lipid Peroxidation (nmol MDA produced/g Hb)		
	GROUP I (Pre-summer)	GROUP II (Summer control)	GROUP III (Summer treatment*)
1	225.38±12.24	338.5±10.46	250.61±11.94
2	209.63±8.81	336.67±15.85	226.02±9.81
3	213.96±13.01	314.8±18.36	246.32±7.15
Overall Mean ± SE	**216.32±6.33^{C}**	**329.99±8.6^{A}**	**240.98±6.07^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Overall mean with different superscripts within groups differ significantly (P<0.05)

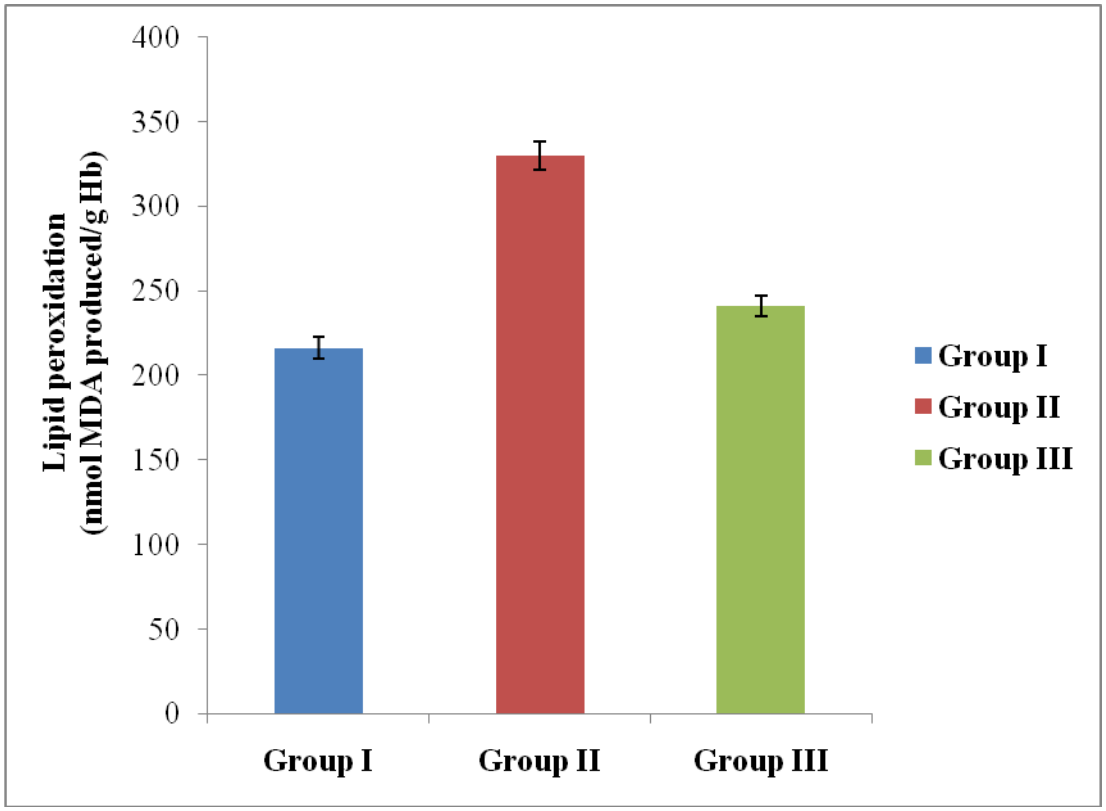

Fig. 11: Effect of yeast culture supplementation on Erythrocytic Lipid Peroxidation (Mean ± S.E.) in buffalo calves during summer season

fatty acid. Similarly Lakhani *et al* (2016) reported significant increase in erythrocytic lipid per oxidation levels in summer stressed buffalos when compared to pre summer group.

Similar results were reported by Altan *et al* (2003) who demonstrated an increase in lipid peroxidation during heat stress. Lower values of Erythrocytic LPO could be due to improved feed utilization and anti-heat stress properties of Yea Sacc^{1026}.

4.4 Physical characteristics

The data of oral administration of Yea Sacc^{1026} on physical characteristics of rumen liquor viz. colour, odour and consistency during summer season in buffalo calves have been presented in Table 12. The results indicated that there was no significant change in colour, odour and consistency of rumen liquor in any of the groups. Similarly, Singh *et al* (2008) observed no significant change in physical characteristics of rumen liquor in buffalo claves supplemented with Yea Sacc^{1026}.

4.5 Ruminal pH

The results of oral administration of Yea Sacc^{1026} on ruminal pH during summer season in male buffalo calves have presented in Table 13 and changes in ruminal pH at different time intervals before and after feeding are shown in graphically Fig. 12. The pH of rumen liquor in Group I, II and III varied from 6.73 ± 0.03 to 7.15 ± 0.05, 6.97 ± 0.03 to 7.32 ± 0.05 and 6.76 ± 0.01 to 7.17 ± 0.03, respectively. The highest value of pH was observed at 0 hr. and lowest at 3 hr. post feeding interval in all groups. The overall mean ruminal pH increased in group II as compared to control group. The pH value approached to normal level in group III after administration of Yea Sacc^{1026}. The increase in pH in group II could be due high level of ammonia nitrogen and lower levels of total volatile fatty acids. Yadav *et al* (2013) reported that the rise in pH during summer season may be attributed to detrimental effect of summer (high temperature) on rumen micro flora and reduced VFA production.

The ruminal pH decreased significantly ($P<0.05$) in Yea Sacc^{1026} supplemented group, which might be due to improved fermentation and increase utilization of ammonia for microbial protein synthesis. Similar were the findings of Singh and Singh (2015), Mutsvangwa *et al* (1992), Corona *et al* (1999) and Mruthunjaya *et al* (2010). However, Hucko *et al* (2009) and Lopuszanska and Krzyst of (2011) observed no significant effect on pH after giving Yea-Sacc^{1026}. This may be due to difference in physiological status of the experimental animals used, which has a significant effect on animal metabolism.

Table 12: Effect of yeast culture supplementation on physical characteristics of rumen liquor in buffalo calves during summer season

Physical characteristics	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
COLOUR	Greenish to Yellowish green	Greenish to Yellowish green	Greenish to Yellowish green
ODOUR	Aromatic	Aromatic	Aromatic
CONSISTENCY	Viscous	Viscous	Viscous

Table 13: Effect of yeast culture supplementation on ruminal pH in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)
0 (Before feeding)	7.15 ± 0.05^{aB}	7.32 ± 0.05^{aA}	7.17 ± 0.03^{aB}
1	6.87 ± 0.04^{bcB}	7.17 ± 0.04^{bA}	6.93 ± 0.03^{cB}
2	6.82 ± 0.04^{dcB}	7.03 ± 0.05^{cA}	6.83 ± 0.03^{dB}
3	6.73 ± 0.03^{dB}	6.97 ± 0.03^{cA}	6.76 ± 0.01^{eB}
4	6.85 ± 0.03^{bcB}	7.03 ± 0.04^{cA}	6.86 ± 0.01^{dB}
5	6.93 ± 0.04^{bcB}	7.15 ± 0.04^{bA}	6.94 ± 0.01^{cB}
6	6.99 ± 0.04^{bB}	7.23 ± 0.04^{abA}	7.03 ± 0.01^{bB}
Overall Mean ± SE	**6.87 ± 0.02^{B}**	**7.13 ± 0.02^{A}**	**6.93 ± 0.02^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

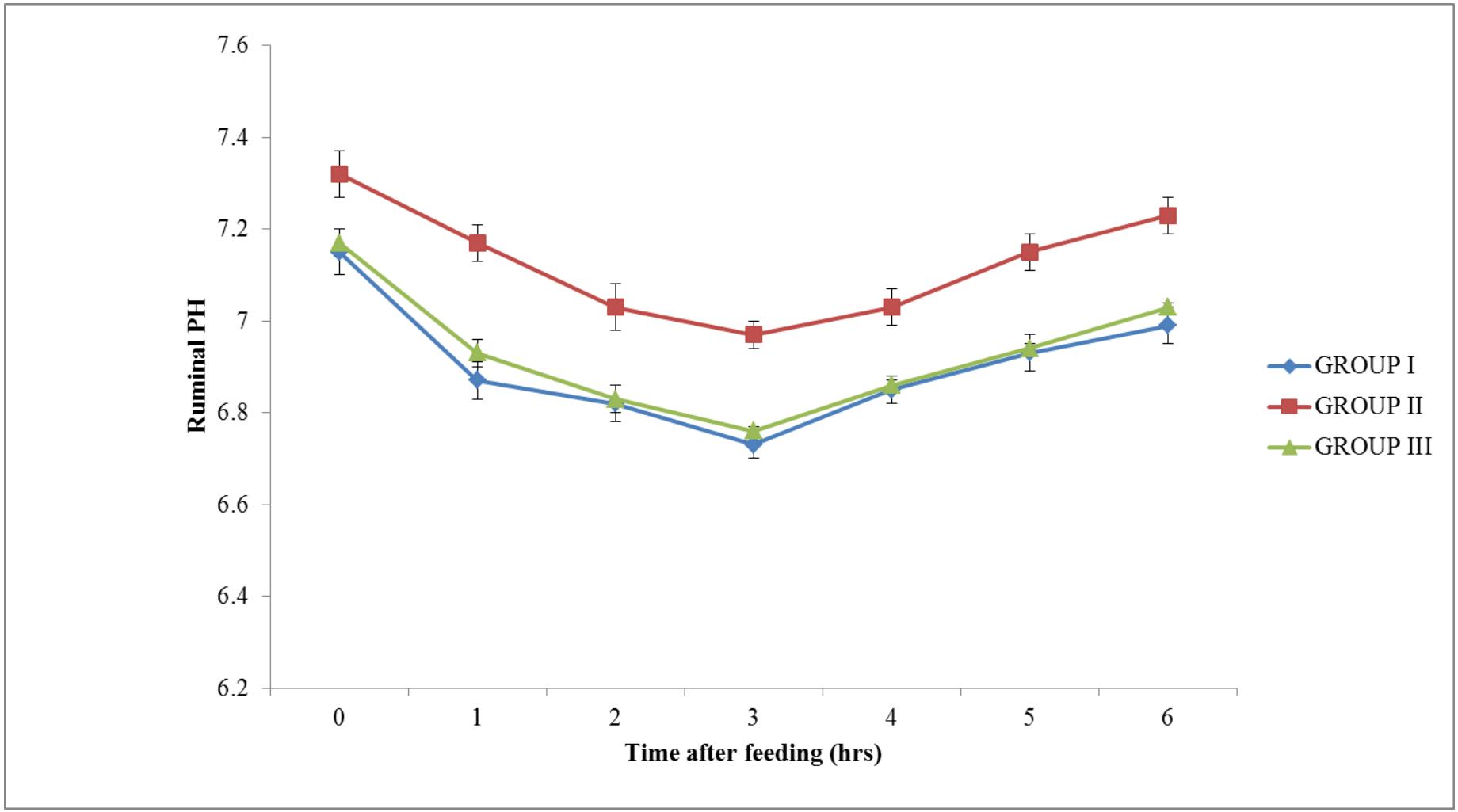

Fig. 12: Effect of Yeast culture supplementation on ruminal pH in buffalo calves during summer season

4.6 Sedimentation activity test (SAT)

The data of oral administration of Yea Sacc^{1026} on SAT during summer season in male buffalo calves have been presented in Table 14 and graphically in Fig.13.

