Received: 10 December 2020 | Accepted: 7 May 2021

DOI: 10.1111/jpn.13579

ORIGINAL ARTICLE

Journal of Animal Physiology and Animal Nutrition WILEY

Effect on physiological and production parameters upon supplementation of fermented yeast culture to Nicobari chickens during and post summer

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Funding information
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Abstract

Nicobari is an indigenous bird reared for meat and eggs. This study evaluated the effect of heat stress on plasma levels of leptin, growth hormone and their receptors, liver AMP kinase, plasma cholesterol and lipid peroxide (MDA). The laying period coincided with the post summer period. The birds were equally divided into three groups, control group was offered ad libitum feed and treatment groups were supplemented with fermented yeast culture at 700 mg (T1) and 1.4 g/kg (T2) of feed/day. The levels of plasma Leptin and GH hormones were higher ($p < 0.05$) in the control group when compared to the treatment groups. The expression of the hormone receptors was higher in the brain, and MMP3 gene expression in the magnum was lower in the treatment group. Plasma cholesterol, MDA and AMP kinase were significantly higher ($p < 0.05$) in the control group. Fermented yeast culture supplementation decreased feed intake and increased egg production parameters, which indicates a greater efficiency of supplementation. Supplementation reduced the severity of necrosis of villi in the jejunum when compared to control. In conclusion, higher ambient temperature during summer had negative effect on production parameters through modulation of physiological parameters which could be ameliorated by supplementation of FYC.

KEYWORDS
gene expression, hormone, metabolites, Nicobari chicken, yeast culture

1 | INTRODUCTION

Indigenous chicken raised by farmers provides them benefits, such as a source for meat and eggs, and at the same time, the commercialization of the products improves the economic situation of farmers. Poultry rearing and marketing of products appear to be the most profitable enterprise in the agricultural sector, and in India, it is the third largest egg producer. Nicobari is one of the indigenous breed of chickens (Yadav et al., 2017). Nicobari fowl is an endangered breed of the Andaman and Nicobar Islands and produces the highest number of eggs among the indigenous chicken breeds of India (Kundu et al., 2012). The feather colour includes three different types namely, black, brown and white. This breed is also known for its resistance to diseases (De et al., 2013). The egg production characteristics are not only determined by their number but also dependant on the chicken's weight.

Indigenous chickens play an important role in the lives of rural population because of their adaptability to harsh climates (Hoffman, 2013; Padhi, 2016). However, there are several reports regarding exotic birds that are known for their higher production level that do not perform the same under tropical conditions. The main aim was to study the physiological performance of Nicobari under farm conditions during summer. It is known that in summer egg production, performance

of laying hens decreases and age at sexual maturity increases (Alkan et al., 2009; Cosmos et al., 2015; Pathak et al., 2013). In the tropical and subtropical regions, heat stress is very common. Reports indicate that chronic heat stress can affect the physiology of poultry, which can subsequently affect production (Kang et al., 2020; Saracila et al., 2020; Wasti et al., 2020). In a previous study, the summer season affected egg weight but did not have any effect on egg number (Alkan et al., 2009; Kang et al., 2020). It has also been observed that fertility decreases after exposure to higher ambient temperature (Nord & Nilson, 2019; Walsh et al., 2019). Leptin hormone gene has been localized in chickens in other tissues including brain (Brown et al., 2009; Serrousi et al., 2017). It is known that leptin and GH regulate ovarian follicle development (Niu et al., 2019; Socha & Hrabia, 2019). It is reported that enhancement of leptin receptor signalling leads to reduction in follicle development, ovarian hormone secretion and further production performance (Lei et al., 2014).

It has been proposed that some feed resources should be included in feed formulation for increasing egg production or weight (Sizova et al., 2020). However, no study has been conducted to observe the effect of the summer season on physiological parameters and its effect on production performance during post summer period, which coincided with the laying period in native Nicobari chickens. Specifically a study on Leptin, GH hormones and their receptors during summer season in this breed has not been conducted. The present study also evaluated the efficacy of FYC in ameliorating the effect of high ambient temperature during summer on the mentioned parameters. It was previously determined that in broilers supplemented with YC at 2.5 g/kg improved the average daily gain and feed conversion (Gao et al., 2009; Zhang et al., 2020). The beneficial effects of YC have been reported as an increase in the number of beneficial bacteria and a decrease in the number of pathological bacteria (Hassanein & Soliman, 2010; Sizova et al., 2020). The aim was to observe whether the supplementation of FYC to the treatment groups (T1&T2) during and post summer will have a beneficial effect on egg production through the parameters mentioned. The commercial product used in this study has not been used for the amelioration of heat stress. It was hypothesized that, during summer, bird's endocrine and metabolic physiology get altered which has negative effect on production and the effect may persist in post summer also. A nutritive supplement like FYC may modulate mentioned parameters and produce positive response on production parameters. In ruminants also, it has been reported that feeding FYC has beneficial effect on nutrient availability, digestive processes and activity of digestive enzymes (Gao et al., 2009; Shen et al., 2009) and immune functions. Higher dose of FYC product other than manufacturer's instructions was used, because higher ambient temperature creates an additional stress to birds and literature is not available with respect to this product.

