Journal: The Journal of Applied Poultry Research
Article doi: 10.3382/japr/pfz018
Article title: Relationship Between Plasma GH, Metabolites, Lipogenic Genes, and MMP3 Expression in PD3 Chicken Line and Role of Fermented Yeast Culture in Alleviating Heat Stress
First Author: Nidamanuri A. Laxmi
Corr. Author: Nidamanuri A. Laxmi

INSTRUCTIONS

We encourage you to use Adobe's editing tools (please see the next page for instructions). If this is not possible, please list clearly in an e-mail. Please do not send corrections as track changed Word documents.

Changes should be corrections of typographical errors only. Changes that contradict journal style will not be made.

These proofs are for checking purposes only. They should not be considered as final publication format. The proof must not be used for any other purpose. In particular we request that you: do not post them on your personal/institutional web site, and do not print and distribute multiple copies. Neither excerpts nor all of the article should be included in other publications written or edited by yourself until the final version has been published and the full citation details are available. You will be sent these when the article is published.

1. **Licence to Publish:** Oxford Journals requires your agreement before publishing your article. If you haven't already completed this, please sign in with your My Account information and complete the online licence form. Details on how to do this can be found in the Welcome to Oxford Journals email.
2. **Permissions: Permission to reproduce any third party material in your paper should have been obtained prior to acceptance. If your paper contains figures or text that require permission to reproduce, please inform me immediately by email.**
3. **Author groups:** Please check that all names have been spelled correctly and appear in the correct order. Please also check that all initials are present. Please check that the author surnames (family name) have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors' full surnames and forenames are tagged correctly, for accurate indexing online.
4. **Figures:** If applicable, figures have been placed as close as possible to their first citation. Please check that they are complete and that the correct figure legend is present. Figures in the proof are low resolution versions that will be replaced with high resolution versions when the journal is printed.
5. **Missing elements:** Please check that the text is complete and that all figures, tables and their legends are included.
6. **Special characters and equations:** Please check that special characters, equations and units have been reproduced accurately.
7. **URLs:** Please check that all web addresses cited in the text, footnotes and reference list are up-to-date.
8. **Funding:** If applicable, any funding used while completing this work should be highlighted in the Acknowledgements section. Please ensure that you use the full official name of the funding body.
9. **Manuscript tagging:** The information in the table that appears before your manuscript contains the manuscript information that will be captured in the tagging online. Please check that this information is accurate as it cannot be changed after publication. If any of the information is incorrect, please mark the changes onto the table.

AUTHOR QUERIES - TO BE ANSWERED BY THE CORRESPONDING AUTHOR

The following queries have arisen during the typesetting of your manuscript. Please answer these queries by marking the required corrections at the appropriate point in the text.

Query No.	Nature of Query	Author's Response
Q1	Author: Please check that all names have been spelled correctly and appear in the correct order. Please also check that all initials are present. Please check that the author surnames (family name) have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors full surnames and forenames are tagged correctly, for accurate indexing online. Please also check all author affiliations.	
Q2	Author: Please provide significance of 1 and 2 in table body.	
Q3	Author: Please provide significance of 1 and 2 in table body.	
Q4	Author: Please provide significance of 1 and 2 in table body.	
Q5	Author: Please provide significance of 1 and 2 in table body.	

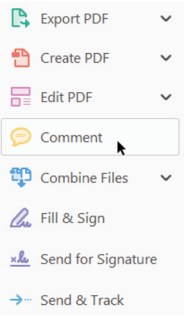
MAKING CORRECTIONS TO YOUR PROOF

These instructions show you how to mark changes or add notes to your proofs using Adobe Acrobat Professional versions 7 and onwards, or Adobe Reader DC. To check what version you are using go to **Help** then **About**. The latest version of Adobe Reader is available for free from get.adobe.com/reader.

DISPLAYING THE TOOLBARS

Adobe Reader DC

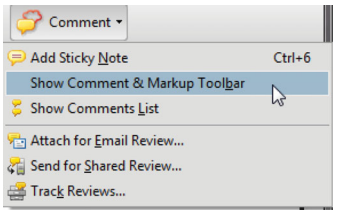
In Adobe Reader DC, the Comment toolbar can be found by clicking 'Comment' in the menu on the right-hand side of the page (shown below).