The SAT values at different time intervals before and after feeding in group I, II and III fluctuated between 10.97±0.34 to 22.32±0.26, 18.42±0.26 to 27.98±0.5 and 11.36±0.18 to 22.31±0.35 min. respectively. The results revealed that value of SAT was highest at 0 hr. and lowest at 3 hr. postprandial in all groups. Similar findings were observed by Radostits *et al* (2000) who reported that normal values of SAT varied between 3 min in animals just fed to 9 min. if last feeding has occurred previously. Singh and Bhatia (2012) also examined the effect of herbally formulated drug on healthy buffalo calves and concluded that the SAT was highest before feeding and there was a sharp decrease at 2 hr after feeding in both control as well as treatment group. The overall mean SAT value was significantly higher in group II as compared to group I and III. The rise in SAT might be due to decreased microbial activity.

There was significant decrease in sedimentation activity test in Group III when compared to group II which might be due to an increase in microbial activity because of supplementation of Yea Sacc^{1026} which significantly increases the microbial population. Similar results were reported by Singh and Singh (2015).

4.7 Methylene blue reduction test (MBRT)

The data of oral administration of Yea Sacc^{1026} on MBRT during summer season in male buffalo calves have been presented in Table 15 and change in values of MBRT at different time intervals before and after feeding are depicted in Fig.14.

The MBRT values at different time intervals before and after feeding in group I, II and III ranged from 2.06 ± 0.19 to 10.29 ± 0.41, 6.45 ± 0.24 to 14.73 ± 0.19, 2.94 ± 0.27 to 10.88 ± 0.43 min. respectively. It reflects from table that lowest value of MBRT was observed at 3hr. and highest at 0 hr. in all groups. Singh *et al* (2016b) observed lowest value of MBRT 3 hr. post feeding and highest at 0 hr. (before feeding) in control and treatment groups during rumen dysfunction and following herbal supplementation. The overall mean value of MBRT in group II (summer

Table 14: Effect of yeast culture supplementation on Sedimentation activity test (minutes) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)
0 (Before feeding)	22.32 ± 0.26^{aB}	27.98 ± 0.5^{aA}	22.31 ± 0.35^{aB}
1	18.07 ± 0.29^{bB}	24.81 ± 0.27^{bA}	18.3 ± 0.26^{bB}
2	14.13 ± 0.25^{dB}	21.23 ± 0.43^{dA}	14.92 ± 0.19^{dB}
3	10.97 ± 0.34^{fB}	18.42 ± 0.26^{fA}	11.36 ± 0.18^{fB}
4	13.18 ± 0.12^{eB}	20.11 ± 0.24^{eA}	13.51 ± 0.22^{eB}
5	14.8 ± 0.12^{dB}	22.9 ± 0.25^{cA}	15.11 ± 0.28^{dB}
6	16.01 ± 0.22^{cB}	24.84 ± 0.28^{bA}	16.57 ± 0.1^{cB}
Overall Mean ± SE	**15.64 ± 0.38^{C}**	**22.90 ± 0.35^{A}**	**16.01 ± 0.37^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

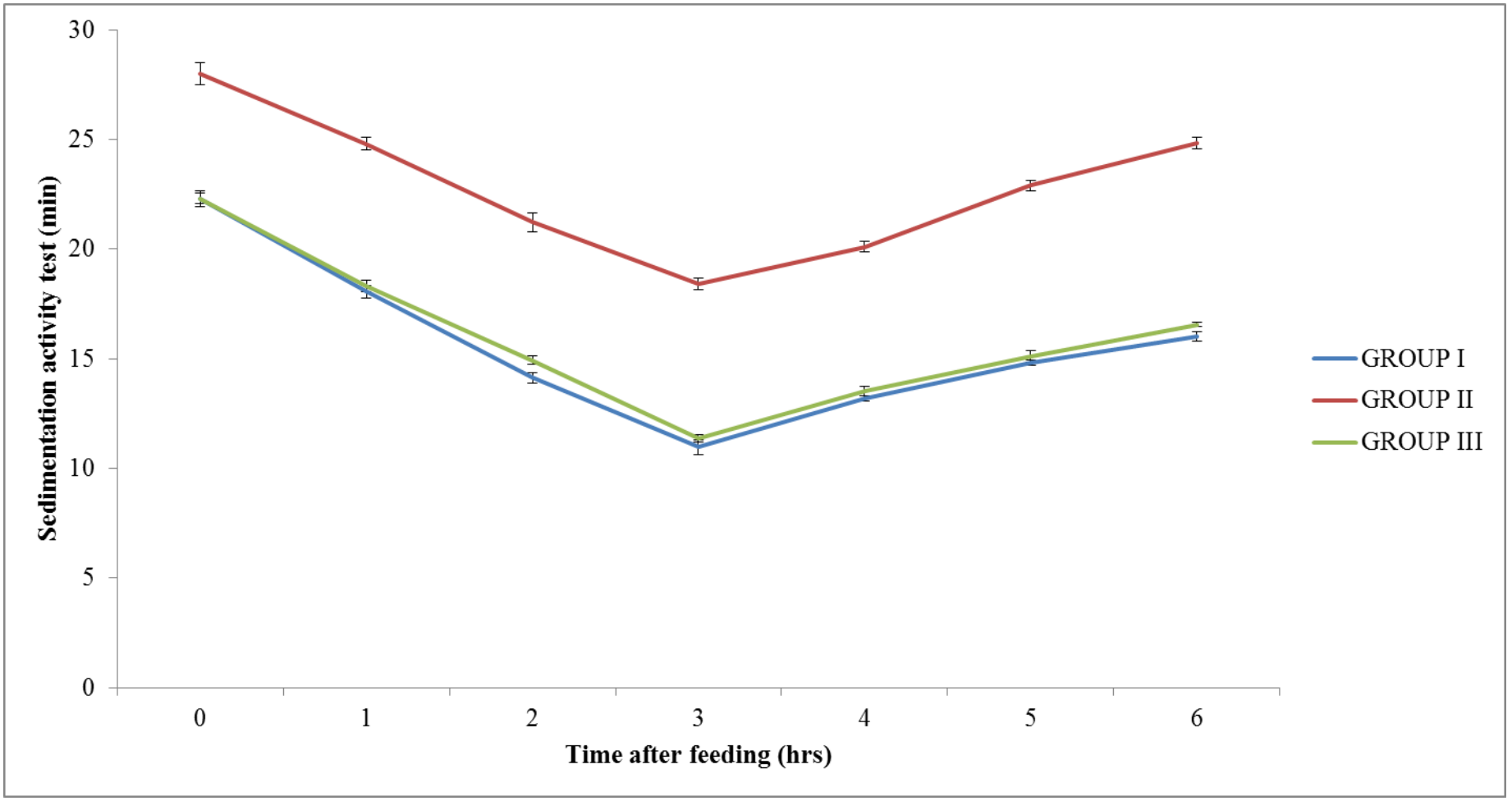

Fig. 13: Effect of yeast culture supplementation on Sedimentation activity test (minutes) in rumen liquor in buffalo calves during summer season

Table 15: Effect of yeast culture supplementation on Methylene blue reduction test (minutes) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)
0 (Before feeding)	10.29 ± 0.41^{Ba}	14.73 ± 0.19^{Aa}	10.88 ± 0.43^{aB}
1	7.57 ± 0.24^{Cb}	10.16 ± 0.15^{Ac}	8.41 ± 0.32^{Bb}
2	4.34 ± 0.19^{Cd}	8.3 ± 0.17^{Ae}	5.77 ± 0.4^{Bc}
3	2.06 ± 0.19^{Cf}	6.45 ± 0.24^{Af}	2.94 ± 0.27^{Be}
4	3.38 ± 0.29^{Ce}	8.27 ± 0.19^{Ae}	4.8 ± 0.33^{Bd}
5	4.98 ± 0.32^{Cd}	9.37 ± 0.12^{Ad}	6.48 ± 0.16^{Bc}
6	6.46 ± 0.19^{Bc}	11.13 ± 0.16^{Ab}	8.22 ± 0.36^{Bb}
Overall Mean ± SE	**5.58 ± 0.30^{C}**	**9.77 ± 0.28^{A}**	**6.78 ± 0.29^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

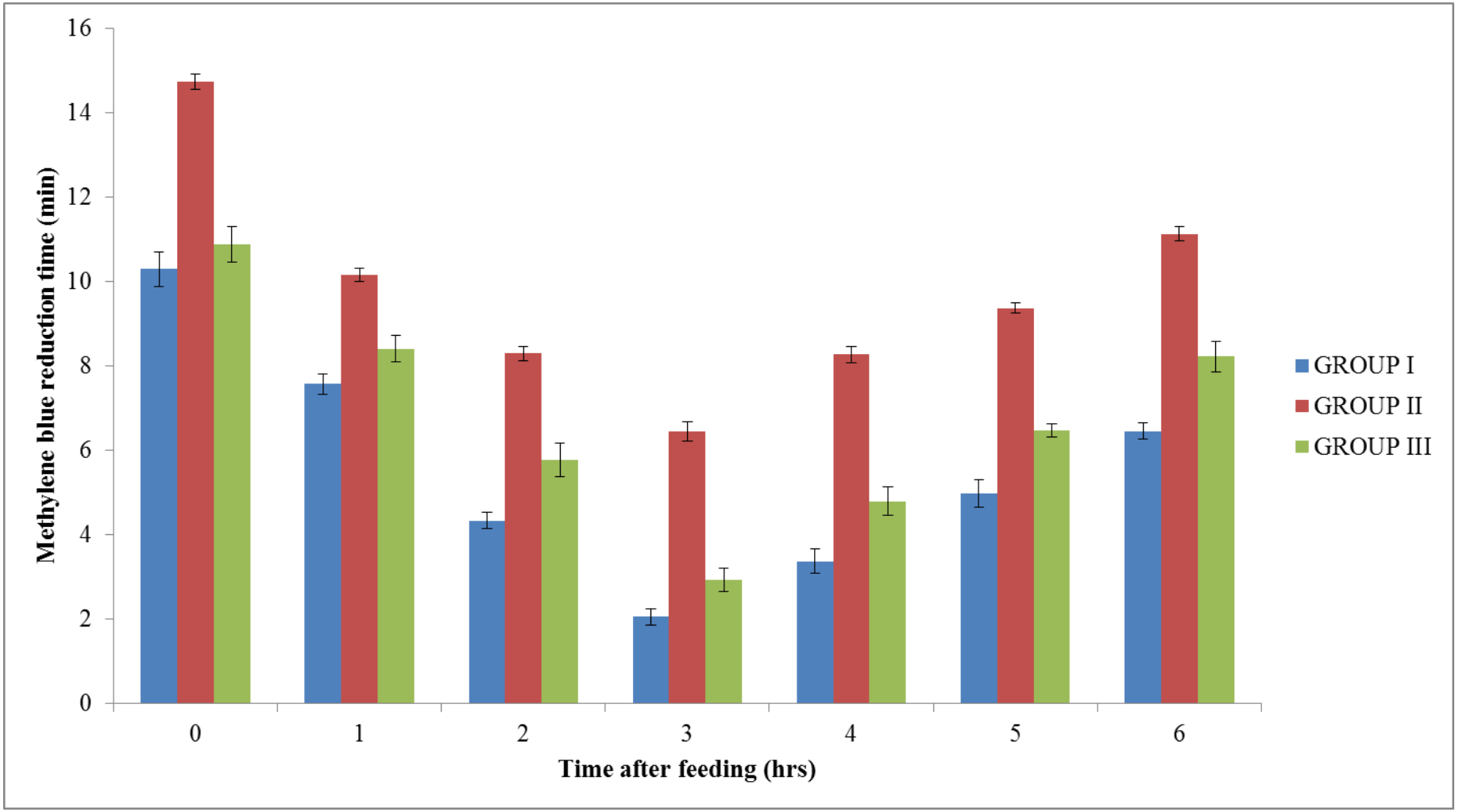

Fig. 14: Effect of yeast culture supplementation on Methylene blue reduction test in rumen liquor in buffalo calves during summer season

control) was significantly higher as compared to pre summer group. However following administration of Yea Sacc^{1026} in group III, the values was restored to normal as in pre summer group. The rise in value of MBRT in group II could be due to decreased fermentation caused by reduced microbial activity.

