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EFFECT OF SUPPLEMENTATION OF FERMENTED YEAST CULTURE (*SACCHAROMYCES CEREVISIAE)* DURING AND POST SUMMER SEASON ON PLASMA HORMONES EGG PRODUCTION POTENTIAL FEED EFFICIENCY OF PD 3 CHICKEN LINE

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ABSTRACT
Heat stress has negative impact on poultry production. Leptin, ghrelin and GH hormones are known to regulate physiological functions of chicken, but their role under heat stress/summer season is not known. The present experiment was conducted to observe the effect of high ambient temperature prevailing during summer season on hormone parameters and egg production in brown egg layers. A hundred number of 16 week old, PD-3 (Dahlem Red) line of chickens were equally allotted to two groups. After duration of eight weeks (April-May) of summer season, experiment was continued till the birds attained 32 weeks of age. The treatment group received 1.25 g of fermented yeast culture commercial product per kg feed as supplement. The feed offered to the control group was devoid of supplement. Leptin, ghrelin, GH, total protein, cholesterol, malondialdehyde and free fatty acids were analysed in blood plasma. Fortnightly body weight and feed intake of the birds were recorded. Egg production and egg weight were also recorded. Supplementation of fermented yeast culture decreased the concentration of plasma leptin ($P < 0.05$), ghrelin ($P < 0.05$), GH ($P < 0.05$), cholesterol ($P < 0.01$) and MDA ($P < 0.05$) during summer season. It decreased feed intake ($P<0.01$) of birds only during post summer season. In the supplemented group the egg production increased from 26-31 weeks of age and FCR was significantly less during post summer season. It was observed that fertility and hatchability of eggs was also more for the supplemented group. In conclusion providing fermented yeast culture to the hens during peri and post summer period improved feed efficiency and egg production, thus increasing their production potential by decreasing the level of mentioned plasma hormones and lipid oxidative parameter. This is the first study in PD 3 chicken line under tropical conditions.

KEY WORDS: Egg production, Hormones, Layers, summer season, Fermented Yeast culture.

INTRODUCTION
Heat stress experienced during summer can have a significant impact on layer flocks, but there are different ways by which hens can be kept healthy and productivity can be maintained. When ambient temperatures rise during summer, egg production declines, and flock mortalities increase. High ambient temperature causes stress which in turn affects biological defence mechanisms, including biochemical and metabolic functions. Under high ambient temperature conditions, the body thermoregulatory mechanisms, try to adapt themselves to maintain homeostasis of the body functions. Profound changes occur in the physiological functions (Horowitz, 2002). Endocrine hormone Leptin, plays an important role in coordinating energy homeostasis and its role has been attributed to decrease in feed intake (Rabe *et al*., 2008). Role of appetite regulating hormones Leptin and Ghrelin on feed intake and functions of liver is known, their effect on metabolic and feed intake in chickens during summer season, when high ambient temperature is prevailing is not known, even the level of these plasma hormones during summer season or heat stress is not known. Excess of energy remaining after utilisation should be dissipated as heat. If the surrounding temperature is less, then the heat dissipated is more. If the humidity in the atmosphere is more then, also heat dissipated will be less. As the relative humidity increases, temperature of the bird's body increases, which results in decrease in feed intake and less amount of heat is produced. With increase in temperature, productivity performance decreases (Talukder *et al*., 2010). The hypothalamus plays a vital role in integrating external environment factors and generates appropriate endocrine, metabolic responses and has an influence on feed intake. It was also revealed that hypothalamic and gastrointestinal tract peptides were involved in the appetite regulation in laying hens exposed to heat (Song *et al*., 2012). It has been reported that poultry when subjected to heat stress, it results in 31.6% reduction in feed efficiency, 36.4 % in egg production and 3.41 % in egg weight (Star *et al*., 2009). Ghrelin present in the gastrointestinal tract is regulating feed intake in broilers and layers (Kaiya *et al*., 2009). In rat and humans, growth hormone release from the pituitary is not only regulated by GH releasing hormone but also by ghrelin (Kojima *et al*., 2000). Leptin is a 167 amino acid peptide whose binding sites have been located in hypothalamic neuronal areas (Madiehe *et al*., 2002). Leptin, Ghrelin and their receptors regulate body weight and food intake (Dridi *et al*., 2005). Ghrelin is a 28 amino acid gastrointestinal peptide and is an endogenous ligand for growth hormone secretagogue (Kojima *et al*.,