The toolbar shown below will then display along the top.

Acrobat Professional 7, 8, and 9

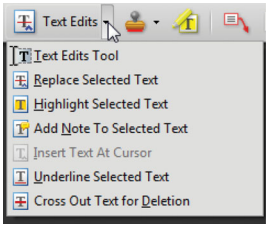
In Adobe Professional, the Comment toolbar can be found by clicking 'Comment(s)' in the top toolbar, and then clicking 'Show Comment & Markup Toolbar' (shown below).



The toolbar shown below will then be displayed along the top.

USING TEXT EDITS AND COMMENTS IN ACROBAT

This is the quickest, simplest and easiest method both to make corrections, and for your corrections to be transferred and checked.



1. Click **Text Edits**
2. Select the text to be annotated or place your cursor at the insertion point and start typing.
3. Click the **Text Edits** drop down arrow and select the required action.

You can also right click on selected text for a range of commenting options, or add sticky notes.

SAVING COMMENTS

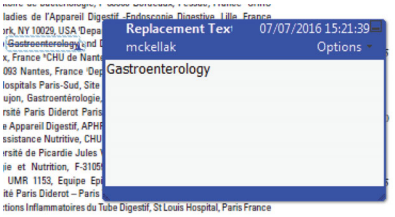
In order to save your comments and notes, you need to save the file (**File, Save**) when you close the document.

USING COMMENTING TOOLS IN ADOBE READER

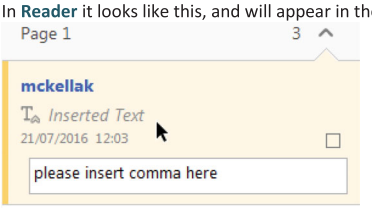
All commenting tools are displayed in the toolbar. You cannot use text edits, however you can still use highlighter, sticky notes, and a variety of insert/replace text options.

POP-UP NOTES

In both Reader and Acrobat, when you insert or edit text a pop-up box will appear. In **Acrobat** it looks like this:



In **Reader** it looks like this, and will appear in the right-hand pane:



DO NOT MAKE ANY EDITS DIRECTLY INTO THE TEXT, USE COMMENTING TOOLS ONLY.

Element	Included in this paper
Manuscript category	Research Report
Funder ID	None
Orcid IDs	None
Contributors	Nidamanuri A. Laxmi, Leslie L. Prince, Ramu Subbiah, Ram K. Mahapatra
Supplementary data files cited	None
Related article(s)	
Corresponding author	Nidamanuri A. Laxmi
Title	Relationship Between Plasma GH, Metabolites, Lipogenic Genes, and MMP3 Expression in PD3 Chicken Line and Role of Fermented Yeast Culture in Alleviating Heat Stress
Keywords	chicken, plasma GH, metabolites, gene expression, yeast culture, MMP3 expression
Collection ID	

Relationship Between Plasma GH, Metabolites, Lipogenic Genes, and MMP3 Expression in PD3 Chicken Line and Role of Fermented Yeast Culture in Alleviating Heat Stress

Nidamanuri A. Laxmi,[1] Leslie L. Prince, Ramu Subbiah, and Ram K. Mahapatra

Directorate of Poultry Research, Rajendranagar, Hyderabad-500030, India

Primary Audience: Animal Science Faculty, Poultry Farmers, Poultry Researchers, Veterinarians

SUMMARY

The present experiment aimed to observe the effect of high ambient temperature during the summer season (April–June) on growth hormone (GH), metabolites, expression of lipogenic, and matrix metalloproteinase (MMP3) genes in different tissues of PD3 chicken line during pre-laying period and their effect on egg production in the post-summer period. We hypothesized that supplementing fermented yeast culture (FYC) to chickens during summer may alleviate heat stress by affecting GH, metabolites, and gene expression levels. Three groups of birds (PD3 line) were considered with an average body weight 900 g at 16 wk. One group served as control which did not receive any dose of supplement (FYC), and the other two groups were supplemented with FYC at the rate of 0.5 g/kg and 1.25 g/kg of feed, respectively, during and post-summer till 32 wk of age. Blood was collected at biweekly intervals, during summer period only. The plasma was separated and stored for estimation of hormone and metabolites. Relative gene expression study was conducted for liver and magnum portion of the reproductive tract. Our results showed that the concentration of GH was significantly ($P < 0.05$) higher in the control group compared to treatment groups. Expression of SCD, FAS lipogenic genes, and MMP3 genes was higher in tissues of control ($P < 0.05$) compared to the treatment groups during summer period. The results indicated that high ambient temperature increased the concentration of GH, cholesterol, MDA, expression of lipogenic, and MMP3 genes in different tissues of chickens during summer (pre-laying) period and supplementation of FYC decreased the level of hormones, metabolites, and gene expression. Therefore, the supplement reduced the effect of heat stress and increased egg production during laying period coinciding with post summer period.