The overall mean value of MBRT was significant lower in group III as compared to group II. Singh (2005) studied the effect of feed additives on rumen profile of buffaloes and found that supplementation of yeast culture significantly lower values of MBRT in rumen liquor. This may be attributed to improved microbial growth with Yea Sacc^{1026} supplementation leading to enhanced microbial activity. Similarly Singh and Singh (2015), who recorded significantly lower values of MBRT in group supplemented with Yea Sacc^{1026} when compared to groups kept exclusively on wheat straw.

4.8 Microbial counts

4.8.1 Total bacterial count

The results of Yea Sacc^{1026}administration on total bacterial count in rumen liquor during summer season in male buffalo calves have been presented in Table 16 and depicted graphically in Fig.15. Total bacterial count in rumen liquor at different hrs. pre and post feeding in group I, II and III varied from 7.36 ± 0.13 to 8.29 ± 0.14, 5.55 ± 0.11 to 6.56 ± 0.09 and 8.1 ± 0.11 to 9.5 ± 0.1 (x10^9/ml), respectively. The results indicated that there was initial drop in bacterial count which was followed by gradual increase and attained peak at 3 hrs. post feeding in all groups. Initial drop in microbial population could be due to dilution effect of feed, water, saliva and attached of ruminal bacteria to incoming food particles. Subsequent increase in total bacterial count may be attributed to growth of rumen micro flora, dislodgment of bacteria from the plant fiber and availability of substrate for synthesis of microbial protein (Gill 1993). However the overall mean exhibits significant decrease in total bacterial count in group II (summer control). Similar findings of inhibition of micro flora activity in group II on prolonged summer stress were also observed by Romero-Perez *et al* (2011).

Table 16: Effect of yeast culture supplementation on Total bacterial count ($x10^9$/ml) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	7.61 ± 0.14^{cdB}	5.86 ± 0.12^{dC}	8.43 ± 0.12^{eA}
1	7.36 ± 0.13^{dB}	5.55 ± 0.11^{eC}	8.1 ± 0.11^{fA}
2	7.83 ± 0.14^{cbB}	6.24 ± 0.11^{bcC}	8.8 ± 0.12^{cdA}
3	8.29 ± 0.13^{aB}	6.56 ± 0.09^{aC}	9.5 ± 0.1^{aA}
4	8.09 ± 0.14^{abB}	6.46 ± 0.09^{abC}	9.22 ± 0.12^{abA}
5	7.92 ± 0.13^{abcB}	6.17 ± 0.08^{bcC}	9.05 ± 0.1^{bcA}
6	7.56 ± 0.14^{cdB}	5.94 ± 0.09^{cdC}	8.57 ± 0.08^{deA}
Overall Mean ± SE	**7.81 ± 0.06^{B}**	**6.11 ± 0.05^{C}**	**8.81 ± 0.06^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

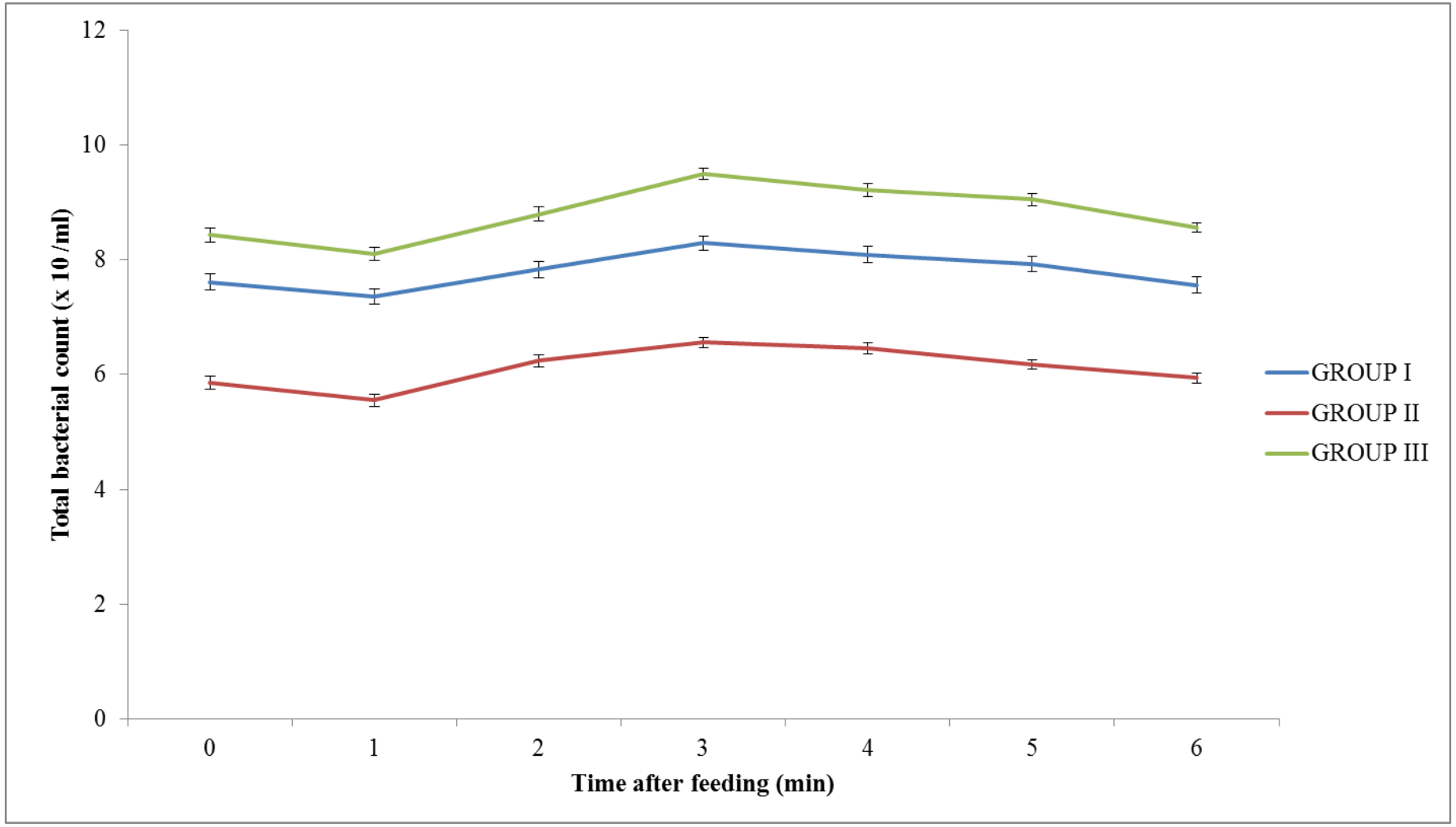

Fig. 15 : Effect of yeast culture supplementation on Total bacterial count (x10^9/ml) in rumen liquor in buffalo calves during summer season

There was a significant increase in total bacterial count in yeast supplemented group as compared to non-supplemented groups. Similar results were found by Kumar *et al* (1994), Wallace (1994), Kaur (2003), Singh *et al* (2008), Singh and Singh (2014) and Lascano *et al* (2009). This increase in bacterial number in the rumen could be attributed to the capacity of yeast to remove oxygen from the rumen. Yeast may scavenge available oxygen on the surfaces of freshly ingested feed particles to maintain metabolic activity in rumen (Chaucheyras-Durand *et al* 2008). This creates better environment for the growth of strict anaerobic cellulolytic bacteria,which stimulates their attachment to forage particles and increases the initial rate of cellulolysis (Roger *et al* 1990). *Saccharomyces cerevisiae* may provide growth factors pro-vitamins and micronutrients that stimulate the growth of bacteria in the rumen (Chaucheyras *et al* 1995).

4.8.2 Total protozoal count

The data of total protozoal count in rumen liquor after administration of Yea Sacc^{1026} in buffalo calves have been presented in table 17 and depicted graphically in Fig.16. The total protozoal count at different time intervals ranged from 2.04 ± 0.08 to 3.11 ± 0.09, 1.55 ± 0.07 to 2.3 ± 0.04, 2.41 ± 0.08 to 3.33 ± 0.06 (x10^5/ml) in group I, II and III, respectively. It is evident from table 17 that there was initial decline in total protozoal count which was followed by gradual increase at 3 hr. post feeding in all the groups. Initial decline in protozoal count could be due to dilution effect of feed, water, saliva and subsequent increase in protozoal count may be attributed to availability of substrate (Sharma *et al* 2009). However the overall mean of total protozoal count in rumen liquor was significantly lower in summer control as compared to pre summer.

The protozoal count was significantly higher in group III as compared to group I and II. Similar findings were reported by Singh and Singh (2014). Feed supplemented with yeast confirms its oxygen-scavenging activity which creates anaerobic environment favouring earlier microbial ecosystem maturation in the young animal (Chaucheyras-Durand and Fonty 2002). Previous reports in dairy cows indicate the significant increase in number of protozoa with supplementation of yeast culture (Mwenya *et al* 2005 and Dolezal *et al* 2011).

Table 17: Effect of yeast culture supplementation on Total protozoal count ($x10^5$/ml) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	2.35 ± 0.09^{eB}	1.79 ± 0.07^{eC}	2.75 ± 0.05^{cA}
1	2.04 ± 0.08^{fB}	1.55 ± 0.07^{fC}	2.41 ± 0.08^{dA}
2	2.68 ± 0.08^{cdB}	2.05 ± 0.06^{cdC}	3.02 ± 0.04^{bA}
3	3.11 ± 0.09^{aB}	2.3 ± 0.04^{aC}	3.33 ± 0.06^{aA}
4	2.98 ± 0.07^{abB}	2.24 ± 0.06^{abC}	3.29 ± 0.06^{aA}
5	2.85 ± 0.08^{bcB}	2.1 ± 0.07^{bcC}	3.06 ± 0.05^{bA}
6	2.6 ± 0.09^{dB}	1.89 ± 0.07^{deC}	2.83 ± 0.04^{cA}
Overall Mean ± SE	**2.66 ± 0.05^{B}**	**1.99 ± 0.04^{C}**	**2.95 ± 0.04^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