1999). Contradictory reports on the effect of fermented yeast culture / yeast culture supplementation on production parameters in layers are available. No effect on egg production (Nursoy *et al*., 2004) and increase in egg production was also observed (Tangendjaja and Yoon, 2002). Reports are not available, with respect to anorexigenic peptides/hormone level during summer in chickens and consequently their effect on egg production. Hence the present experiment was taken up to observe the effect of heat stress prevailing during summer season on the level of plasma anorexigenic hormones, plasma metabolites, cholesterol and also to observe the effect of fermented yeast culture supplementation in amelioration of heat stress in PD3 (Dahlem Red) chicken line. The effect of summer period was observed on egg production potential. Laying period coincided with the post summer period. This is the first study on effect of supplementation of fermented yeast culture to PD3 chicken line on endocrinological, metabolic and production parameters under tropical conditions.

MATERIALS & METHODS

Approval was taken from Institutional Animal Ethics committee for techniques involved in conducting the present study. The experiment was conducted at poultry farm situated in Directorate of Poultry Research, Rajendranagar, Hyderabad, India. The month of April and May is characterized by *gradually rising* daily high temperatures, with daily highs around 37°C throughout the month, exceeding 41°C or dropping below 34°C only one day in ten. The month of June is characterized by *rapidly falling* daily high temperatures, with daily highs decreasing from 37°C to 31°C over the course of the month, exceeding 38°C or dropping below 29°C only one day in ten (https://weatherspark.com /averages/ 33946/ 6/ Hyderabad -Andhra-Pradesh-India). A total of 100 number of PD 3 line chickens which were 16 weeks of age were procured from our institute farm, they are basically lineage of Dahlem Red breed. Till 19 weeks of age, they were provided with growers feed (Table 1) and then, from 20 week onwards, they were provided with layers feed (Table 1). Birds with uniform body weight ranging between 850-950 g were taken for the present study. The birds were divided in to two groups, one served as control and the other served as treatment/supplemented group. Initially a pilot study was conducted with two different doses (0.5mg/kg and 1.25mg/kg) of fermented yeast culture, it was observed that, the lower dose did not have any significant effect on the mentioned parameters when compared between the control and supplemented group. Hence the dta for the lower dose has been omitted. The supplemented group in addition to normal feed, was provided with fermented yeast culture (*Saccharomyces cerevisiae*) @ 1.25g/kg feed. Each group had ten replicates containing five birds in each replicate. The summer period was considered from last week of April till last week of June (8 weeks). Though it was summer season, the profile of the temperature was not following in the same trend, hence profile of the variations in temperature is given in Fig. 1.The birds attained 24 weeks of age only by the end of summer period, hence, the study on egg production parameters continued in the post summer period, till the birds attained 32 weeks of age. Each hen was placed in a single cage, feed and water was supplied *ad libitum*. Constituents of feed are given in Table-1. CP and ME of the grower's feed was16% and 2800kcal/kg whereas for layer's feed was 14% and 2500kcal/kg respectively. Body weight was recorded at fortnight interval. Weekly feed intake was estimated. In the laying period, eggs were collected daily and weight was recorded. A 3 ml of blood from six birds of each group at random, was collected from brachial vein at weekly interval during eight weeks of summer season only. During laying period, blood was not collected from birds, as it may cause stress to birds. The collected blood was immediately transported on ice to lab. The blood samples were centrifuged at 3000 rpm for 15 min; plasma was separated and stored at -40^{o}C for assay of parameters, like hormones, metabolites, cholesterol, MDA etc. From 24 till 32 weeks of age, studies were restricted to egg production parameters, feed and body weight parameters. Supplementation of fermented yeast culture continued during this tenure also. Blood samples were not collected during laying period, as it may create additional stress to the birds.

Estimation of plasma leptin, ghrelin and GH

All the three hormones in plasma were estimated with the help of commercially available kits purchased from BioGene Biotech, Shanghai, China. The assay procedure was based on competitive binding assay. The minimum detection limit was 0.5 ng , 0.5ng , and 2.5ng/ml for leptin, ghrelin and GH respectively. The absorbance of the color solution obtained at the end of the reaction was read at 450 nm. The intra and inter assay coefficients of variation were were less than 6%.