2 | MATERIALS AND METHODS

A total of 90 Nicobari chickens were maintained at the Directorate of Poultry Research Farm, Rajendranagar, Hyderabad. The experiment during the summer period occurred from the third week of April until the third week of May (4 weeks). The peak temperature in the shed ranged between 35–37.9°C and RH ranged between 46%–56%. Experiment was conducted on the birds whose age at the beginning of the experiment was 19 weeks and who were 22 weeks of age (7–28 days of the experiment) by the end of the summer. The initial body weights of C, T1 and T2 pullets were 1074.10 ± 11.64, 1080.73 ± 11.64 and 1100.40 ± 11.64 g respectively (Table 8). Further experiment continued during the post summer period which also coincided with the laying period for an additional period of 10 weeks. Three groups were maintained with thirty birds in each group. Each group had five replicates with six birds in each replicate. The control group was offered feed ad libitum which was devoid of supplement. The constituents of the feed are given in Table 1. The diet was based on a maize–soybean diet. The basal diet offered to the birds was standardized based on the diet reported by Panda et al., (2012). The control and experimental groups were offered water *ad libitum*. Fermented yeast culture (FYC, Unigrow, *Saccharomyeces Cerevisiae*) which is a commercial product was supplemented throughout the experimental period. The yeast culture product was purchased from Nurture Organics, Karol Bagh, New Delhi. The fermented product was a dried product composed of yeast and the medium on which it is grown, preserving the fermenting activity of the yeast, contained 3×10^5 cfu/g of the product, it is also a source of amino acids,

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
De Oiled Rice Bran	15.3	0.49
Stone grit	1.86	10.9
Di Calcium Phosphate	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
Choline chloride	0.1	0.1
Toxin binder	0.1	0.1
Tylosin	0.05	0
Coccidostat	0.05	0
Calculated values		
ME (kcal/kg)	2707.90	2626.93
Crude protein (%)	18.22	16.91
Lysine (%)	0.86	0.83
Methionine (%)	0.37	0.35
Calcium (%)	1.05	3.90
Available phosphorus (%)	0.40	0.36

TABLE 2 Sequence of the primers

Genes	Sequence of forward (F) and Reverse (R) primers	Size of the PCR product	Annealing temperature
Leptin R	F 5′-CGGACTACCTCATGAAGATCCTGAC-3′	162 bp	55°C
	R 5′-GCCAATGGTGATGACCTGACCATC-3′		
GH R	F 5′-TCAGAAAGGATGGATTACTCTGGAGT ATG-3′	161 bp	55°C
	R 5′-CGGAGGTACGTTGTCTTGATCGGAC-3′		
MMP3	F 5′-TACCAGATGTCCGCTCATACAGCA-3′	162 bp	55°C
	R 5′– TCACCACTGTAGAGCCTGATGAAC-3′		

Abbreviations: F, Forward primer; R, Reverse primer.

minerals, cell wall extract and vitamins. The two treatment groups were offered supplement at a concentration of 700 mg/kg (T1) and 1.4 g/kg (T2) of feed and a dose of 700 mg/kg was recommended by the manufacturers. The calculated feed values during the grower's phase (19–21 weeks of age) the CP of the diet was 18.22% and ME was 2707.9 kcal/kg. During layer's phase (22–32 weeks), CP was 16.9% and ME was 2626.9 kcal/kg. Birds were kept in individual cages. The body weight was recorded at fortnight interval and blood samples were collected (in tubes rinsed with EDTA) from the brachial vein at weekly interval only during the summer period for the estimation of hormones and metabolites. The samples were immediately brought to the laboratory and centrifuged at 1107 g for 15 min. The supernatant containing the plasma was separated and stored at −20°C. The Leptin and GH hormones were estimated, in the blood samples collected at 7 days interval from six birds at random from each group. The analysis was done using competitive enzyme immunoassay method, via commercial EIA kits (Blue Gene Biotech; Chicken Elisa kits). Horse Radish Peroxidase enzyme was used in the enzyme immunoassay method. At the end of the assay, the absorbance of the formed coloured product was measured at 450 nm in a spectrophotometer. The concentration of Leptin and GH hormones in the samples was estimated using a standard curve prepared with the absorbance values for the different concentrations of the given standards along with the kit. The intra- and inter-assay coefficient of variation was <8%.

Estimation of liver AMP kinase and plasma melanodialdehyde (MDA) was conducted using commercially available kits (Blue Gene Biotech). MDA was assayed based on the reaction of MDA with a chromogenic agent thiobarbituric acid (TBA) at high temperature under acidic conditions to form MDA-TBA adduct. The absorbance of the complex formed at the end of the reaction was measured at 540 nm. Liver tissue AMP Kinase was also estimated by a competitive enzyme immunoassay method using Horse Radish Peroxidase enzyme. The absorbance of the coloured complex formed at the end of the reaction was measured at 450 nm on an ELISA reader. Plasma cholesterol was estimated according to the method of Zlatkis et al., (1953).

Feed intake was estimated at weekly intervals during summer and at fortnight interval for the post summer period. The body weight (BW) was recorded at fortnight intervals. Recording of body weight was initiated at the end of the 17th week. The egg numbers and egg weight were recorded daily for 20 eggs selected at random from each group. The laying period coincided with the post summer period which was divided into two phases, 24–27 and 28–32 weeks of age. The results for the egg weight are given from 28 to 33 weeks of age of the birds. From 24 to 28 weeks, the difference in the egg weight was not significant and hence the results for that period are not provided.

For quantification of the hormone receptors, five birds from each group selected at random were sacrificed by cervical dislocation. The liver, brain, magnum and jejunum tissues were excised. After collection of the tissues, except for jejunum, approximately 100 g of the tissue was taken, cleaned and rinsed in saline and was further stored at −40°C for gene expression studies. For the brain tissue, the samples from a group were pooled together.

From liver, brain and magnum tissues, the total RNA was extracted using TRIZOL (In Cell Technologies) and further converted to c DNA using a First-Strand c DNA Synthesis Kit (Thermo Fisher Scientific).

The c DNA was then amplified with respective primers whose sequence is given in Table 2. The primers were synthesized at Chromous. As an internal standard, Actin was utilized as housekeeping gene. Once the PCR protocol with different primers was standardized, the c DNA was subjected to Q-PCR for quantification of the Leptin, GH receptors and Matrix Metalloproteinase-3 gene with SYBR Green kit (Invitrogen, Bioservices India). The temperature cycles were as follows: 94°C for 5 min., followed by 40 cycles of 94°C for 5 s, 55°C for 10 s, 72°C for 10 s and 72°C for 5 min. The SYBR green fluorescence was measured at the end of each cycle. Oligonucleotide sequences of primers, annealing temperatures and the sizes of the expected PCR products are shown in Table 2. All primer concentrations were optimized. All measurements were carried out in triplicate, and the average values were obtained. The values were normalized to mRNA expression of avian β-actin. The relative expression levels were calculated with the $2-\Delta\Delta Ct$ method.