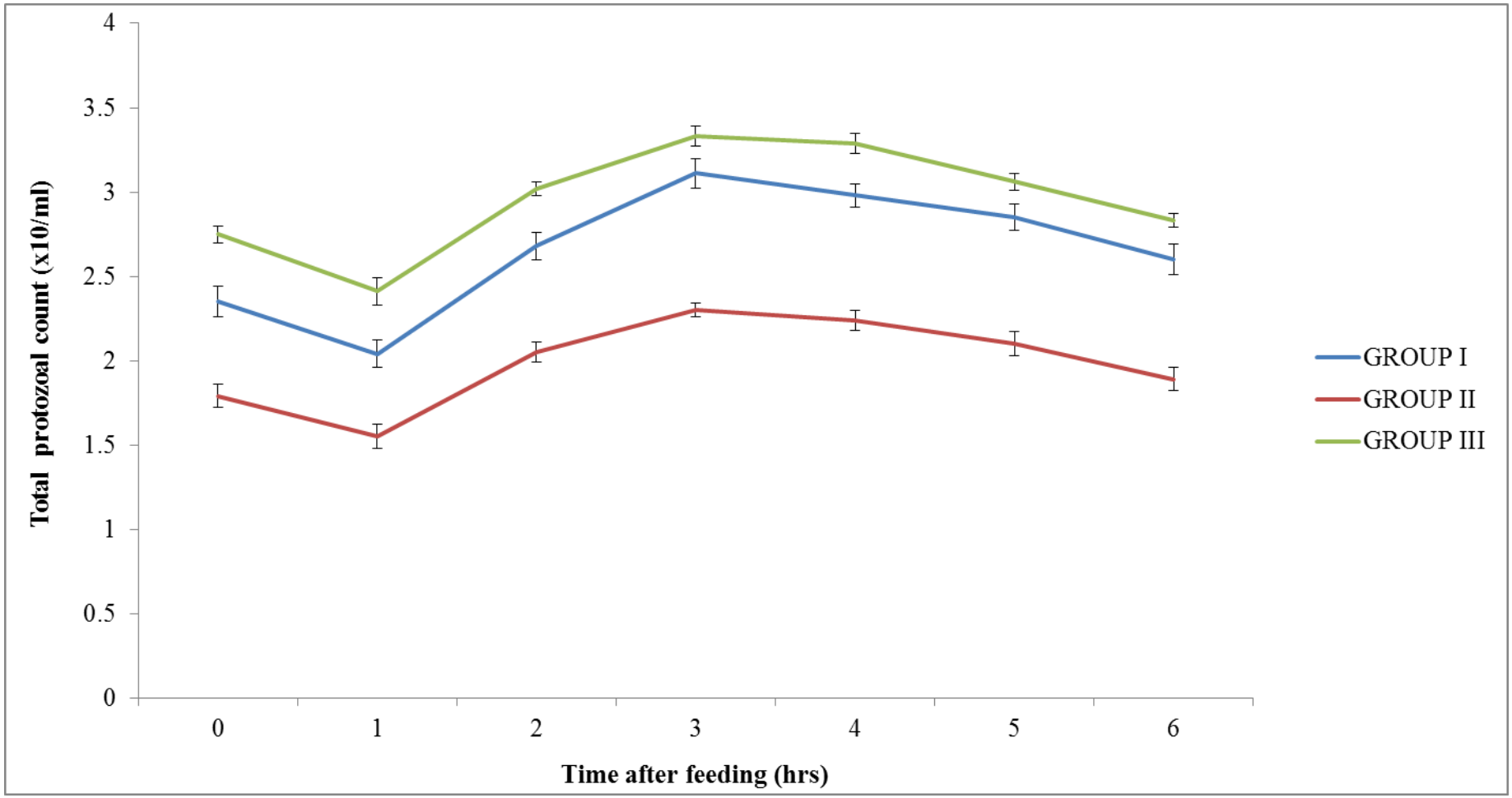

Fig. 16: Effect of yeast culture supplementation on Total protozoal count (x10^5/ml) in rumen liquor in buffalo calves during summer season

4.8.3 Differential protozoal count

The results of oral administration of Yea Sacc^{1026} on holotrich and entodinomorphs percentage during summer season in buffalo calves have been presented in table(s) 18 and 19 and change in percentage of holotrich and entodinomorphs at different time intervals before and after feeding are depicted in Fig.17 and 18 respectively. Percent holotrichs showed progressive rising trend up to 2 hr. post feeding followed by decline in population up to 4 hr post feeding and then again increase in percentage population up to 6 hr. post feeding in all groups, where as entodiniomorph population decreased upto 2 hr post feeding, further it was followed by rising trend upto 4 hr post feeding in all the groups and again represented declining trend up to 6 hr post-prandial. Iqbal (1989) found maximum percentage of holotrichs at 2 hrs post feeding with declining trend for rest of the time intervals after feeding. Post feeding variations in the percentage of holotrichs might be due to sequesterization.

Holotrichs ordinarily sequester on the wall of reticulum and migrate rapidly from reticulum to rumen after feeding and sequester again on the reticular wall after few hrs in response to a chemical stimulus originating from diet (Sharma *et al* 2009). There was significant increase in percentage of holotrichs and significant decrease in percentage entodinomorphs during summer season. However following Yea Sacc^{1026} supplementation there was optimization of population of Holotrichs and Entodinomorphs with no significant difference from pre summer and summer control.

4.9 Total volatile fatty acids (TVFA)

The results of oral administration of Yea Sacc^{1026} on total volatile fatty acids in rumen liquor during summer season in male buffalo calves have been presented in Table 20 and change in total volatile fatty acids at different time interval before and after feeding are shown graphically in Fig.19.

Total volatile fatty acids (TVFA) in rumen liquor in group I, II and III ranged between 80.92 ± 0.95 to 109.25 ± 1.13, 73.83 ± 1.02 to 99.42 ± 0.56 and 89.58 ± 0.84 to 117.5 ± 0.65 mEq/L respectively. It appears from table that there was progressive increase in levels of volatile fatty acids from 0-3 hrs. Post feeding in all groups and decline at 6 hr. Similar results have been reported by Singh *et al* (2016a). The lowest value was observed at 0 hr and highest at 3hr post feeding. An initial increase

Table 18: Effect of yeast culture supplementation on Holotrichs percentage in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	19.62 ± 0.84^{dB}	24.82 ± 0.86^{dcA}	18.57 ± 0.86^{eB}
1	27.7 ± 0.63^{abA}	27.8 ± 0.66^{abA}	28.13 ± 0.47^{bA}
2	28.81 ± 1.3^{aA}	29.26 ± 0.9^{aA}	30.2 ± 0.6^{aA}
3	25.26 ± 0.8^{bcA}	26.43 ± 1.22^{bcdA}	25.81 ± 0.63^{cA}
4	22.48 ± 0.95^{bcA}	23.98 ± 1.03^{dA}	23.79 ± 0.51^{dA}
5	24.88 ± 1.19^{bcA}	25.7 ± 0.58^{bcdA}	26.4 ± 0.47^{bcA}
6	26.84 ± 1.34^{abA}	27.1 ± 0.58^{abcA}	27.87 ± 0.87^{bA}
Overall Mean ± SE	**25.08 ± 0.50^{B}**	**26.44 ± 0.36^{A}**	**25.82 ± 0.45^{AB}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant (P<0.05) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant (P<0.05) difference between groups.

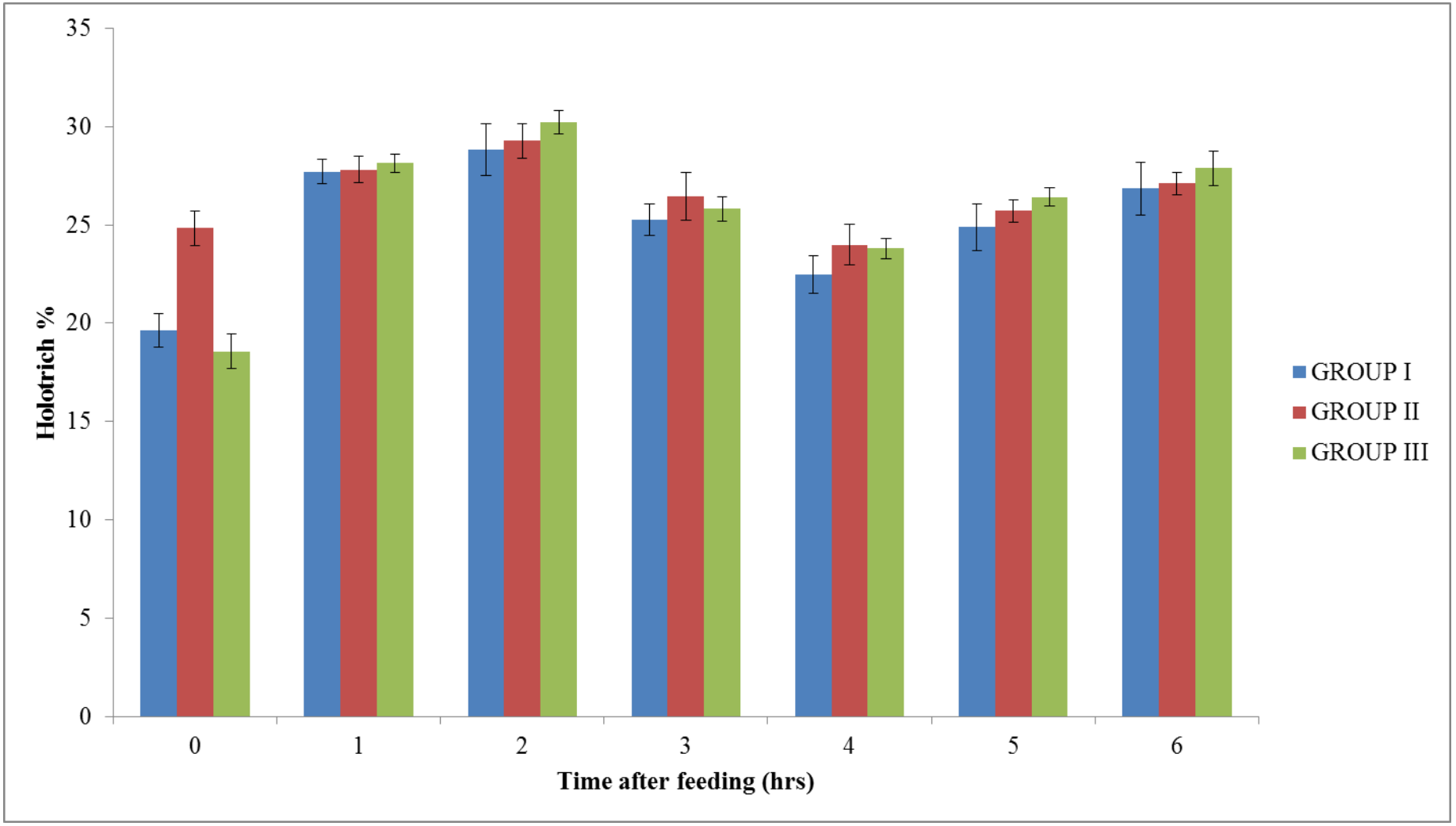

Fig. 17: Effect of yeast culture supplementation on Holotrichs percentage in rumen liquor in buffalo calves during summer season

Table 19: Effect of yeast culture supplementation on Entodiniomorphs percentage in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	80.38 ± 0.84^{aA}	76.02 ± 1.03^{aB}	81.43 ± 0.86^{aA}
1	72.3 ± 0.63^{deA}	66.86 ± 5.45^{bA}	71.87 ± 0.47^{dA}
2	71.19 ± 1.3^{eA}	70.28 ± 0.69^{abA}	69.8 ± 0.6^{eA}
3	74.79 ± 0.83^{bcdA}	73.57 ± 1.22^{abA}	74.19 ± 0.63^{cA}
4	77.57 ± 0.95^{abA}	75.19 ± 0.86^{aA}	76.26 ± 0.51^{bA}
5	75.58 ± 1.19^{bcA}	74.3 ± 0.58^{aA}	74.06 ± 0.59^{cA}
6	73.16 ± 1.34^{cdeA}	72.9 ± 0.58^{abA}	72.13 ± 0.87^{dA}
Overall Mean ± SE	**74.99 ± 0.50^{A}**	**72.73 ± 0.87^{B}**	**74.25 ± 0.45^{AB}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