Estimation of plasma free fatty acids (FFA), triglycerides and cholesterol

Plasma free fatty acids (FFA), triglycerides and cholesterol were estimated with the help of commercially available kits purchased from BioAssay Systems, CA 94545, USA. EZassay^{TM} TBARS estimation kit for lipid oxidation was purchased from Hi Media Co. Pvt., Ltd., and Mumbai, INDIA. The assay is based on the reaction of malondialdehyde (MDA) with a chromogenic agent thiobarbituric acid (TBA) at high temperature and acidic conditions to form a color complex whose absorption was read at 532 nm.

Statistical analysis

Statistical analysis was carried out using unpaired't' test for comparison of different parameters between the two groups and two way ANOVA for comparison with respect to days using Graph Pad Prism version 5.0.

TABLE 1: Composition of feed (in percentage) for layers

Components of diet	Layer grower	Layer breeder
Maize	56.05	61.39
Soyabean meal	24.09	24.74
DORB	15.3	0.49
Stone grit	1.86	10.9
DCP	1.66	1.5
Salt	0.35	0.35
Sodium bicarbonate	0.1	0.1
DL-methionine	0.11	0.1
L-Lysine	0	0
Trace minerals	0.1	0.1
Vitamin premix	0.015	0.1
B complex	0.015	0.1
Antibiotic	0.05	0.05
Choline chloride	0.1	0.1
Toxin binder	0.1	0.1
Tylosin	0.05	0
Coccidostat	0.05	0

RESULTS

Effect of supplementation of fermented yeast culture to layers on plasma parameters is given for the period during summer season (8 weeks, birds age- 16-24 weeks, is expressed in days) only. Results of egg production parameters for post summer season is given in weeks (Birds age- 24-32 weeks). Blood samples were not collected during this tenure. The average day temperature during summer period in the experimental shed ranged between 36.9°C and 29°C (Fig 1). Relative humidity ranged between 46% and 56%. During post summer season temperature ranged between 30°C and 26°C whereas relative humidity ranged between 50% - 70%. During summer season, mortality rate in control group was observed to be 13% where as in treatment group it was as less as 3.7%. This further increased to 20% and 10% in respective groups for the entire study period.

FIGURE 1: Profile of average day temperature represented for a week during summer (56 days, eight weeks)

Effect of summer season on blood plasma body weight and feed parameters

It was observed that level of plasma hormones leptin, ghrelin and GH was higher ($P<0.05$) during summer period in the control group (Fig 2 a, b, c). On supplementation of fermented yeast culture @1.25 g/kg from 16 to 24 weeks of age, the concentration of all the three hormones in plasma decreased ($P < 0.05$, Fig 2 a, b, c). The decrease in the level of leptin and ghrelin hormones did not bring about any significant difference in the feed intake of the birds and consequently difference in the body weights of the birds when compared between the groups (Fig 3 a, b). In the control group, the highest concentration of leptin and ghrelin was observed at 21d where as for GH it was observed at 14d after the initiation of the experiment. Later till 56d or eight weeks of experiment, the level of the plasma hormones of the control group decreased, but, was always greater than the concentration of the respective hormones of the treatment group (Fig 2 a, b, c). As the ambient temperature decreased, the level of the plasma hormones also decreased.