Further histopathological studies of the jejunum were conducted. From the birds of each group, a jejunum portion of the digestive tract was excised and cleaned after sacrifice. The jejunum extends from the distal portion of the duodenal loop to Meckel's diverticulum. The contents of the jejunum were gently removed. The tissue was then rinsed in saline and fixed in 10% formaldehyde. The intestinal segments were kept in Bouin's solution and dehydrated in

a graded series of alcohol. Finally, each specimen was embedded in paraffin wax. The horizontal sections of 4 μ thickness were made. Further, the sections were fixed on glass slides and stained with haematoxylin–eosin. The slides were then observed under a light microscope at 400× magnification for observation of the morphology and necrosis of the villus. The severity of the necrosis was graded as medium (++), mild (+) and normal (Inchroen et al., 2010; Lensing, 2012).

Approval from the Institutional Animal Ethics Committee was obtained No. IAEC/DPR/4/2016 to conduct the present experiment.

2.1 | Statistical analyses

The effects of the diet treatment on hormones and metabolites were examined using one-way ANOVA. Data were analysed by an analysis of variance (ANOVA) using a general linear model procedure (GLM) of SPSS 10 for Windows. Further pairwise comparisons were conducted by Duncan's Multiple Range Test (SPSS 10). A *p*-value of 0.05 or less was considered significant unless otherwise stated. Further Polynomial contrast analyses were also done whose components are Linear and Quadratic. A *p*-value of 0.05 or less was considered significant.

3 | RESULTS AND DISCUSSION

As analysed by the one-way ANOVA, it was observed that, during the first 3 weeks (21 days), the concentration of plasma leptin was significantly higher ($p < 0.05$) in the control group, when compared to the T1 and T2 group (Table 3). Both doses of FYC were equally significant in decreasing the plasma level of leptin. In the last week of the summer period, the concentration of plasma Leptin hormone was not significantly different when compared between different groups. Supplementation of 1.4 g/kg of FYC had a significant linear effect ($p < 0.01$) in decreasing the level of plasma leptin till 21 days

TABLE 3 Plasma levels of leptin, GH, malondialdehyde, cholesterol and liver AMP kinase in Nicobari chicken during summer season

Days	C	T1	T2	SEM	Contrast (*p*-Value)
Leptin (ng/ml)					
7	2.63^{a}	1.71^{b}	1.75^{b}	0.051	L-$p < 0.01$, Q-$p < 0.01$
14	2.15^{a}	1.33^{b}	1.31^{b}	0.026	L-$p < 0.01$, Q-$p < 0.01$
21	1.78^{a}	1.29^{b}	1.28^{b}	0.072	L-$p < 0.01$, Q-$p < 0.013$
28	1.19^{a}	1.09^{a}	1.15^{a}	0.030	L-$p < 0.03$, Q- < 0.036
GH (ng/ml)					
7	4.32^{a}	3.68^{b}	2.73^{c}	0.104	L-$p < 0.01$, Q-ns
14	3.89^{a}	3.06^{b}	2.77^{c}	0.080	L-$p < 0.01$, Q-$p < 0.013$
21	3.66^{b}	3.56^{b}	3.76^{b}	0.034	L-ns, Q-ns
28	3.78^{b}	3.71^{b}	3.60^{b}	0.071	L-$p < 0.03$, Q-ns
MDA (μM/ml)					
7	26.93^{a}	21.16^{b}	22.60^{b}	0.570	L-$p < 0.01$, Q-$p < 0.01$
14	23.89^{a}	17.17^{b}	16.58^{b}	0.509	L-$p < 0.01$, Q-$p < 0.01$
21	19.16^{a}	18.48^{ab}	17.64^{b}	0.205	L-$p < 0.01$, Q-ns
28	18.87^{a}	18.54^{a}	18.39^{a}	0.206	L-ns, Q-ns
Cholesterol (μg/ml)					
7	923.72^{a}	920.82^{a}	934.32^{a}	9.52	L-ns, Q-ns
14	946.10^{a}	890.36^{b}	854.35^{b}	14.59	L-$p < 0.01$, Q-ns
21	1042.05^{a}	909.32^{b}	910.92^{b}	24.59	L-$p < 0.01$, Q-$p < 0.05$
28	1089.87^{a}	874^{b}	855.01^{b}	18.67	L-$p < 0.01$, Q-$p < 0.01$
AMP kinase enzyme (ng/mg)					
7	4.7^{a}	3.74^{b}	3.66^{b}	0.111	L-$p < 0.01$, Q-$p < 0.01$
14	4.88^{a}	3.29^{b}	2.71^{c}	0.110	L-$p < 0.01$, Q-$p < 0.01$
21	5.85^{a}	3.01^{b}	2.57^{c}	0.121	L-$p < 0.01$, Q-$p < 0.01$
28	5.04^{a}	3.25^{b}	2.44^{c}	0.154	L-$p < 0.01$, Q-$p < 0.02$

Note: Values are represented as Mean ± SEM. 7-28 days represents 18–21 weeks of age of Nicobari chicken. In a row, values with different superscripts are significantly different from each other at least at $p < 0.05$. C-Control (0 g/kg), T1-Treatment 1 (700 mg/kg), T2-Treatment 2 (1.4 g/kg). $N = 6$. Components of Contrast—Linear and Quadratic.