Key words: chicken, plasma GH, metabolites, gene expression, yeast culture, MMP3 expression

2019 J. Appl. Poult. Res. 0:1–10
http://dx.doi.org/10.3382/japr/pfz018

DESCRIPTION OF PROBLEM

Physiological stress causes deleterious effects on the production performance of poultry [1, 2]. Poultry growth performance is not only inherited but also greatly affected by the change in the environmental conditions [3]. Heat is the main source for oxidative stress in domestic birds that leads to impairment in biological, metabolic, and endocrine systems, health disorders, and

[1]Corresponding author: antianand@gmail.com

lower production rates, which ultimately leads to economic loss [4]. Growth hormone (GH) is synthesized and released from the somatotroph cells of the anterior pituitary gland and is involved in a variety of biological processes. Previous research reported that extra-pituitary GH is expressed in the ovaries of Hy-Line hens during growing period (between 10–16 wk of age) and also at the onset of egg laying stage [5]. An earlier study [6] reported that GH might have local autocrine and paracrine functions. Moreover, GH affects the whole body as well as at the tissue level and has a regulatory role in growth and development of animals [7, 8]. When GH binds to its receptor, it further controls the functions of other organs and tissues. GH also regulates lipid metabolism in adipose tissue and liver, which is referred to as hepatic metabolism [9, 10].

Changes in secretion and/or metabolism of hypothalamic neurotransmitters increase the secretion of GH by increasing the secretion of GHRH or by decreasing the secretion of somatostatin [11]. In a previous study, no difference was observed in GH or T4 concentration among the fast and slow-growing group of birds [12]. A previous study reported that in case of 28 d old black boned chickens, the plasma level of GH decreased under heat stress when compared to chicken exposed to thermo neutral conditions. A similar finding was reported by another study stating the adverse effect of heat stress on expression of growth-related genes, and also the GH receptor in case of quails [13].

The concentration of malondialdehyde (MDA) in blood and tissues is used as biomarkers of lipid peroxidation [14, 15, 16]. Significant increases in plasma MDA occurred in heat stressed layers [17].

Expression of Fatty Acyl Synthase (FAS) and Stearoyl CoA Desaturase (SCD) genes regulate lipogenesis. SCD is ubiquitously expressed in chicken tissues with highest levels marked in proventriculus followed by ovary, hypothalamus, kidney, liver, and adipose tissue in the female chickens [18]. Stromelysin-1 (MMP-3) and Stromelysin-2 (MMP-10) showed the same structural design as collagenases that could degrade many different extracellular matrix components [19, 20]. Matrix metalloproteinases (MMPs) are primarily known for their ability to degrade extracellular matrix, but they can also degrade the non-matrix proteins [21].

Previous research showed that β-glucan, a major component of yeast cell wall, inhibited the expression of intestinal genes associated with cholesterol and fatty acid synthesis [22]. Further based on that, treatment for lowering cholesterol levels in the body can involve the use of β-glucan, i.e., a polysaccharide derived from wheat, seaweed, fungi, and yeast [23].

Hence, the present study aimed at studying the plasma levels of different parameters and relationship between GH, metabolites and expression of lipogenic, and MMP3 genes in different tissues of chickens along with supplementation of fermented yeast culture (FYC) during and post-summer season to determine effects of heat stress. If supplementation of FYC proves to be beneficial in negating heat stress effects, it can be given as a supplement to chickens during summer season.