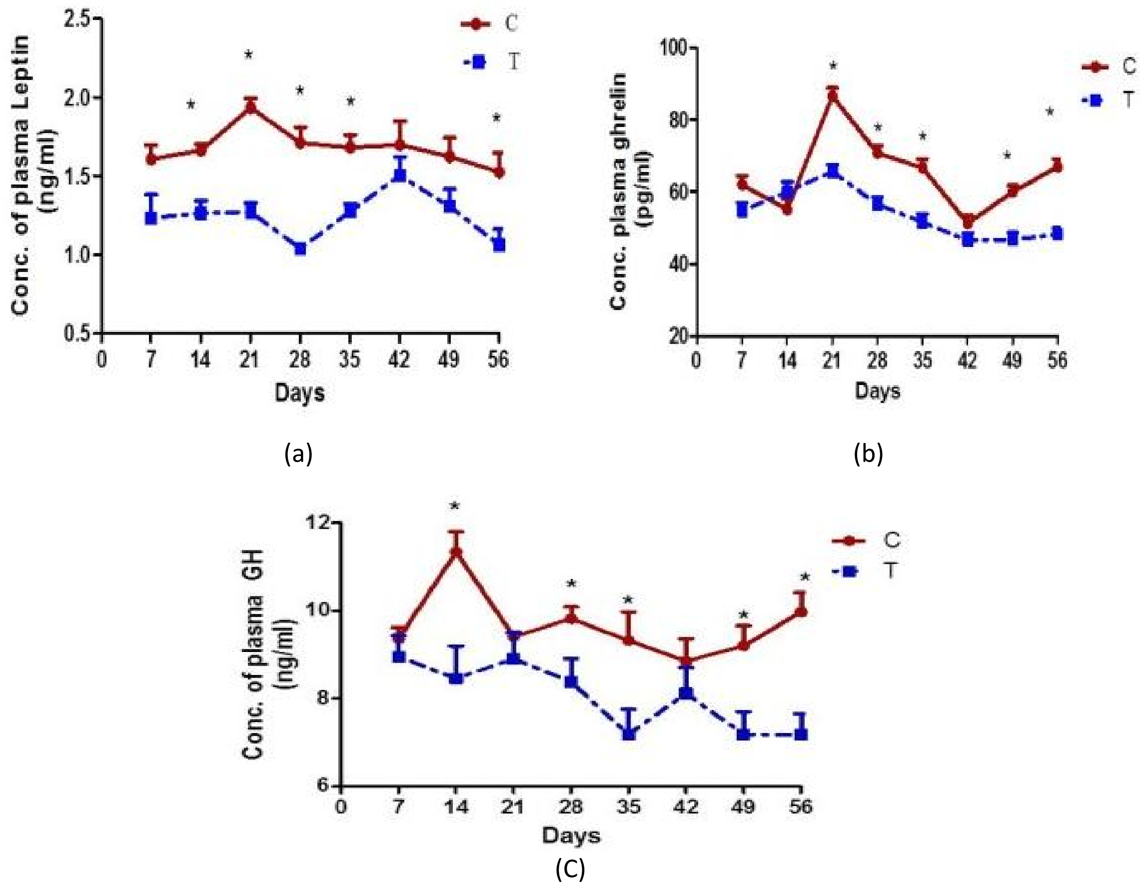

FIGURE 2: Profile of plasma leptin (a), ghrelin (b) and GH (c) during summer season (56 d ,8 weeks) in PD 3 chicken line. C- Control, T- Treatment, Values are exhibited as Mean±SE, and are significant atleast at the level of *P<0.05, Age of the birds during this period was 16-24 weeks.

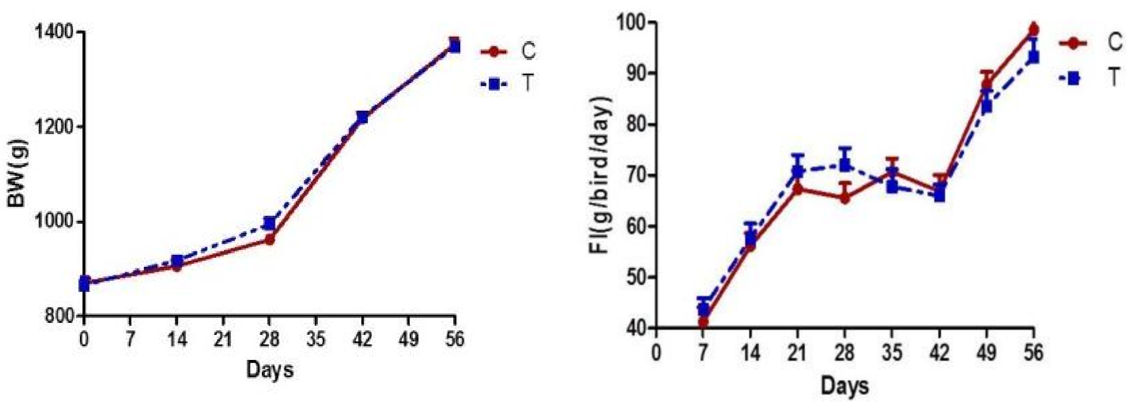

FIGURE 3: Weekly average body weight (a) and feed intake(b) of chickens during summer(56 d ,8 weeks), Values are exhibited as Mean ± SE . C-control, T- treatment, BW-body weight, FI- Feed intake. Age of the birds during this period was 16-24 weeks.