Abbreviation: SEM, Standard Error of the Mean.

and then the trend became quadratically significant at 28 days with 700 g being most effective dose. Leptin exerts its effect by binding to a receptor which belongs to the cytokine receptor superfamily (Newman & Gonzalez-Perez, 2013). In pigeons, it has been reported that, heat stress elevates plasma leptin (Al-Azraqi, 2008). Furthermore, its expression at the level of the ovary (Niu et al., 2019**)** suggests that Leptin might directly act on the ovary to regulate chicken reproductive function. A higher concentration of plasma Leptin in the control group might have caused a decrease in the percentage of egg production (Lei et al., 2014).

The concentration of plasma GH was significantly different ($p < 0.05$) at 7 and 14 days. The concentration being greater in the control group. However, at 21 and 28 days the concentration of GH was not significantly different between the groups, during the experimental tenure. This indicates that the concentration of GH was greater in the C group in the initial 14 days, but later on, the plasma levels of treatment groups increased and were not significantly different from control group, which indicates that the effect of FYC was not present after 14 days (Table 3). Furthermore, the supplementation of FYC decreased the level of plasma Leptin and GH in the T1 and T2 groups which was dose dependent (Table 3). Polynomial contrast analysis indicated that 1.4 g/kg dose showed linear effect on decreasing ($p < 0.01$) plasma levels of GH. GH affects the whole body at the tissue level and has a regulatory role in the growth and development of animals (Luna et al., 2014). When GH binds to its receptor, it further controls the functions of other organs and tissues. GH also regulates lipid metabolism in the adipose tissue and liver, which is referred to as hepatic metabolism (Waters et al., 2012). Previous studies reported that buffaloes sampled in hot humid conditions had a higher concentration of GH than those sampled in winter (Haque et al., 2018). The supplementation might have acclimatized the birds to heat stress which might have led to a decrease in the concentration of plasma GH. The results on hormones indicate that supplementation of FYC decreased the level of hormones when compared with the control group. The higher concentration of Leptin and GH hormones was not the same as that observed in the PD 3 line (Anand Laxmi et al., 2017, 2019). Overall, the response of Nicobari chickens to heat stress was less. This may be because it is a native bird. The supplementation might have acclimatized the birds to heat stress which might have led to decrease in concentration of plasma GH. In the present study, it was observed that in the control group even when the level of circulatory hormones during summer was more, there was not significant difference on feed intake between the groups. Similar reports are available in layers (Mahdavi et al., 2005).

The results on the concentration of plasma MDA of the C group at 7 days were significantly more ($p < 0.01$) from the concentrations observed for the T1 and T2 groups, but the difference was not significant between the two latter groups. The lower dose of FYC (700 mg/kg) was more efficient in decreasing the level of MDA although the difference between the T1 and T2 groups was not significant. The results were the same at 14 days. However, at 14 days, a higher dose (T2) was more effective ($p < 0.05$) in decreasing the plasma level of MDA. At day 21, the concentration of plasma MDA in the C group was significantly different from the concentration observed of the T2 group, but the difference was not significant when compared to T1. A higher dose of FYC supplement was more effective in decreasing the concentration of plasma MDA. But at 28 days, the concentrations of plasma MDA in both groups were not different from each other which indicated that in the C group, the plasma MDA concentration decreased by the 28 days of the experiment (Table 3). Hence, the supplementation of FYC did not have any additional effect over the C group. Polynomial contrast analysis indicated that higher dose of FYC had a significant ($p < 0.01$) linear effect on plasma MDA levels which were in decreasing trend. Lipid peroxidation is a biomarker of oxidative stress and is clinically employed to investigate the effects of free radicals (Surai et al., 2019). MDA, Protein carbonyl etc. have been used as indicators of oxidation of protein, lipid, DNA and carbohydrate respectively. Heat stress (HS) causes oxidative stress, cellular changes and changes in gene expression levels in an attempt to reduce the harmful effects of ROS (Habashy et al., 2018, 2019). It is known that fermented yeast culture reduces MDA levels (Matur et al., 2011). In addition, heat stress enhances reactive oxygen species production and induces oxidative stress, which can lead to cytotoxicity, decreased vitamin concentrations, changed enzyme activity and the resultant production parameters (Akbarrian et al., 2016) and lipid peroxidation (Estaviz., 2015). The present study also supports the fact that higher ambient temperature increases plasma MDA levels during summer season and it could be decreased upon supplementation of FYC.

The concentration of plasma cholesterol was also greater in the control group which was significantly different ($p < 0.05$) from 14 to 28 days of the experiment during summer when compared between the groups. The concentration was also higher in the control group when compared to either of the treatment groups. The decrease in the plasma cholesterol level was dose dependant. At 7 days, the difference in the concentration of cholesterol between the groups was not significant. However, the concentration was less in the T1 and T2 group when compared to the C group (Table 3). It was reported earlier that a higher ambient temperature increases the concentration of plasma cholesterol (Ismail et al., 2013). The results of the previous work indicate that the component of yeast inhibits synthesis or lowers cholesterol levels in broilers (Shareef & Al-Dabbagh, 2009). Polynomial contrast analysis revealed that till 14 days of treatment, higher dose (T2) of FYC had a significant ($p < 0.01$) linear effect, in decreasing levels of plasma cholesterol which persisted at 28 days also.

The concentration of liver AMP kinase enzyme was significantly more in the control group ($p < 0.05$) when compared to either of the treatment groups (T1 and T2) throughout the period of the study. However, the difference in the concentration of the enzyme was not significant when compared between the treatment groups at 7 days. Yet from 14 to 28 days, the higher dose T2 was more efficient in decreasing the level of liver AMP kinase (Table 3). By polynomial contrast analyses, it was observed that FYC (1.4 g) dose had linear effect ($p < 0.01$) in decreasing liver AMP kinase enzyme levels at different

day intervals. This indicates that the supplementation of FYC reduced the requirement of the AMP kinase enzyme in the treatment groups. The concentration of the liver AMP kinase requirement was less with supplementation of a higher dose (T2) of FYC and indicated that there was sufficient energy in the T2 group of birds (Table 3). AMPK is a serine/threonine kinase composed of a catalytic alpha and regulatory beta and gamma subunits (Hardie, 2011). In situations of high ATP use, the AMP levels increase and activate AMPK (Kahn et al., 2005; Violet, 2017) by promoting net phosphorylation of the α subunit, and also by allosteric activation of phosphorylated AMPK (Sanders et al., 2007; Winder & Thomson, 2007). AMPK regulates many metabolic and physiological processes, and activity is increased during inflammation (Jeon et al., 2016; Mihaylova & Shaw, 2011). A greater amount of AMP kinase in the liver homogenate of the control group when compared to the supplemented groups indicates low ATP levels, stressed and inflammatory liver tissue. The concentration of AMP kinase in the tissue was inversely related to the dose of FYC which indicated a lesser requirement of ATP in the supplemented groups.