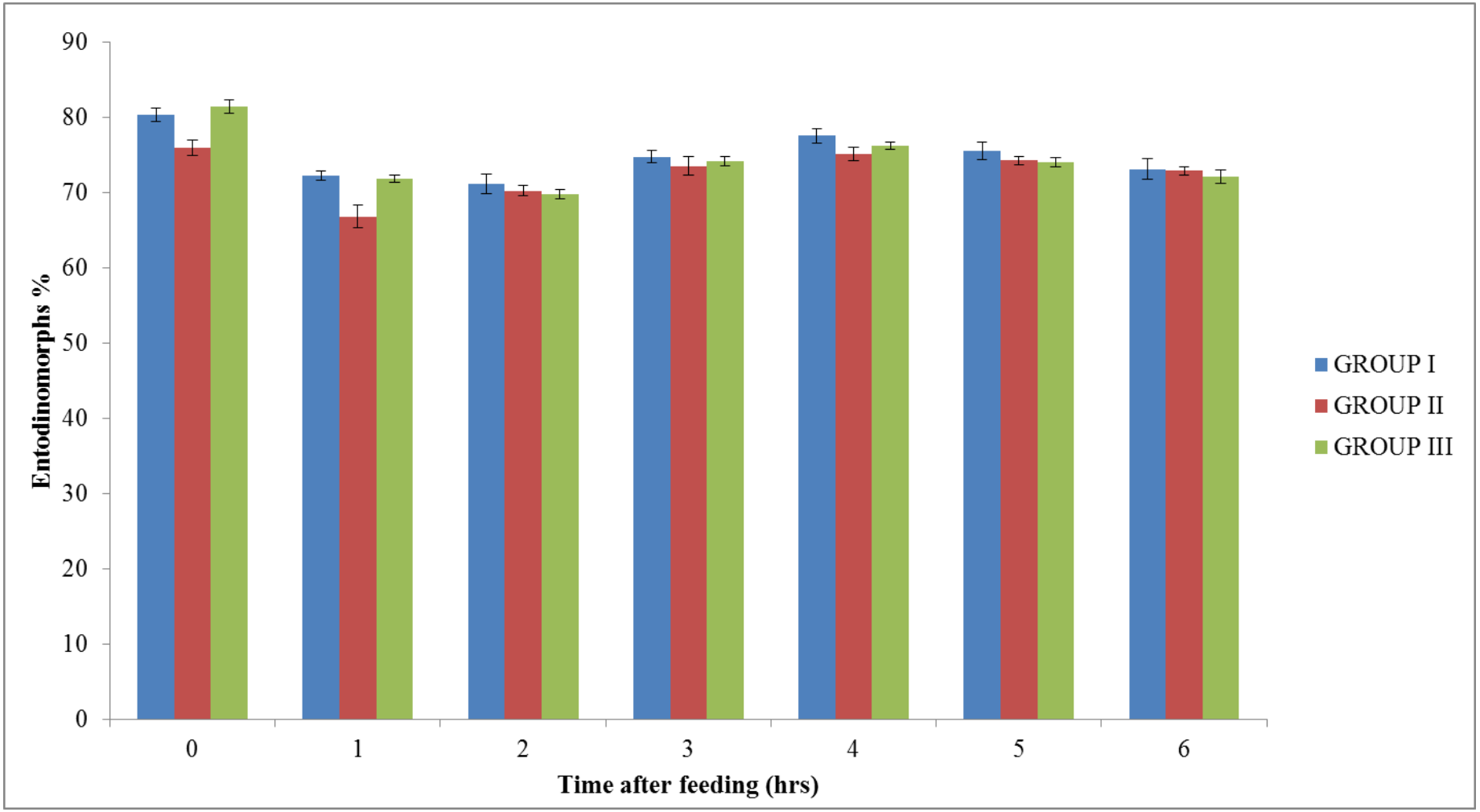

Fig. 18: Effect of yeast culture supplementation on Entodiniomorphs percentage in rumen liquor in buffalo calves during summer season

Table 20: Effect of yeast culture supplementation on Total volatile fatty acids concentration (mEq/L) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	80.92 ± 0.95^{dB}	73.83 ± 1.02^{fC}	89.58 ± 0.84^{eA}
1	90.5 ± 0.83^{cB}	82.17 ± 0.85^{eC}	98.42 ± 1.13^{cA}
2	97.83 ± 0.83^{bB}	91.25 ± 0.8^{cC}	107.83 ± 1.11^{bA}
3	109.25 ± 1.13^{aB}	99.42 ± 0.56^{aC}	117.5 ± 0.65^{aA}
4	98.42 ± 0.84^{bB}	93.5 ± 0.63^{bC}	109.67 ± 1.37^{bA}
5	90.33 ± 0.76^{cB}	86.75 ± 0.71^{dC}	100.75 ± 1.02^{cA}
6	81.67 ± 1.28^{dB}	81.5 ± 0.68^{eB}	94.75 ± 1.02^{dA}
Overall Mean ± SE	92.70 ± 1.08^{B}	86.92 ± 0.91^{C}	102.64 ± 1.05^{A}

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

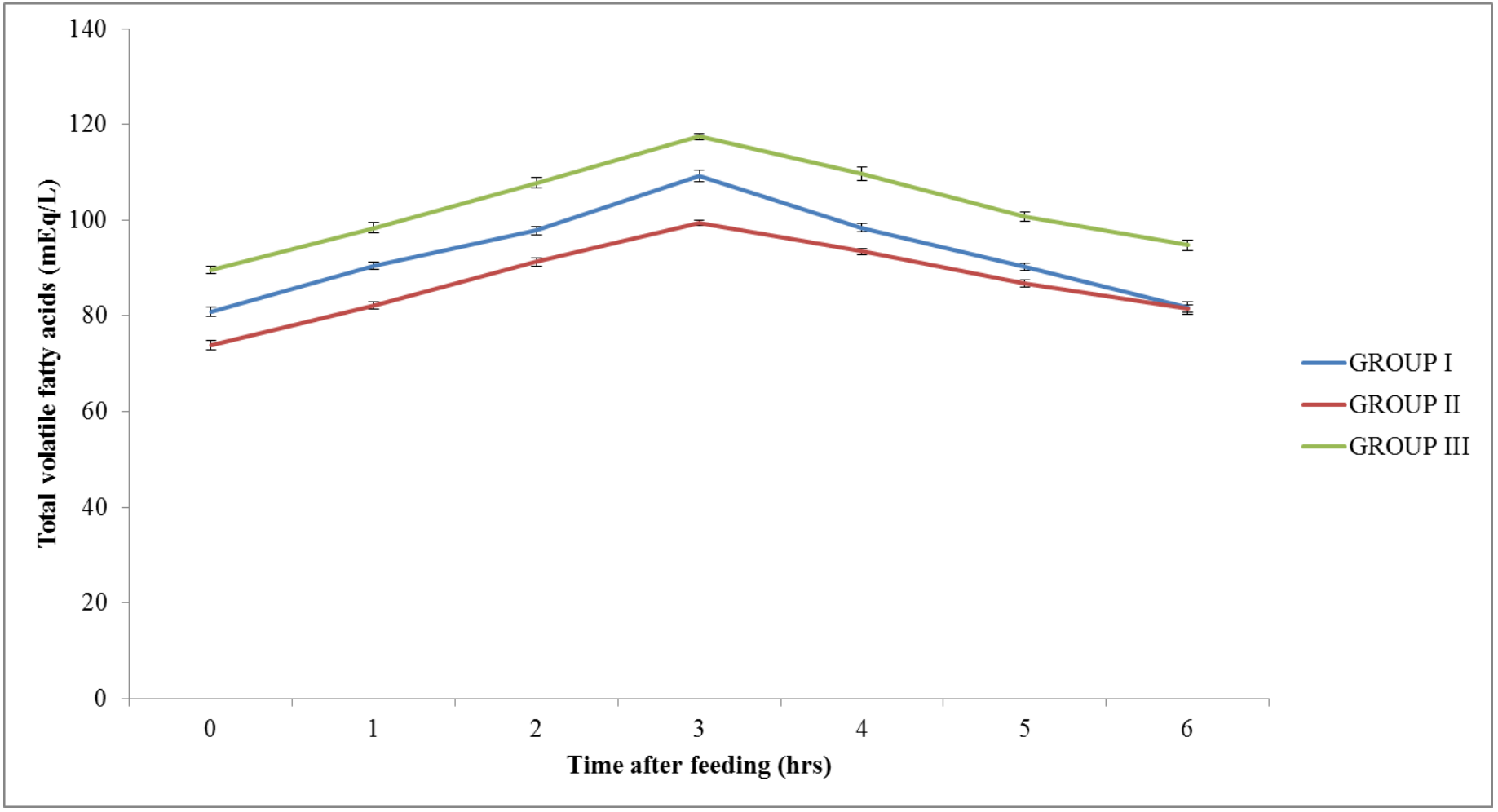

Fig. 19: Effect of yeast culture supplementation on Total volatile fatty acids concentration (mEq/L) in rumen liquor in buffalo calves during summer season

in concentration of TVFA in all the groups may be attributed to increase in fermentation rate due to increased availability of nutrients (Wanapat *et al* 2013); (Jain *et al* 2005). Maximum concentration of total volatile fatty acids at 3 hrs post feeding could be ascribed to maximum microbial fermentation of carbohydrates and catabolism of amino acids leading to formation of volatile fatty acids. However, decline in total volatile fatty acids at 6 hr post-prandial may be due to absorption of total volatile fatty acids through rumen wall into blood stream and decrease in availability of nutrients for microbial fermentation (Sharma *et al* 2009).

During heat stress present study revealed significantly lower levels of total volatile fatty acids with an overall mean value of 86.92± 0.91 as compared to conventional diet (92.70 ± 1.08) and Yea Sacc^{1026} supplementation (102.64 ±1.05). Similarly, decrease in TVFA in rumen liquor during summer were also reported by Beed and Collier (1986). Nonaka *et al* (2008), Kelly *et al* (1967) and Tajima *et al* (2007) who reported that heat stress reduced the total production of total volatile fatty acid concentration and lower the ruminal pH.

However, the levels of total volatile fatty acids were significantly higher in group III than that of group II. (Mruthunjaya *et al* 2010) reported that the higher values of TVFAs in rumen liquor could be attributed to effect of Yea Sacc^{1026} supplementation on viable or total bacterial population specifically cellulolytic bacteria, which in turn, enhanced the fermentation in the rumen and resulted in increased production of total volatile fatty acids. Results of the studies conducted by Moya *et al* (2009), Lopuszanska and Krzysztof (2011) and Singh and Singh (2014) with yeast supplementation are in accordance with the present findings.

4.10 Individual Volatile Fatty Acids IVFA

The results of oral administration of Yea Sacc^{1026} on molar percentage of acetic acid, propionic acid, butyric acid in rumen liquor during summer season in male buffalo claves have been presented in Tables 21, 22 and 23 respectively. Changes in individual volatile fatty acids at different time interval before and after feeding have been presented graphically 20, 21 and 22 respectively.