The pattern of feed intake did not correlate with the anorexigenic property of the hormones during heat stress or summer season. The greater concentration of the hormones did not cause decrease in the feed intake (g/bird/d) of the control group. Feed intake for both the groups increased with increase in the age of the birds. It was also inversely related with the increase in average day temperature recorded between 28 and 35 days of the experiment (Fig 3 b). As, there were intermittent rains, the temperature decreased from 36-30^{o}C between 14-21d of the experiment. From 21-35 d of experiment, when the ambient temperature increased from 29^{o}C- 32^{o}C, a decrease in feed consumption was observed for both control and treatment groups, the decrease observed was more for the control group(Fig1, 3b). The difference in feed intake was not significant between the groups. Post 35d of the experiment, the feed consumption increased with the increase in the age of the birds, but during this tenure the day temperature also reduced from 32^{o} to 29^{o} C. In the treatment group, feed intake of birds decreased when compared with the control, but the difference was not significant (Fig 3b).

The difference between the concentration of total plasma protein and Free Fatty Acids (FFA) between the groups was not significant (Fig4 a, b). The maximum decrease in the concentration of protein and increase in the concentration of free fatty acids was observed at d 21 for both the groups when the temperature decreased from 36.9° to 31°C. Post 21d of the study tenure, concentration of plasma protein and free fatty acids of both supplemented and control group increased steadily. It was observed that concentration of these two parameters was always less for the control group. The increase in day temperature (29-32°C) between 21-35d, did not affect the concentration of these parameters. Supplementation of fermented yeast culture also could not bring about significant difference in the concentration of these parameters between the groups. The pattern of cholesterol profile observed was different from profile of the above mentioned plasma parameters.

Till 21st d of the experiment , the difference in the concentration of plasma cholesterol between the groups was not significant, but it increased in the control group, from 28th day till the end of the experiment and was significantly different (P<0.01) from the estimated concentration of the treatment group (Fig 4d). Plasma MDA level which indicates lipid oxidation level was significantly more in the control group (P < 0.01) during summer period (Fig 4 c) indicating more oxidation of lipid in the control group .