During summer at 14 days, the difference in the bodyweights between the treatment (either T1 or T2) and control groups was significant ($p < 0.05$), greater for the treatment (T1), whereas the difference in the bodyweights between the treatment (T1 and T2) groups was not significant, which indicated a beneficial effect of a lower dose (T1) of supplementation of the yeast culture on bodyweight (Table 4). The difference in body weights at 28 days revealed that the C group body weight was significantly less when compared to the T1 and T2 ($p < 0.05$) groups. The body weight of the T1 group was higher ($p < 0.05$) than the T2 group (Table 4). The lower dose (T1) had a greater beneficial effect in increasing the body weight during summer. Polynomial contrast analyses showed significant ($p < 0.01$) quadratic effect on body weight with supplementation of FYC. A dose level higher than 700 g/kg tended to reduce performance.

During the post summer period, the age of the birds ranged from 24 to 32 weeks. At 24 weeks, the body weight of the T1 group was more ($p < 0.05$) compared to the C and T2 group. The body weight of the T2 group was more ($p < 0.05$) when compared with the C group. The lower dose given for the T1 group was more effective in increasing the body weight when compared to the C and T2 group. At 26 weeks, the body weight of the T1 group was still higher ($p < 0.05$) than the C or T2 group. The difference in the body weights of the C and T2 groups was not significant (Table 5). At 28 weeks, the trend in increase in body weight of the three groups was T1 > T2 > C and the difference was significant ($p < 0.05$). At 30 and 32 weeks, trend for body weights was C,T2 < T1. T1 dose was observed to be more efficient than the T2 dose in increasing body weight. The difference in the body weights between T1 and C as well as T1 and T2 groups was significant ($p < 0.05$), whereas the difference observed between the C and T2 groups was not significant (Table 5). Polynomial contrast analyses showed significant ($p < 0.01$) quadratic effect on body weight with supplementation of FYC. A dose level higher than 700 g/kg tended to reduce performance (Table 5). Beneficial effects upon supplementation of yeast culture have already been reported. (Yalcin et al., 2010; Zhang et al., 2020).

The difference in the quantity of the feed intake between the groups was not significant until 21 days. At 28 days, it was observed that FI in groups supplemented with T1 ($p < 0.05$) and T2 ($p < 0.05$) was more when compared to the C group (Table 6). This may be due to a decrease in temperature from the initiation of the experiment. FCR with respect to body weight was significantly more for the control group (5.95) when compared to the T1 (4.85) and T2 groups (5.00). Upon contrast analyses, it was observed that FYC exerted linear ($p < 0.05$) effect on feed intake with higher dose of FYC being more effective.

However, during post summer, supplementation until 24 weeks decreased ($p < 0.05$) the feed intake of birds of the T1 and T2 groups when compared with the C group. Overall, the difference between the T1 and T2 groups was not significant. Similar results were also obtained at 26th week. At 28 weeks, the FI of all the three groups when compared were significantly different ($p < 0.05$) from each other (C > T1 > T2). At 30 weeks, the FI of the C group was significantly different from T1 and T2, but the difference observed between T1 and T2 was not significant. At 32 weeks, similar results were obtained (Table 7). Contrast analyses revealed that during post summer period also, linear effect ($p < 0.01$) was observed with higher dose of FYC exerting decrease in FI (Table 7).

The difference in the number of eggs laid in terms of eggs/hen/day when compared between the groups was also more for treatment groups. A dose of 700 mg (T1) was more effective in increasing the percentage of number of eggs when compared to T2 and control group. The difference between the two later groups was not significant (Table 8). The same results were observed for egg weights (T1 > C,T2; $p < 0.05$) at 29 and 31 weeks of age (Table 8). But at 32 weeks of age, the egg weights were significantly different

TABLE 4 Body weight (g) of chickens during summer season

Days	C	T1	T2	SEM	*p*-Value	Contrast (*p*-Value)
0	1074.10[a]	1080.73[a]	1100.40[a]	11.64	ns	L-ns, Q-ns
14	1118.09[a]	1168.40[b]	1199.04[b]	8.305	$p < 0.05$	L-ns, Q-$p < 0.01$
28	1194.44[a]	1298.86[b]	1234.38[c]	12.47	$p < 0.05$	L-$p < 0.03$, Q-$p < 0.01$

Note: Values are represented as Mean ± SE. 7-28 days represents 18–21 weeks of age of nicobari chicken. In a row, values with different superscripts are significantly different from each other. C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg). *N* = 10. Components of Contrast—Linear and Quadratic.

Abbreviation: SEM, Standard Error of the Mean.

TABLE 5 Body weight (g) of chickens during post summer season

Weeks	C	T1	T2	SEM	*p*-Value	Contrast (*p*-Value)
24	1248.34[a]	1310.97[b]	1271.37[a]	7.83	$p < 0.05$	L-$p < 0.047$, Q-$p < 0.01$
26	1250.08[a]	1398[b]	1300.42[c]	9.85	$p < 0.05$	L-ns, Q-$p < 0.01$
28	1308.92[a]	1436.76[b]	1353.03[c]	10.27	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.01$
30	1343.86[a]	1444.88[b]	1384.14[a]	10.62	$p < 0.05$	L-$p < 0.012$, Q-$p < 0.01$
32	1394.73[a]	1517.14[b]	1416.37[a]	10.43	$p < 0.05$	L-ns, Q-$p < 0.01$

Note: Values are represented as Mean ± SE. 24–32 weeks represents age of the nicobari chickens. In a row, values with different superscripts are significantly different from each other. C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg). *N* = 10. Components of Contrast—Linear and Quadratic.