MATERIALS AND METHODS

Before conducting the present study, approval was taken from the Institutional Animal Ethics committee for techniques involved. The experiment was conducted at poultry farm situated in ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad, India. The study was conducted in summer season during months of April May and June which is characterized by gradual rise in the daily temperatures, with maximum temperature around 37°C throughout the month that may reach 41°C or drop below 34°C only 1 d in 10. The month of June is characterized by a rapid decrease in daily high temperatures, with maximum temperature ranging between 37°C to 31°C throughout the month, exceeding 38°C or dropping below 29°C only 1 d in 10 [24]. Summer period was from last week of April till last week of June. For studies on egg production parameters, post summer period extended till last week of August when the temperature ranged between 29°C–32°C. The relative humidity throughout the experimental period ranged between 46%–70%. Temperature and humidity in the shed were recorded daily

Table A. Composition of Feed (in Percentage) for Layers.

Components of diet	Layer grower	Layer breeder
Maize	56.05	61.39
Soybean 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
Choline chloride	0.1	0
Toxin binder	0.1	0
Tylosin	0.05	0
Coccidostat	0.05	0

with digital environmental thermometer. A total of 150 PD3 line chickens from the lineage of Dahlem Red breed, aged 16 wk, were selected for the current study.

Grouping and Management of Chickens

The chickens were divided into 3 groups, one served as control, and the other 2 served as treatment/supplemented groups. Each treatment had 10 replicates with 5 chickens in each replicate. Chickens with uniform body weight ranging between 850–950 g, at 16 wk of age were considered for the present study. Till 19 wk of age, they were provided with growers feed (Table A), and then, from 20 wk onwards, they were provided with layers feed (Table A). Control group had access to feed and water *ad libitum*. Basal diet was offered to hens as per the composition followed by Panda *et al.* (2012) [22]. The supplemented groups were fed with FYC (*Saccharomyces cerevisiae*) at 2 different concentrations 0.5 g/kg (T1) and 1.25 g/kg feed (T2) in addition to basal diet (based on maize and soy feed). The summer period was considered from last week of April till last week of June (8 wk). The birds attained 23 wk of age only by the end of summer period (8 wk). Hence, the study on egg production parameters continued in the post-summer period, coinciding with the laying period, which lasted until the chickens attained 32 wk of age.

Analysis of Plasma GH and Metabolites

GH in plasma was estimated by competitive enzyme immunoassay method, using commercial EIA kit (Blue Gene Biotech, Shanghai, China). The assay procedure was based on competitive binding assay with a minimum detection limit of 2.5 ng/ml. The absorbance of the color solution obtained at the end of the reaction was read at 450 nm. The intra and interassay coefficients of variation were less than 6%.

Plasma cholesterol was estimated based on the method of Zlatkis *et al.* (1953) [23]. EZassay^{TM} TBARS estimation kit was used for estimation of MDA (lipid oxidation)[d]. The assay is based on the reaction of MDA with a chromogenic agent, thiobarbituric acid (TBA), at high temperature and acidic conditions to form a color complex whose absorption was recorded at 532 nm.

Gene Expression Studies

For gene expression studies, liver and magnum portion of the reproductive tract were excised from 5 birds from all the three groups during summer period. The tissues were homogenized, and RNA was extracted with Trizol (InCell Technologies, Hyderabad, INDIA). The purity and integrity of RNA were analyzed by estimating the ratio of ODs recorded at 260 and 280 nm, which ranged 1.9–2.0. Further RNA samples were also run on 1.0% agarose gel. The RNA was subjected to cDNA synthesis using first strand cDNA synthesis kit (Thermo Fischer Scientific, Bangalore, INDIA). PCR technique was standardized, subjecting cDNA to amplification with respective Actin, SCD, FAS, and MMP3 primers (Table B). The primers were got synthesized by Chromous Co. Bangalore, India. The PCR products were analyzed by electrophoresis in 2% (wt/vol) agarose gel (Figure 4) using ethidium bromide as the stain. Further qPCR was then performed using a Step One Plus Real-Time PCR System (ABI) with Power SYBR Green PCR Master Mix (Thermo Fischer Scientific, Bangalore, INDIA). Actin gene was kept as the housekeeping gene. PCR cycling conditions included predenaturation at 94 °C for 5 min followed by 40 cycles of denaturation at 94°C for 10 s, annealing at 55°C for 10 s, and extension at 72°C

Table B. Sequence of Primers for Gene Expression Study.