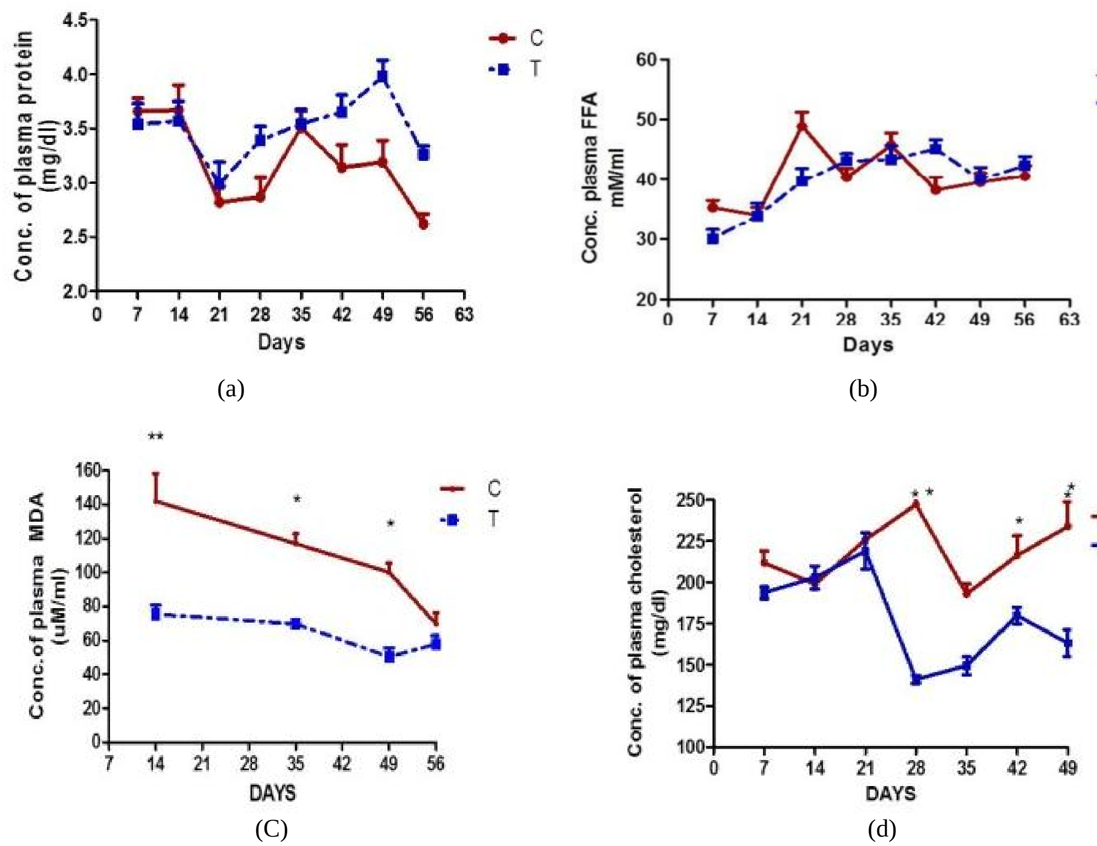

FIGURE 4: Profile of plasma proteins (a), Free fatty acids (b), MDA (c) and Cholesterol (d) during summer(8 weeks, 56d) in PD3 chicken line, Values are exhibited as Mean ±SE. *P<0.05, **P<0.01. Age of the birds during this period was 16-24 weeks.

Effect on egg production parameters and feed intake post summer season

During post summer season, the experiment continued, till the birds attained 32 weeks of age. A significant decrease (P < 0.01) in feed intake (Fig 5) was observed for the supplemented group. Birds of both the groups started laying eggs at 20 weeks of age. The body weight (Fig 5) and egg weight of both the groups did not differ significantly. Attainment of 50% egg production was earlier in supplemented group. Maximum egg production percentage was observed at 30 weeks of age which was again greater for the supplemented group (68.16 vs. 64.08). The egg production was significantly greater for the supplemented group from 26-30 weeks of age (Table 2). Feed conversion ratio (FCR, 3.59 vs.3.05; 26-32 weeks) was observed to be significantly less (P < 0.05) for the supplemented group when compared with the control group. The percentage of fertility and hatchability estimated for 28-31 weeks was also greater for the supplemented group (Table 3).

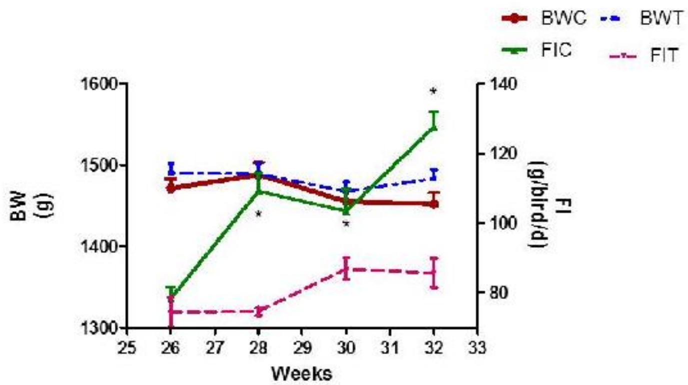

FIGURE 5: Body weight (BW) and feed intake (FI) of PD 3 chicken line during post summer season (Age of the birds 26-32 weeks). Values are represented as Mean ± SE. FI values between groups was significantly different at * P<0.01. C-Control, T- Treatment

TABLE 2: Comparison of egg production potential between the groups

Weeks	Control (%)	Treatment (%)
24	36.55	40.78
25	44.69	48.88
26	47.61	50.64
27	54.18	59.94
28	58.66	63.67
29	60.75	65.35
30	64.08	68.16
31	62.91	66.08
32	62.94	66.74

Values are represented as average for one week.
Attainment of 50% egg production potential earlier in treatment group.