Table 21: Effect of yeast culture supplementation on Acetic acid (molar %) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	63.73 ± 0.31^{cB}	70.62 ± 0.24^{cA}	60.51 ± 0.12^{bC}
1	63.94 ± 0.3^{cB}	70.95 ± 0.31^{bcA}	61.04 ± 0.78^{bC}
2	64.79 ± 0.31^{bB}	71.98 ± 0.3^{abA}	61.15 ± 0.18^{abC}
3	65.66 ± 0.19^{aB}	72.34 ± 0.37^{aA}	62.19 ± 0.16^{aC}
4	65.52 ± 0.23^{abB}	72.21 ± 0.38^{aA}	62.15 ± 0.3^{aC}
5	65.4 ± 0.23^{abB}	72.19 ± 0.5^{aA}	62.2 ± 0.29^{aC}
6	65.06 ± 0.22^{abB}	71.26 ± 0.31^{abcA}	60.89 ± 0.15^{bC}
Overall Mean ± SE	**64.87 ± 0.12^{B}**	**71.65 ± 0.15^{A}**	**61.45 ± 0.15^{C}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

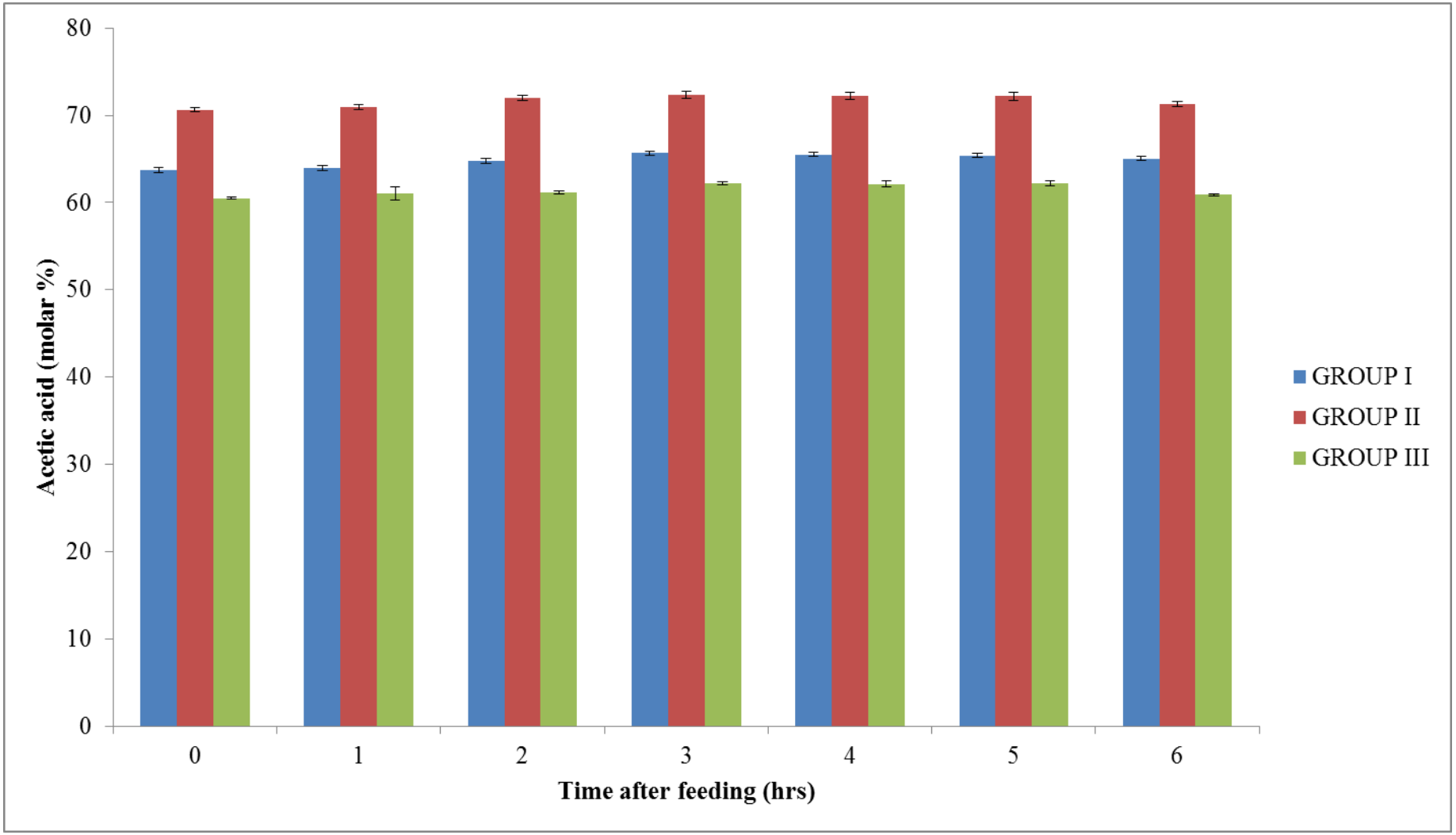

Fig. 20: Effect of yeast culture supplementation on Acetic acid (molar %) in rumen liquor in buffalo calves during summer season

Table 22: Effect of yeast culture supplementation on propionic acid (molar %) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	25.48 ± 0.3^{bcB}	17.98 ± 0.35^{aC}	28.05 ± 0.2^{cA}
1	26.06 ± 0.59^{abcA}	18.06 ± 0.33^{aC}	28.12 ± 0.2^{bcA}
2	26.59 ± 0.37^{abB}	19.22 ± 0.39^{aC}	29.19 ± 0.31^{aA}
3	26.95 ± 0.52^{aB}	20.41 ± 16.87^{aA}	29.19 ± 0.32^{aA}
4	25.43 ± 0.28^{bcB}	19.08 ± 0.32^{aC}	28.8 ± 0.18^{abA}
5	25.41 ± 0.29^{bcB}	17.88 ± 0.33^{aC}	28.44 ± 0.17^{bcA}
6	25.22 ± 0.26^{cB}	17.78 ± 0.32^{aC}	28.1 ± 0.19^{bcA}
Overall Mean ± SE	**25.88 ± 0.16^{A}**	**20.77 ± 2.42^{B}**	**28.55 ± 0.1^{A}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

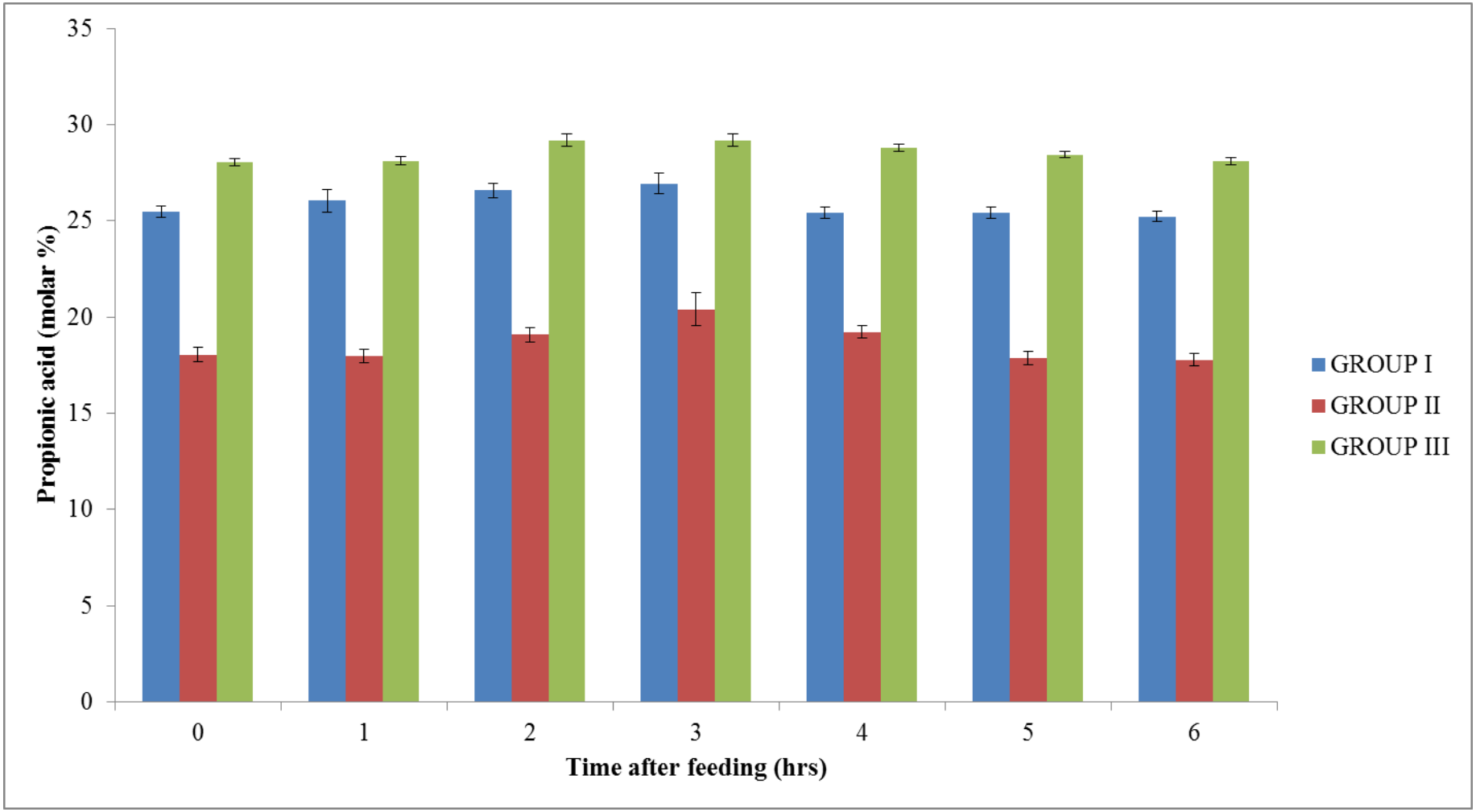

Fig. 21: Effect of yeast culture supplementation on propionic acid (molar %) in rumen liquor in buffalo calves during summer season

Table 23: Effect of yeast culture supplementation on Butyric acid (molar %) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	9.02 ± 0.31^{aA}	9.62 ± 0.38^{aA}	9.6 ± 0.38^{aA}
1	9.2 ± 0.34^{aA}	9.76 ± 0.32^{aA}	10.05 ± 0.23^{aA}
2	9.24 ± 0.29^{aB}	10.03 ± 0.32^{aA}	10.3 ± 0.3^{aA}
3	9.59 ± 0.27^{aA}	10.19 ± 0.33^{aA}	10.21 ± 0.45^{aA}
4	9.28 ± 0.23^{aA}	10 ± 0.08^{aA}	9.86 ± 0.2^{aA}
5	9.28 ± 0.17^{aB}	9.98 ± 0.09^{aAB}	9.81 ± 0.4^{aA}
6	9.15 ± 0.44^{aB}	9.83 ± 0.16^{aA}	9.61 ± 0.19^{aA}
Overall Mean ± SE	$\mathbf{9.25\pm0.11^{B}}$	$\mathbf{9.92\pm0.1^{A}}$	$\mathbf{9.92\pm0.12^{A}}$