Abbreviation: SEM, Standard Error of the Mean.

TABLE 6 Feed intake (g) of chickens during summer season

Days	C	T1	T2	SEM	*p*-Value	Contrast (*p*-Value)
7	55.28[a]	55.41[a]	56.55[a]	0.735	ns	L-ns, Q-ns
14	56.31[a]	55.83[a]	56.91[a]	0.560	ns	L-ns, Q-ns
21	57.46[a]	56.85[a]	55.98[a]	0.490	$p < 0.05$	L-$p < 0.043$, Q-ns
28	60.49[a]	63.49[b]	62.51[c]	0.811	$p < 0.05$	L-$p < 0.01$, Q-ns

Note: Values are represented as Mean ± SE. 7–28 days represents 18–21 weeks of age of nicobari chicken. In a row, values with different superscripts are significantly different from each other. C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg). *N* = 10. Components of Contrast—Linear and Quadratic.

Abbreviation: SEM, Standard Error of the Mean.

TABLE 7 Feed intake (g) of chickens during post summer season

Weeks	C	T1	T2	SEM	*p*-Value	Contrast (*p*-Value)
24	61.42[a]	58.42[b]	58.30[b]	0.501	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.026$
26	62.32[a]	57.82[b]	57.22[b]	0.589	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.012$
28	62.12[a]	56.95[b]	58.01[c]	0.356	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.01$
30	69.77[a]	60.00[b]	61.87[b]	0.735	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.01$
32	72.44[a]	65.72[b]	63.06[b]	0.541	$p < 0.05$	L-$p < 0.01$, Q-$p < 0.01$

Note: Values are represented as Mean ± SE. 24–32 weeks represents age of the nicobari chickens. In a row, values with different superscripts are significantly different from each other. C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg). *N* = 10. Components of Contrast—Linear and Quadratic.

Abbreviation: SEM, Standard Error of the Mean.

TABLE 8 Egg production and egg weight during post summer period (laying period)

	C		T1		T2		SEM	
Weeks	Egg production (%)	Egg weight (g)	Egg production (%)	Egg weight (g)	Egg production (%)	Egg weight (g)	Egg production (%)	Egg weight (g)
26	28.84[a]	–	38.99[b]	–	29.99[a]	–	0.56	–
27	44.02[a]	–	48.23[b]	–	46.23[c]	–	0.45	–
28	54.28[a]	40.80[a]	56.56[b]	40.72[a]	54.23[a]	40.72[a]	0.48	0.270
29	55.85[a]	40.23[a]	58.23[b]	42.88[b]	56.42[a]	42.88[b]	0.58	0.391
30	55.98[a]	40.47[a]	61.98[b]	40.77[a]	58.23[c]	40.77[a]	0.52	0.302
31	60.31[a]	40.84[a]	64.88[b]	42.24[b]	58.20[c]	42.24[b]	0.61	0.478
32	59.02[a]	41.99[a]	63.87[b]	43.05[b]	60.04[a]	43.05[b]	0.60	0.605

Note: Age of nicobari chickens was 24–32 weeks. In a row, values with different superscripts are significantly different from each other ($p < 0.05$). C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg). *N* = 20.

Abbreviation: SEM, Standard Error of the Mean.

($p < 0.05$) when compared between T1 and T2 groups and also when compared with control (C < T2 < T1) (Table 8). The age at sexual maturity was greater for the control (160 days) and T2 group (168 days) when compared to the T1 group (152 days). A quadratic effect ($p < 0.01$) of FYC was observed on number (percentage) and weight of eggs. The higher dose (T2) tended to reduce the performance. Zhang et al., (2020), Yalcin et al., (2010) and Blount (2016) reported an improvement in feed efficiency and egg components quality with supplementation of the yeast culture (YC). The hatchability and fertility of the eggs were also observed to be greater for the T1 group when compared to the T2 and for the eggs collected during 27–29 weeks of age. The same was observed for results on fertility for eggs collected during 30–32 weeks of age, without any significant difference between the groups for the hatchability parameter (Table 9). Similar results with respect to egg production were reported by Kidd et al., (2013).

Histopathological studies of the jejunum of the control group revealed mild (+) (60%) and medium (++) (20%) necrosis during summer stress conditions, and in the remaining (20%), necrosis of villi was not observed or was normal. The villi of the jejunum of the T1 and T2 group of the chickens was normal (80%) while in the rest (20%), a mild necrosis condition (+) was observed (Figure 1). YC is known to increase the availability of beneficial compounds and decrease the availability of stress factors (Giannenas et al., 2018) in the gut. The severity of necrosis being greater in the control group might have affected digestion and the absorption of nutrients and subsequently affected production. A beneficial effect of yeast products on gut function in broilers has also been reported by Muthusamy et al., (2011). Yeast products affect nutrient digestibility (Shin et al., 2005) and intestinal mucosal development (Song et al., 2014). A previous study showed that heat stress-related effects in the jejunum were prevented in chickens fed a diet containing galacto oligosaccharides (Varasteh et al., 2015). It has also been reported that hyperthermia resulted in increased permeability and marked intestinal epithelial damage (Tabler et al., 2020). Hence, supplementation of FYC was observed to be beneficial in reducing the severity of necrosis which might have helped in better absorption of nutrients and increase in production performance.