Genes	Sequences
Matrix metalloproteinase 3	5′-TACCAGATGTCCGCTCATACAGCA-3′- FP
	5′-TCACCACTGTAGAGCCTGATGAAC-3′- RP
Fatty acid synthase	5′-GATCTGGAGGCTCGTGTCAATGCT-3′- FP
	5′- GTCTTTGCCCGCATCAGTGTCACG-3′- RP
Stearoyl CoA desaturase	5′-CTCCATGGCCTTCCAGAATGACATC-3′- FP
	5′-TAAATCACTCAGGTCCAGCTTCTG-3′- RP
Actin	5′-GGACTACCTCATGAAGATCCTGAC–3′- FP
	5′– GCCAATGGTGATGACCTGACCATC–3′- RP

FP- forward primer, RP- reverse primer

Gene	Size of the PCR product
1 PCR amplicon of SCD	190 bp
2 PCR amplicon of FAS	176 bp
3 PCR amplicon of MMP3	162 bp
4 PCR amplicon of Actin	160 bp
5 L	DNA ladder

for 10 s and final extension at 72°C for 5 min. Relative mRNA expression of genes in liver and magnum samples was performed in triplicates for MMP3 gene whereas relative gene expression studies of SCD and FAS genes, was performed for liver tissue only and was analyzed based on the $2-\Delta\Delta CT$ method. Statistical analysis of the qPCR data was also performed.

Hypothesis

Summer season negatively affects the production parameters of livestock. The hypothesis states that supplementation of FYC may reduce the adverse effects of heat stress experienced during the summer season via GH, metabolites, expression of MMP3 and SCD, and FAS lipogenic genes. This, in turn, may increase egg production in PD3 layers [25].

RESULTS AND DISCUSSION

Level of plasma GH was compared between the three groups, i.e., Control, T1 (0.5 g), and T2 (1.25 g), for the summer season. It was observed that during summer season, the level of plasma GH was significantly higher ($P < 0.05$) in the control group, when compared to the other 2 groups (Table 1). The Mean±SE rectal temperature of the birds for 8 wk in the T1 and T2 group was 107.2 ± 0.02 and 106 ± 0.02 respectively, whereas that of the control was 107.9 ± 0.03°F.

Mortality rate was 13%, 9%, and 5% in control, T1 and T2 groups, respectively. Rectal temperature and mortality rate was lower for the T2 group when compared to the respective values for T1 and control groups.

Table 1. Concentration of Plasma GH (ng/ml) During Summer Season.

Days	Control	T1	T2
15	$100.49^{a} \pm 0.636$	$97.76^{b} \pm 0.636$	$89.14^{c} \pm 0.636$
30	$119.65^{a} \pm 0.752$	$109.72^{b} \pm 0.752$	$58.74^{c} \pm 0.752$
45	$110.97^{a} \pm 1.09$	$99.51^{b} \pm 1.09$	$59.65^{c} \pm 1.09$

[1]T1 (0.5) and T2 (1.25) are supplemented groups.
[a-c]Mean±SE values with different superscripts are significantly different from each other within a row at $P \leq 0.05$.
[2]Number of birds in each group was 10. (Age of the birds is 18–22 wk).

During summer season, the difference in feed intake and body weight between the groups was not significant (data not shown). On the 15th day, it was observed that the level of plasma cholesterol was significantly greater in control ($P < 0.05$) when compared to the T1 and T2 group. The difference between the T1 and T2 was not significant indicating that difference in the dose of the supplement did not bring out significant difference in the plasma cholesterol level. The concentration of plasma MDA and cholesterol was significantly greater ($P < 0.05$) at 30 and 45 d of the experiment in the control group when compared to the T1 and T2 group (Tables 2 and 3). However, post 15 d at 30 and 45 d, the decrease in the concentration of cholesterol was found to be dose-dependent. It was observed that the concentration of plasma MDA, the lipid oxide parameter, was significantly higher for the

Table 2. Concentration of Plasma Cholesterol (mg/dl) During Summer Season.

Days	Control	T1	T2
15	$227^{a} \pm 1.47$	$205^{b} \pm 1.47$	$204^{b} \pm 1.47$
30	$246.54^{a} \pm 0.981$	$209.831^{b} \pm 0.981$	$180.03^{c} \pm 0.981$
45	$235.43^{a} \pm 1.04$	$206.98^{b} \pm 1.04$	$160.03^{c} \pm 1.04$

[1]T1 (0.5) and T2 (1.25) are supplemented groups.
[a-c]Mean ± SE values with different superscripts are significantly different from each other at $P \leq 0.05$ within a row.
[2]Age of the birds is 18–22 wk.