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

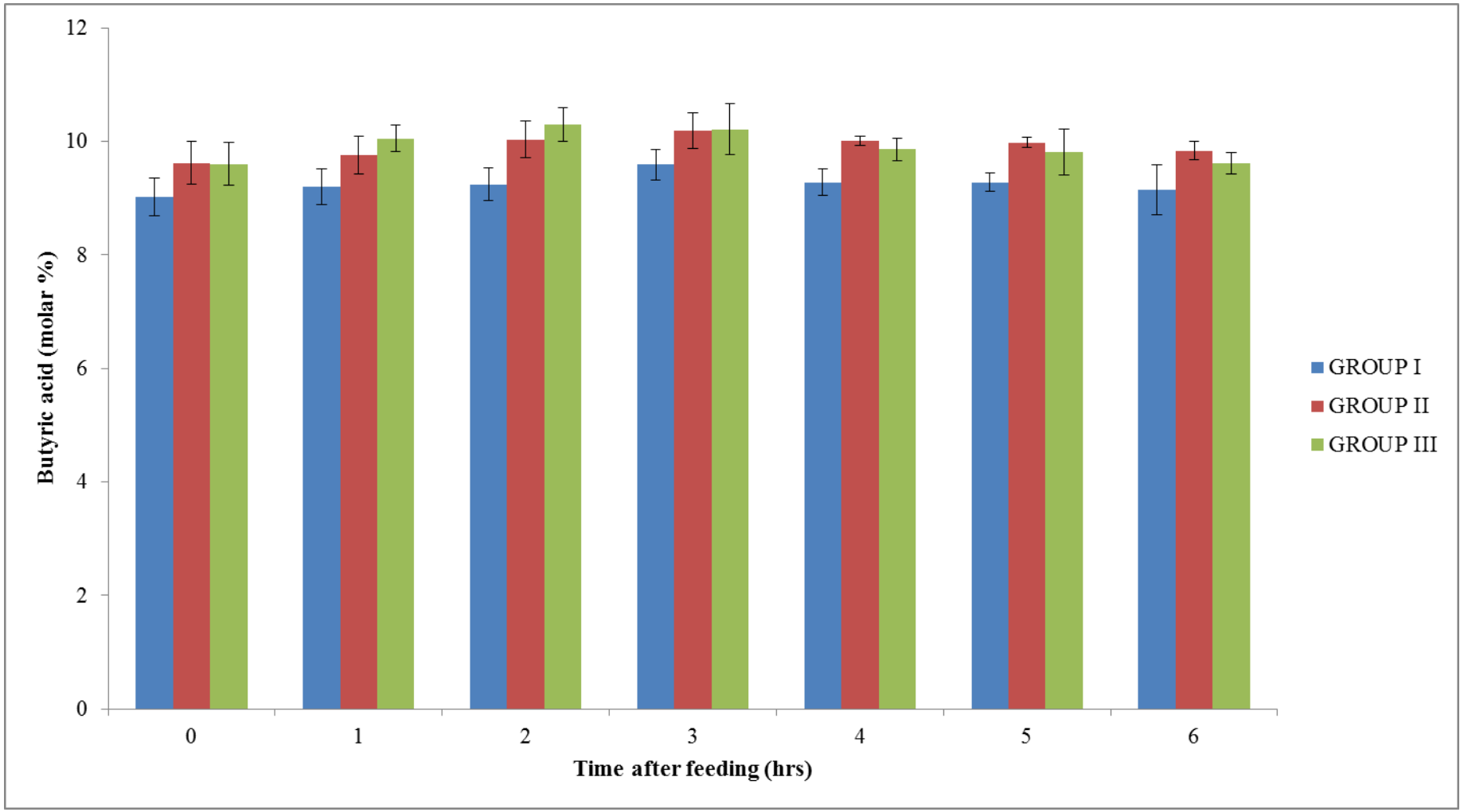

Fig. 22: Effect of yeast culture supplementation on Butyric acid (molar %) in rumen liquor in buffalo calves during summer season

Overall mean molar percentage of acetic acid and butyric acid were significantly higher during summer season when compared with pre summer group whereas over all mean of molar percentage propionic acid decreased during summer season. It is clear from data that these values were restored to normal after supplementation of Yea Sacc^{1026} in treatment group except butyric acid which did not show any change. Singh *et al* (2008) found that after Yea Sacc^{1026} supplementation in healthy buffalo claves molar percentage of propionic acid levels was increased.

4.11 Ammonia nitrogen (NH_3-N)

The results of oral administration of Yea Sacc^{1026} on ruminal ammonia nitrogen during summer season in male buffalo calves have presented in Table 24 and changes in concentration of ammonia nitrogen at different time intervals before and after feeding are depicted in Fig.23. The ammonia nitrogen concentration in group I, II and III at different time intervals ranged between 6.53±0.47 to 12.6±0.42, 16.8±0.34 to 24.47±0.3 and 8.05±0.39 to 16.1±0.37mg/dl, respectively. It is reflected from the table that there was an increase in level of ammonia nitrogen after feeding and attained the peak at 3 hrs. post feeding in all three groups. However overall mean also showed a significant increase in ammonia nitrogen concentration during summer stress and significant drop in group III compared to group I and II. Rise in ammonia nitrogen level during summer season could be due to lack of utilization of ammonia nitrogen as substrate for microbial protein synthesis reduced by microbial population.

Initial post-prandial increase in ammonia nitrogen levels could be attributed to increased availability of substrate, which on proteolysis and deamination leads to the formation of ammonia in the rumen (Singh *et al* 1996). Post-prandial decline in NH3-N level in all the groups from 2 to 6 hr might be due to direct absorption of NH3-N through ruminal wall or the onward passage along with digesta from rumen or incorporation of nitrogen in the synthesis of microbial proteins (El-Galil *et al* 2011; Singh and Bhatia 2012).

Table 24: Effect of yeast culture supplementation on Ammonia nitrogen (mg/dl) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	7.82 ± 0.56^{deC}	18.43 ± 0.57^{dA}	10.62 ± 0.44^{dB}
1	10.5 ± 0.53^{bcC}	22.05 ± 0.46^{bA}	14.12 ± 0.47^{bB}
2	11.2 ± 0.34^{bC}	22.87 ± 0.31^{bA}	13.77 ± 0.34^{bB}
3	12.6 ± 0.42^{aC}	24.47 ± 0.3^{aA}	16.1 ± 0.37^{aB}
4	9.45 ± 0.46^{cC}	20.65 ± 0.46^{cA}	11.9 ± 0.37^{cB}
5	8.05 ± 0.52^{dC}	19.02 ± 0.36^{dA}	9.8 ± 0.34^{dB}
6	6.53 ± 0.47^{eC}	16.8 ± 0.34^{eA}	8.05 ± 0.39^{eB}
Overall Mean ± SE	**9.45 ± 0.28^{C}**	**20.61 ± 0.31^{A}**	**12.05 ± 0.32^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

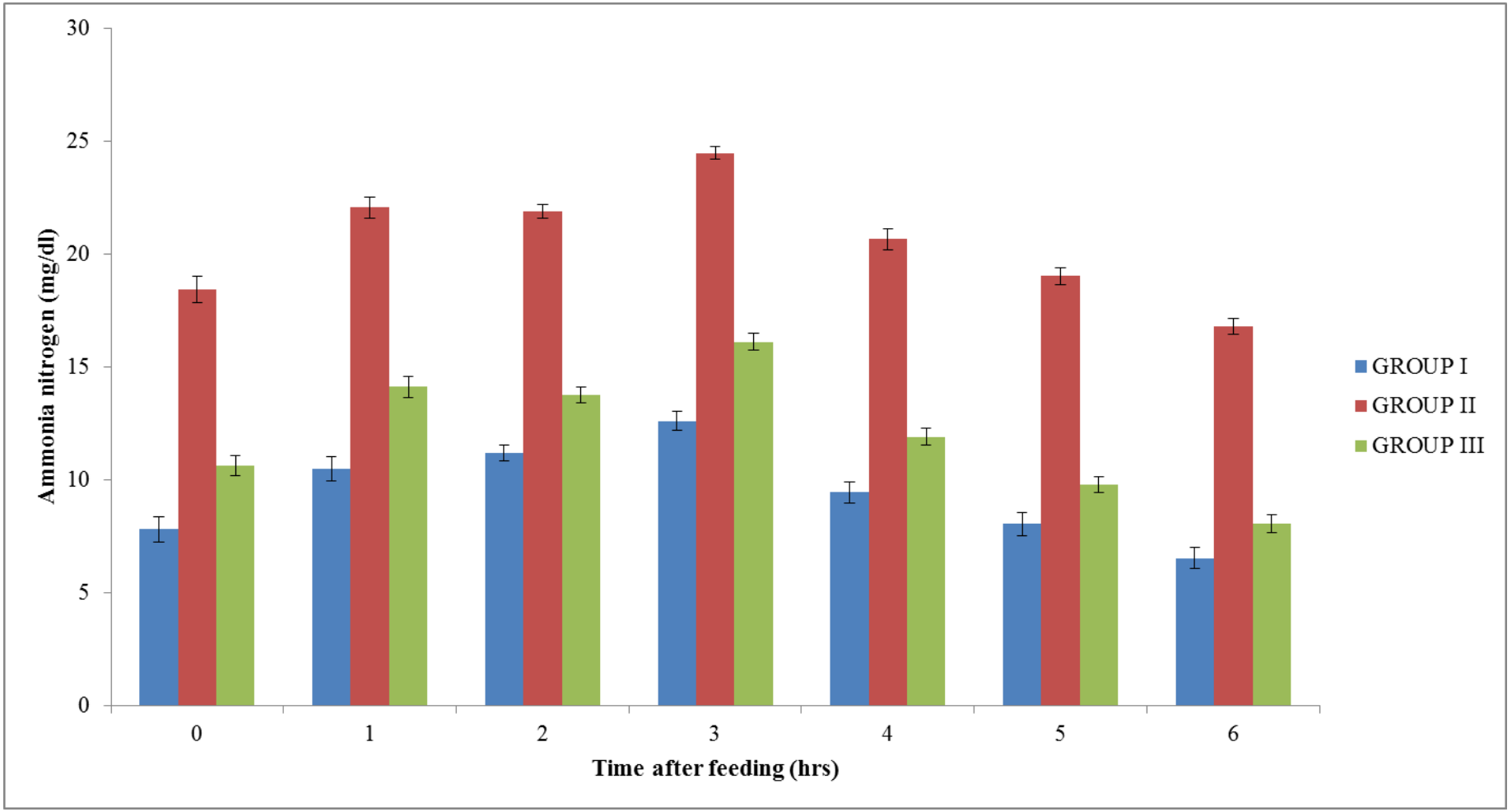

Fig. 23: Effect of yeast culture supplementation on Ammonia nitrogen (mg/dl) in rumen liquor in buffalo calves during summer season

The overall mean value of ammonia nitrogen in group II (20.61±0.31) was found significantly higher than group I (9.45±0.28) and Group III (12.05±0.32). Similar results were reported by (Salles *et al* 2010) who reported that the heat-stressed animals presented higher levels of ammonia nitrogen in the rumen liquor during summer season. According to Nocek and Russell (1988), the interaction between the carbohydrates and the protein in the rumen metabolism is particularly intense, and if there is any kind of deficiency or inefficiency of the feed protein use in the rumen, the carbohydrate digestibility may decrease. If the diet carbohydrate is insufficient to support the microbial growth, the nitrogen will be lost as rumen NH3 and its concentration will increase in the rumen. As there was a decrease on feed ingestion by the animals under heat stress, carbohydrate in the rumen decreased to support microbial growth causing increase in rumen liquor ammonia nitrogen increase.

In present study overall mean values of summer treatment is lower than summer control. This could be due to increase microbial population which leads to increase utilization of ammonia nitrogen for microbial protein synthesis. Similar results were reported by Kumar (1994), Enjalbert *et al* (1999) and Singh *et al* (2016a).

4.12 Total nitrogen

The results of oral administration of Yea Sacc^{1026} on total nitrogen concentration during summer season in male buffalo calves have presented in Table 25 and changes in concentration of total nitrogen at different time intervals before and after feeding are depicted in Fig.24. The total nitrogen in rumen liquor at different hrs. post feeding in group I, II and III varied from 57.07 ± 0.77 to 65.8 ± 0.57, 65.22 ± 1.36 to 90.3 ± 1.21 and 60.08 ± 0.87 to 72.8 ± 1.23 mg/dl respectively. It appears from the table that there was significant increase in level of total nitrogen and attained the peak levels at 3hr. post prandialy which is followed by gradually decrease at 6 hr. post feeding in all the groups. Similar results were obtained by Singh *et al* (2016a) and Singh *et al* (2008). However overall mean indicated rise in total nitrogen concentration during summer season compared to control and significant decrease with Yea Sacc^{1026} treatment compared to summer control. This trend of change in total nitrogen concentration in different groups are similar as for ammonia nitrogen which is a faction of total nitrogen.