TABLE 3: Effect of supplementation of FYC on fertility and hatchability

	Egg set (nos.)	Fertility %	Hatchability %
28-29 weeks			
Control	212	77	93.9
Treatment	248	83	97
30-31 weeks			
Control	206	80	92.1
Treatment	220	86	99.5

DISCUSSION

The present experiment on chickens was initiated during the months of summer; supplementation was initiated in the summer itself. Since egg production time coincided with the post summer period, supplementation was continued in this phase also. In India, after summer, during July- September, rainy season begins and humidity increases (from 46% - 70%) and hence though temperature decreases, animals are under stress in this phase also. There are different effects of chronic and acute heat stress. Very little information is available on endocrine factors and their relationship with physiological functions, metabolites etc. in chickens especially PD 3 line, under heat stress conditions or summer season. This is the first study on plasma hormone parameters in PD 3 chicken line (Dahlem Red) during summer season and the effect of supplementation of FYC during and post summer season on egg production. Dairy calves supplemented with yeast products did not require medical attention often, indicating improvement in health and performance (Magalhães *et al*., 2002; Anand Laxmi *et al*. ,2012; Anand Laxmi *et al*., 2016). In the present situation also supplementation might have improved health status reducing mortality.

The effect of certain hormones can change according to the physiological status of the animal (Bauman *et al*., 1982). The higher temperature prevailing during first 15 d of the experiment caused an increase in the level of hormones in the control group, but supplementation caused decrease in the plasma level of these hormones. Higher GH levels have never been always good indicators of growth, but it is positively correlated with conversion of dietary protein to body protein (Decuypere *et al*., 2005). Hence, in the present study higher level of plasma GH might have helped in the conversion of dietary protein in to body protein and hence, it might not have resulted in the significant reduction in the body weight of the hens of the control group, besides prevailing higher ambient temperature. It is reported that no effect was observed on feed intake upon supplementation with yeast or yeast products in layers (Mahdavi *et al*., 2005). In the present study also supplementation of fermented yeast culture, during summer season did not result in any significant change in feed intake in pullets. Probiotic supplementation helps in protein and energy retention, in the present study also supplementation of fermented yeast culture might have improved the protein retention capability in the supplemented group, and this might have resulted in greater concentration of plasma protein but was not significantly different from the control group. Since the age of the birds was also increasing with increase in the period of the study tenure, a clear relation between temperature and feed intake cannot be explained.

Though there was no significant difference between the plasma FFA levels between the groups, the highest level of plasma GH in control group at 21 d corresponded with the highest level of plasma FFA at 21d. In the treatment group also maximum GH level was observed at 21 d and then a rise in FFA level was observed. This indicates that increase in concentration of GH might have preceded lipolysis with sudden decrease in temperature inside the shed. In rats and humans it is suggested that GH release from the pituitary is not only regulated by GHRH but also by ghrelin (Kojima *et al*., 2000). The higher level of plasma GH in the control group might have been triggered by the higher plasma level of circulating ghrelin during the summer period. It is stated that adaptation to high temperature might not reflect differences in plasma level of metabolites (Song *et al*., 2012). In the present study also, it appeared that birds of control group at a later stage, were getting adapted to high ambient temperature, which did not reflect changes in plasma FFA. However level of plasma cholesterol was observed to be significantly higher ($P < 0.05$) in the control group, during summer season and is positively related to the increased level of plasma hormones. A further research is required in this area. Cotton seed meal fermented by yeasts reduced plasma cholesterol (Nie *et al*., 2013). In the present study also supplementation decreased level of plasma cholesterol during summer period. Reports are available with respect to higher expression level of ghrelin in jejunum, stomach and hypothalamus of heat stressed layers (Song *et al*., 2012) but no reports are available with respect to circulatory ghrelin. The anorexigenic effect of ghrelin might be due to its action on target organs duodenum and jejunum and induce its effect on feed intake (Lei *et al*., 2013). Higher levels of ghrelin might have decreased feed intake in the control group for a short period during summer, the inhibitory effect on feed intake might have decreased in the later period due to decrease in the concentration of hormone at a later stage. Supplementation of FYC to treatment group also decreased feed intake as observed during last 15d of summer period (42-56d) of study and hence no significant difference in feed intake between the groups could have been observed during summer period. Only one report in humans is available, where greater level of plasma ghrelin is reported under chronic heat stress (Lutter *et al*., 2008). In pigeons it has been reported that, heat stress elevates plasma leptin (Al-Azraqi, 2008). Similar reports are available in mice where higher plasma leptin level was observed during heat stress and was not related to feed intake (Morera *et al*., 2012). In chickens leptin is known as anorexigenic hormone, in the control group even though level of plasma leptin was significantly more it was not able to bring about decrease in feed intake when compared with the supplemented group. Supplementation of yeast culture might have also caused decrease in feed intake. Hence no significant difference on feed intake could be observed when compared between the groups. Further, studies have to be conducted under controlled conditions to come to any conclusion on the relation between plasma hormones and feed intake under high ambient temperature conditions.