Table 3. Concentration of Plasma Malondialdehyde (uM/ml) During Summer Season.

Days	Control	T1	T2
15	$100.89^{a} \pm 0.970$	$99.22^{a} \pm 0.970$	$89.95^{b} \pm 0.970$
30	$118.92^{a} \pm 1.61$	$106.96^{b} \pm 1.61$	$59.30^{c} \pm 1.61$
45	$117.62^{a} \pm 1.19$	$101.06^{b} \pm 1.19$	$57.37^{c} \pm 1.19$

[1]T1 (0.5) and T2 (1.25) are supplemented groups.
[a-c]Mean ± SE with different superscripts are significantly different ($P \leq 0.05$) from each other within a row. [2]Number of birds in each group was 10. (Age of the birds is 18–22 wk).

control ($P < 0.05$) when compared to the concentration observed for the other 2 groups.

The decrease in the concentration of plasma MDA was directly related to the supplement dose. Our results also showed that the FYC supplementation decreased the level of plasma cholesterol after 15 d of the experiment. In dairy cows, previous studies reported that cows sampled in summer had higher concentration of GH than those sampled in winter [26, 27]. Acclimation to heat stress might have led to decrease in concentration of plasma GH. Opposite results were reported in case of buffaloes [28]. It has been reported by a previous study [29] that during acute physical stress in humans, plasma GH level increases. In the present study, supplementation of FYC (T1 and T2 group) might have made the chickens acclimatize to the hot season or higher temperature, which decreased the plasma GH concentration. Previous studies also reported [30] that heat stress during summer caused increase in plasma MDA levels. In the present study also, during summer season, the prevailing high ambient temperature resulted in lipid peroxidation and higher plasma MDA level in the control group.

The β-glucans present in the yeast cell wall induced cholesterol-lowering effects [31]. In rats, it has been reported that β-glucan extract from S. cerevisiae can reduce total cholesterol approaching normal values at doses of 10 mg of 32.79% in blood plasma and 33.71% in the liver [32]. Since FYC is a source of less number of live yeast cells and source of other factors like vitamins, minerals, and amino acids, supplementation of FYC along with the diet lowered cholesterol and lipid peroxidation as indicated by lesser concentration of plasma MDA in the treatment group, thus resulting in the significant differences between the treatments and control group. The treatments also reduced the expression of lipogenic genes FAS (T1, $P < 0.05$ and T2, $P < 0.05$) and SCD (T1, $P < 0.05$ and T2, $P < 0.01$) as shown in Figures 1 and 2, when compared to their respective control group during summer season. Decrease in the FAS gene expression directly relates to the FYC dose. Both the doses of FYC were equally effective in reducing the expression of FAS gene whereas effect on SCD gene was dose-dependent. Higher dose was more effective (T1, $P < 0.05$ and T2, $P < 0.01$) in reducing the expression of SCD gene. Cholesterol can control the synthesis of monounsaturated fatty acids in liver by regulating the expression of the SCD genes [33].

Earlier research showed that heat stress and disturbances in lipid metabolism reflect in increased serum triacylglycerol, total cholesterol levels [34], and increased hepatic lipogenic enzymes activities like maleic enzyme, acetyl CoA carboxylase, and fatty acid synthase [35]. Disturbance in lipogenesis occurs as a result of up-regulation of hepatic lipogenic enzymes through an increase in mRNA gene expression level of these enzymes [36], which also leads to metabolic disorders. In the present study also, we observed a significant increase in the level of plasma cholesterol and up regulation of lipogenic genes (Figures 1 and 2) in the control group during summer season. Previous studies reported that β-glucan; component of yeast cell wall inhibits the expression of intestinal genes associated with cholesterol and fatty acid synthesis [37]. Besides that, hyperlipidemia is associated with higher level of cholesterol and MDA, which further leads to oxidative stress [38]. Higher concentration of cholesterol and MDA may induce more lipid synthesis in control group of chickens. Enzymes, vitamins,