Table 25: Effect of yeast culture supplementation on Total nitrogen level (mg/dl) in rumen liquor in buffalo calves during summer season

Sampling time after feeding (hrs)	GROUP I (Pre-Summer)	GROUP II (Summer Control)	GROUP III (Summer Treatment)*
0 (Before feeding)	57.07 ± 0.77^{dC}	65.22 ± 1.36^{eA}	60.08 ± 0.87^{fB}
1	61.83 ± 0.81^{bcC}	80.43 ± 1.3^{cA}	69.07 ± 1.37^{bcB}
2	65.8 ± 0.57^{aC}	86.1 ± 1.15^{bA}	70.35 ± 1.02^{abB}
3	65.8 ± 1.02^{aC}	90.3 ± 1.21^{aA}	72.8 ± 1.23^{aB}
4	63 ± 0.97^{bC}	81.43 ± 1.49^{cA}	66.68 ± 1^{cdB}
5	60.2 ± 0.81^{cC}	75.77 ± 1.7^{dA}	64.05 ± 1.01^{deB}
6	57.75 ± 0.85^{dC}	72.57 ± 1.59^{dA}	61.72 ± 1.01^{efB}
Overall Mean ± SE	**61.64 ± 0.47^{C}**	**78.83 ± 1.00^{A}**	**66.39 ± 0.69^{B}**

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals.
Mean with different superscripts (a, b, c) vertically represent significant ($P<0.05$) difference within groups.
Mean with different superscripts (A, B, C) horizontally represent significant ($P<0.05$) difference between groups.

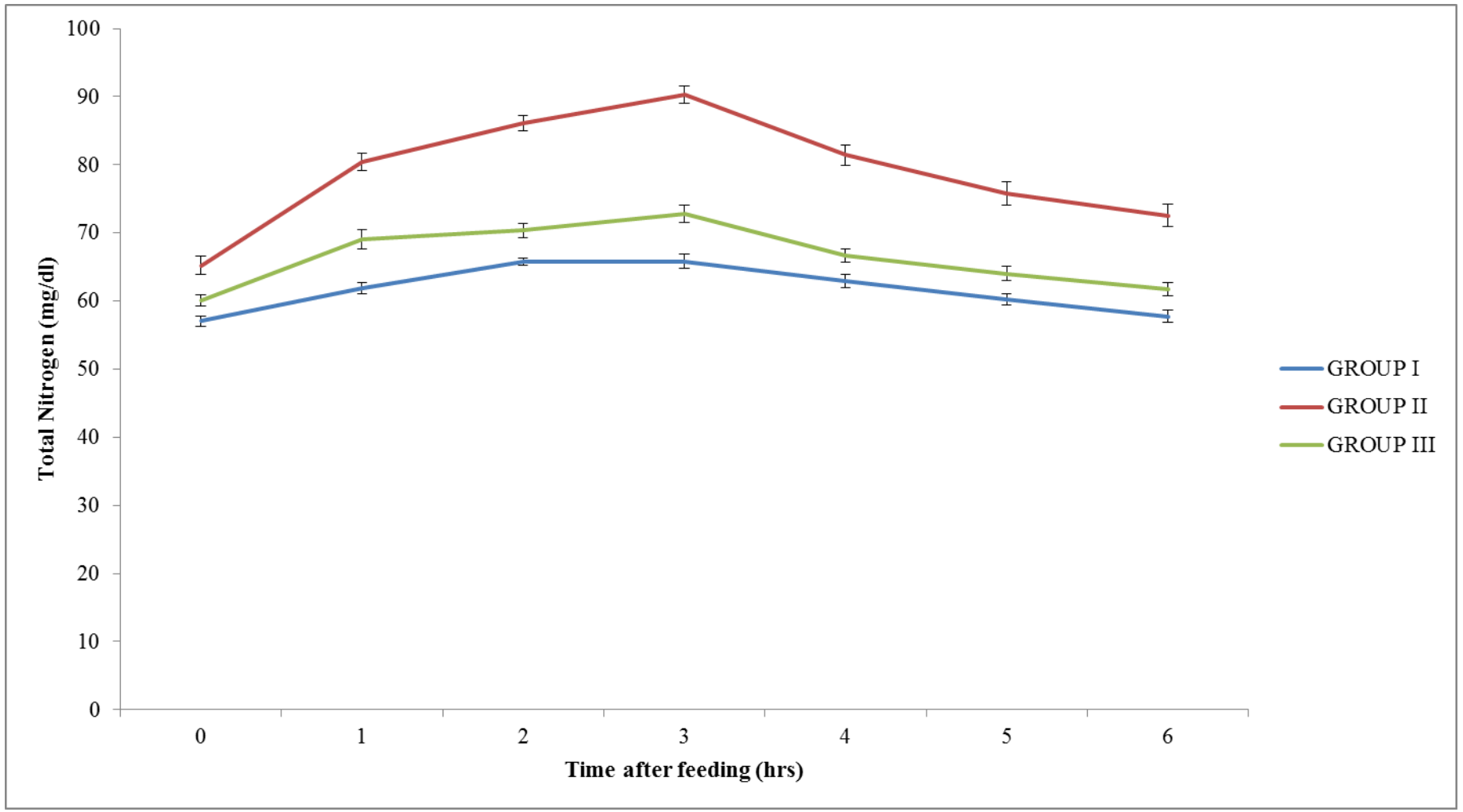

Fig. 24: Effect of Yeast culture supplementation on Total nitrogen level (mg/dl) in rumen liquor in buffalo calves during summer season

CHAPTER V

SUMMARY AND CONCLUSION

This study was conducted on eight apparently healthy male buffalo calves of 10-12 months age. Animals were divided into 3 groups, Group I (pre summer group), Group II (summer control), Group III (summer treatment). The study was conducted during two seasons, i.e. Pre-summer (March-April) and Summer (June to August) with same animals. The animals of all the groups were fed conventional diet comprising of wheat straw, concentrate, mineral mixture and green fodder but the animals of Group III were supplemented with Yea Sacc^{1026} @ one bolus (consisting of 25 billion live yeast cells) / animal / day for 21 days after acclimatization with summer season for one month. Rumen liquor samples were collected for 3 consecutive days at different time intervals (0,1,2,3,4,5,6 hr) after the period of microbial adaptation and were analyzed for physical characteristics, pH, SAT, MBRT, total bacterial count, total protozoal count, differential protozoal count, total volatile fatty acids, Individual volatile fatty acids, ammonia nitrogen and total nitrogen.

Three blood samples were collected from each animal at weekly interval in Pre-Summer group, Summer group and Summer Treatment group. In Summer Treatment group animals the first sampling was done after one week of start of supplementation. During investigation various physiological parameters, blood biochemical, haematological parameters were examined.

The results revealed that

1. Rumen liquor was greenish to yellowish green in colour, aromatic in odour and viscous in consistency during Pre-summer which did not changed during heat stress and after supplementation with Yea Sacc^{1026}.
2. Ruminal pH showed significant decrease with feeding of Yea Sacc^{1026} during summer season as compared to summer control.
3. There was significant decrease in overall SAT values in Yea Sacc^{1026} supplemented animals during summer season as compared to control group. SAT values were significantly declined from 0 to 3 hrs post feeding followed by an increase at 6 hr post feeding in all the groups.

4. There was significant decrease in overall mean MBRT values in Yea Sacc^{1026} supplemented group as compared to control group. Highest values were obtained at 0 hr and lowest at 3 hrs post feeding in all the groups.
5. Total bacterial count found lowest at 0 hr, followed by progressive increase which attained peak levels at 3 hrs postprandial and then gradually decline upto 6 hrs after feeding in all the groups. However, overall mean values revealed significant increase in total bacterial count with Yea Sacc^{1026} supplementation.
6. Total protozoal count was significantly decreased in animals of control group although within physiological limits and increased when maintained on Yea Sacc^{1026} supplementation which is very similar to level of pre summer group. Max. count was recorded at 3 hrs postprandial irrespective of diet.
7. Total volatile fatty acids in the rumen liquor were significantly increased though within physiological limits during feeding of Yea Sacc^{1026} supplementation than conventional feeding and summer stressed animals. Peak was observed at 3 hrs postprandial in all the groups.
8. Acetic acid and butyric acid was significantly higher during summer season whereas propionic acid decreased, these values were restored to normal after Yea Sacc^{1026} supplementation but butyric acid did not show any change.
9. There was significant decrease in ammonia nitrogen and total nitrogen levels in animals after supplementing Yea Sacc^{1026} when compared to heat stressed animals.
10. The mean respiration rate, heart rate and rectal temperature were significantly higher in Group II as compared to Group I, confirming the adverse effect of summer stress. In treatment group (Group III), the supplementation of Yea sacc^{1026} caused a significant decrease in heart rate and rectal temperature in summer stressed buffalo calves.
11. The mean packed cell volume and hemoglobin were found to be lower in summer control group (Group II) as compared to pre-summer group (Group I). There was significant increase in PCV and Hb in Group III as compared to Group II.
12. The mean plasma glucose concentration was found to be higher in summer stressed buffalo claves (Group II) as compared to pre summer group (Group I).

Plasma glucose concentration in treatment group buffalo calves (Group III) was significantly lower as compared to Group II (summer control group) but there was no significant difference between Group I and Group III suggesting the positive effect of supplementation of Yea sacc ^{1026} in relieving the effect of heat stress in buffalo calves.

13. The mean plasma total protein concentration was found to be lower in Group II as compared to Group I . Supplementation of Yea sacc^{1026} in Group III produced an increased in plasma protein concentration.
14. Plasma cholesterol level was similar in all groups (Group I, II and III). So, cholesterol level was neither affected by summer stress nor by Yea Sacc^{1026} supplementation in buffalo calves.
15. LPO level was significantly higher in summer stressed buffalo claves (Group II) as compared to pre summer buffaloes (Group I) indicating the adverse effect of summer stress on buffalo calves. In Group III, LPO was significantly lowered and was comparable with Group I which show that Yea Sacc^{1026} supplementation have beneficial effect on relieving summer stress in buffalo calves.
16. There was a significant increase in plasma urea level during summer as compared to pre summer. On supplementation of Yea Sacc^{1026}, there was significant decrease in plasma urea level in Group III.
17. Plasma creatnine concentration was significantly increased in group II as compared to Group I, but after Yea Sacc^{1026} supplementation these values were not brought to normal.

CONCLUSION

It can be concluded from the present investigation that oral administration of Yea Sacc^{1026} can ameliorate the adverse effect of heat stress on rumen profile, physiological and metabolic status of buffalo calves during summer season by enhancing the microbial population, optimizing the concentration of rumen metabolites and reversing the affect of heat stress on hematological and blood biochemical parameters.

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ANNEXURE- I

Weekly recording of temperature, relative humidity and temperature humidity index (THI) of Buffalo calves shed

Seasons	week	Temperature of Shed °C			Relative humidity	THI	Mean THI
		Max. Temperature	Min. Temperature	Av. Temperature			
Presummer	1	32.2	19.8	26.00	30.20	71.16	68.5
	2	31.5	20.4	31.00	31.00	71.28	
	3	33.1	21.0	27.05	30.85	72.35	
Summer	1	34.3	24.1	29.15	74.00	81.13	83.5
	2	34.2	27.3	30.70	85.00	85.33	
	3	35.1	27.4	31.25	76.00	84.72	

VITA

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