Measurement of MDA levels in plasma or serum indicates, index of lipid peroxidation and is a non-invasive biomarker of oxidative stress, often clinically employed to investigate radical-mediated effects (Meagher *et al*., 2000). Supplementation of yeast or yeast products can delay or even inhibit oxidation processes and thus restore and maintain homeostasis in the organism (Xie *et al*., 2015). Supplementation of fermented yeast culture decreased plasma MDA levels, with no significant effect on protein carbonyls, exhibiting efficacy of supplementation, in reducing lipid peroxidation levels from the beginning of the experiment. Similar result with no effect, on protein carbonyls was observed, in broilers when subjected to acute and chronic heat stress (Beauchemin *et al*., 2008). As described by others, supplementation of yeast culture decreased concentration of plasma MDA in goats indicating alleviating effect on oxidative stress (Mandal *et al*., 2009).

Live yeast products and their derivatives (*i.e.*, yeast cell wall products) are currently utilized in food animal production for a variety of reasons encompassing performance enhancement and overall benefits to animal health and well-being (Wang *et al*., 2017). On supplementation of fermented yeast culture during post summer, though the feed intake decreased significantly, the body weight and egg weight of the birds were not affected, when compared between the groups. These results are in accordance with the studies of Liu *et al*. (2002), Nursoy *et al*. (2004). But results are contradictory, with reports of Bidura *et al.* (2016), where they observed an increase in egg weight of Lohmann Brown layers. The differential effects observed may be due to difference in the time of experiment, the dose and the type of yeast culture supplemented. Supplementation of FYC during post summer period or during laying period might have had direct effect on developmental capacity of follicles, which cannot be ruled out. This might have also been due to decrease in the level of plasma hormones, observed during the summer period which might have influenced the egg production parameters in the post summer period. Such studies have not been reported earlier. An increase in percentage of egg production observed in the supplemented group from 26-30 weeks in the present study is in accordance with other studies (Bidura *et al*., 2016). As it is known that yeast culture supplementation improves beneficial bacterial population in the gut, this might have increased feed efficiency of the birds during laying period in the present study. It has been similarly reported (Pan *et al*., 2014).Yeast culture provides beneficial growth factors, vitamins and amino acids, which may serve as stimulants for beneficial bacteria in the gut as well as for the bird (Ahmad, 2006). In the present study also, fermented yeast culture may be a source of all these parameters and may be supporting health of the bird by alleviating negative consequences of the stress (Lensing *et al*., 2012). Fertility and hatchability of the eggs are also adversely affected by stress (Rozenboim *et al*., 2007). In the supplemented group, greater percentage of fertile and hatched eggs may be due to the effect of yeast culture and its fermented products. Supplementation of yeast culture significantly increased hatchability and fertility in quails (Yildrim *et al*., 2003). Study conducted (Hayat, 1992), in white turkey hens stated improvement only in number of fertile eggs while other parameters, body weight, egg weight, hatchability were not affected when fermented yeast culture was supplemented at 5% level. The heat production of birds decreases with lower feed consumption and this may also be one of the reasons for increase in egg production and earlier attainment of 50% egg production in the supplemented group during laying period. Decrease in feed intake with no effect on body and egg weight and maintained egg production indicates that supplementation did not cause decrease in body reserves. Hence, it can be concluded that supplementation of fermented yeast culture improves egg production potential and FCR in PD 3 chicken line during post summer period by positively affecting mentioned plasma parameters during summer season.

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