TABLE 9 Effect of summer season on fertility and hatchability trait of eggs (post summer season) for different groups

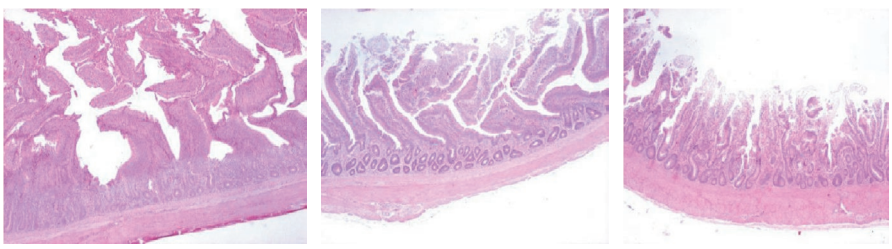
	C	T1	T2	SEM	*p*-Value
Fertility (%) (28–29 weeks)	75.69[a]	80.23[b]	77.56[a]	0.75	$p < 0.05$
Hatchability (FES) (%)	89.99[a]	92.87[b]	90.21[a]	0.68	$p < 0.05$
Fertility (%) (30–32 weeks)	79.23[a]	83.98[b]	80.31[a]	0.58	$p < 0.05$
Hatchability (FES) (%)	91.02[a]	92.56[a]	90.91[a]	0.43	ns

Note: Age of the nicobari chickens was 28–32 weeks. In a row, values with different superscripts are significantly different from each other. C—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg).

Abbreviations: %, percentage; SEM, Pooled Standard Error of the Mean.

The PCR products obtained after amplification with respective primers for Leptin, GH and MMP3 are provided in Figure 2. In the supplemented group of Nicobari birds, the receptors for Leptin and GH were upregulated in the brain only at 20 days interval in relation to the lower concentration of respective circulatory hormones (Figures 3 and 4). It is reported that reduction in hormone levels may be to influence metabolism and neuroendocrine functions that will save energy during exposure to stress (Khan et al., 2012) and upregulation of receptors indicate greater sensitivity or synthesis (Arbuthnot & Munoz, 2010) for maintaining the homeostasis of the functions in the body. In the present experiment also, the similar results were observed in the yeast supplemented group. The expression patterns of leptin, GH and their receptors have been reported in different tissues of chicken including brain (Harvey et al., 2014; Serrousi et al., 2016). Hence, autocrine function of leptin and GH also cannot be ruled out.

Downregulation of Leptin and GH receptors in the control group indicates less activity of hormones at the receptors in the control group. This may be due to refractoriness and regulation of receptors in presence of higher concentration of circulatory hormones, which was also reported by Brooks and Waters (2010) and Arbuthnott and Garcia-Munoz (2010). Hence, it might have not affected feed intake during the summer season, but in the post summer season, it increased feed intake in the control group when compared with the supplemented group. However, gene expression studies were also restricted for the summer period only. In other tissues liver and magnum, supplementation of FYC did not result in any significant effect on the expression of these receptors when compared to the control group (Figures 3 and 4). However, gene expression studies for receptors were not conducted for the post summer period. MMPs are the enzymes that break down



(a) Normal **(b)** Mild **(c)** Medium

FIGURE 1 Histomorphology of the jejunum exhibiting (a) Normal villi (b) mild (+) and (c) medium (++) necrosis. Magnification—400×

ECM and connective tissues to facilitate tissue remodelling (Loffek et al., 2011; Wong et al., 2016). It was observed that higher concentration of all the plasma hormones caused higher expression of MMP3 gene in the magnum of the control group when compared to the T1 and T2 groups (Figure 5). However, egg production was less in the control group and indicated less beneficial effect. MMPs synthesis is regulated primarily at the transcriptional level but also at post-transcriptional level (Yan & Boyd, 2007). Although MMPs are known to be involved in cell biological processes and organ development, this can also lead to inflammatory conditions under stressful conditions. In inflammatory disorders and upon activation by ROS, MMP3 is over expressed (Manka et al., 2019). In the present study, higher MDA levels in the control group might have also led to a higher expression ($p < 0.05$) of the MMP 3 gene in magnum tissue of the control group. Similar studies were conducted in PD3 chickens, from the lineage of Dehlam Red (Anand Laxmi et al., 2017; Anand Laxmi et al., 2017) during the summer season where supplementation of FYC modulated the expression of the genes or this breed was more responsive to summer stress. Nicobari breed is a native breed of Andaman, India and is well adapted to the tropics. However, the effect of higher ambient temperature on hormones, receptors and metabolites has not been reported. Hence, we wanted to observe the response of plasma hormones, metabolites, the energy sensor AMPK and hormone receptors during summer season. Local breeds contain inherited characteristics pertinent to their particular environments (Hoffmann, 2013). For the first time, expression of Leptin and GH receptors in the liver and magnum portion of the reproductive tract of Nicobari chickens is being reported. Until now, no one has conducted studies on the level of hormones and their receptors in this breed during the summer season and evaluated the role of feed supplement in alleviating heat stress. Besides, by altering the management conditions, nutritional

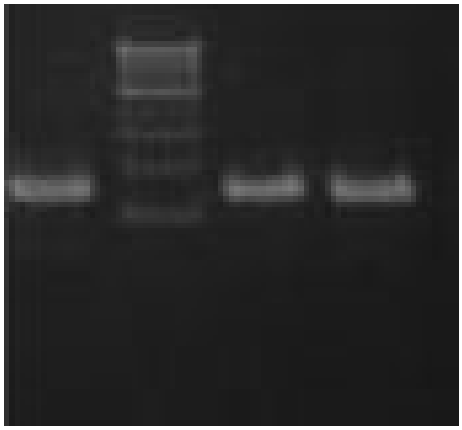


FIGURE 2 PCR products obtained on 1.8% Agarose gel (1) Leptin R, (2) GH R (3) MMP3. PCR product size (Table 2)

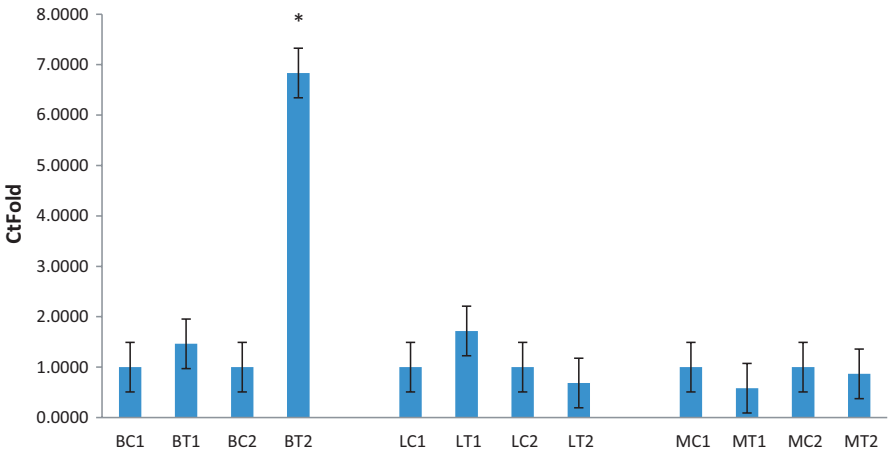


FIGURE 3 Relative gene expression of leptin receptor in B—Brain, L—Liver and M—magnum of Nicobari chicken post 20 days of the experiment during summer season. *$p < 0.01$. $N = 5$, C1 & 2—Control (0 g/kg), T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg)

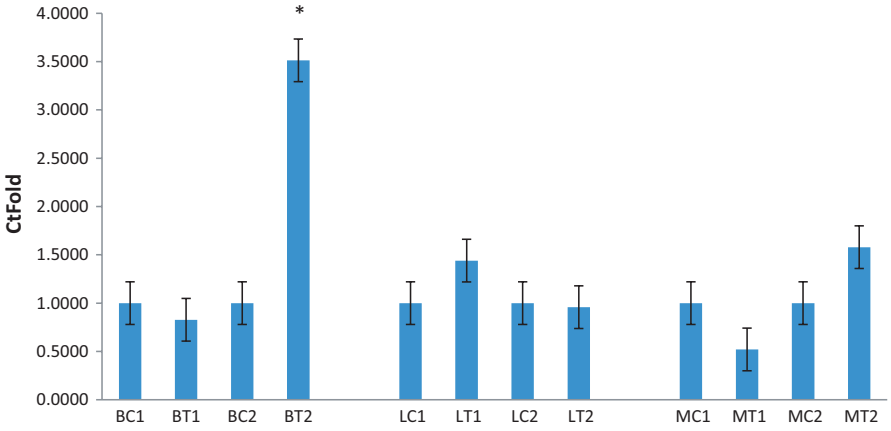


FIGURE 4 Relative gene expression of Growth hormone receptor in B—Brain, L—Liver and M—magnum of Nicobari chicken post 20 days of the experiment during summer season.*$p < 0.05$. $N = 5$, C1 & 2—Control (0 g/kg),T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg)

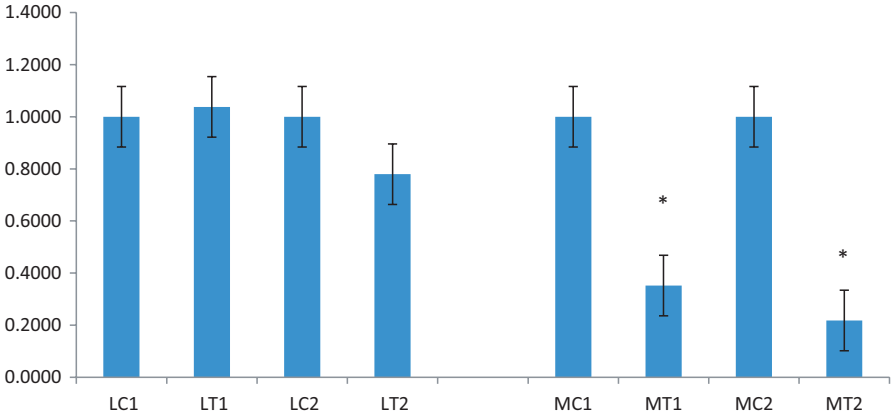


FIGURE 5 Relative gene expression of MMP3 gene in L—liver and M—magnum of Nicobari chicken post 20 days of the experiment during summer season. *$p < 0.05$, $N = 5$, C1 & 2—Control (0 g/kg),T1—Treatment 1 (700 mg/kg), T2—Treatment 2 (1.4 g/kg)

strategies were applied to reduce the stress levels experienced by chickens under higher ambient temperature conditions prevailing during the summer by the addition of salts, minerals or yeast cultures (Lara & Rostagno, 2013). The supplementation of a lower dose of YC (700 mg) was observed to be more beneficial than the dose of 1.4 g supplementation in Nicobari chickens during the summer. In conclusion, exposure to higher ambient temperature during summer impaired the FI and growth performance of layers and these negative effects were related with the greater secretion of leptin and GH hormones and severity of necrosis of the villi in jejunum. It also caused downregulation of respective hormone receptors in brain and up regulation of MMP3 gene in the magnum. These were negated by supplementation of FYC.

This is the first study to show that supplementation of fermented yeast culture to Nicobari chickens during and post summer ameliorated heat stress, by improving production through modulation of mentioned parameters.

ACKNOWLEDGEMENTS

We would like to acknowledge the financial assistance from ICAR-New Delhi for carrying out the research. We would also like to acknowledge Mr. RamaSubbaiah, MSc, assistance in carrying out different experiments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. IAEC No. IAEC/DPR/4/2016.

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How to cite this article: Nidamanuri, A. L., Prince, L. L. L., Mahapatra, R. K., & Murugesan, S. (2021). Effect on physiological and production parameters upon supplementation of fermented yeast culture to Nicobari chickens during and post summer. *Journal of Animal Physiology and Animal Nutrition*, 00, 1–12. https://doi.org/10.1111/jpn.13579