COLOUR ONLINE, B&W IN PRINT

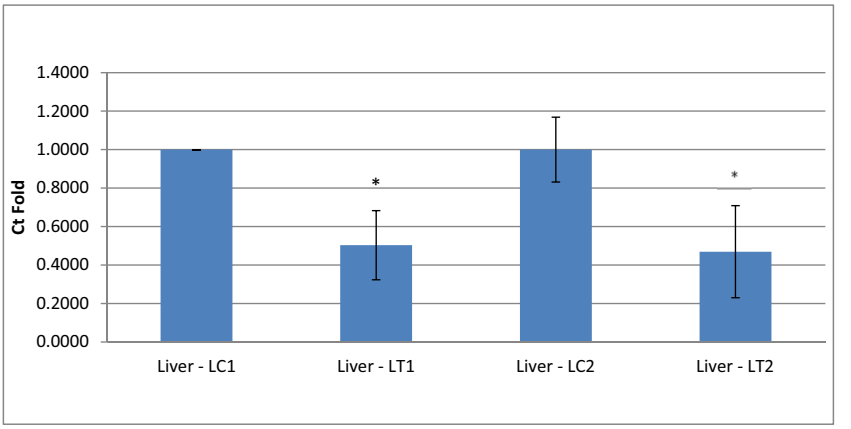


Figure 1. Relative gene expression of FAS gene in liver tissue of PD3 line chicken. C1 and C2–Control for Treatment 1 and 2, respectively. FAS-Fatty Acyl Synthase. Actin gene served as housekeeping gene. $^{*}P \leq 0.05$, N = 5.

COLOUR ONLINE, B&W IN PRINT

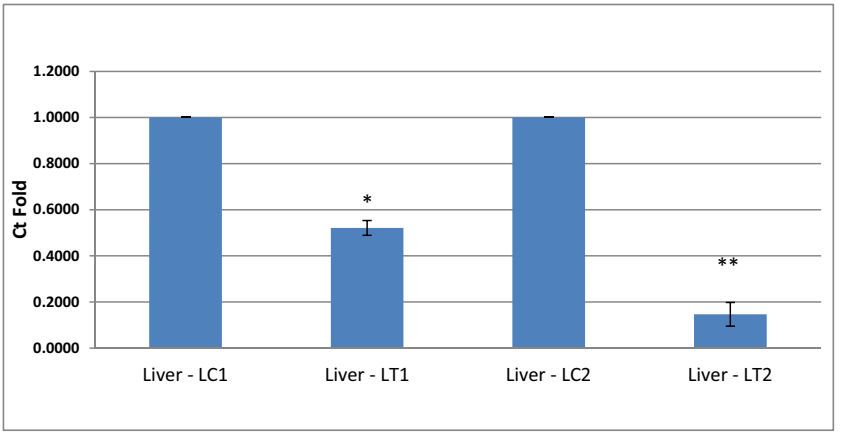


Figure 2. Relative gene expression of SCD gene in liver tissue of PD3 line chicken. C1 and C2–Control for Treatment 1 and 2, respectively. SCD-Stearoyl Co A Desaturase. Actin gene served as housekeeping gene.$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, N = 5.

saccharides, and other metabolites produced from yeast fermentation may benefit growth, metabolism, and health of chickens. Beneficial effects of FYC in ruminants and pigs are well known. It has been reported in pigs that supplementation of FYC resulted in higher ADG, better intestinal health, and immunomodulatory effects [39].

In the present study, the concentration of plasma GH, cholesterol, and MDA was significantly high in the control group of PD3 chickens, which had no inclusion of yeast culture.

Further, supplementation of FYC lowered cholesterol and MDA, often used as markers of heat stress [40]. It has also been observed that supplementation of FYC during summer to pullets with histopathological condition of jejunum, showed improved egg production and lowered stress factors [41]. Studies in rats have indicated that high levels of circulating cholesterol can induce SCD gene expression in the liver [42, 43]. In vivo studies suggested that when liver was challenged with excess of cholesterol, it increased SCD activity for cholesterol esterification for storage in the form of VLDL molecule and transported to other tissues. In the present study, we found higher levels of plasma cholesterol in the control group that likely increased SCD gene expression (Figure 3). The chickens were not hypercholesteremic, but plasma

COLOUR ONLINE, B&W IN PRINT

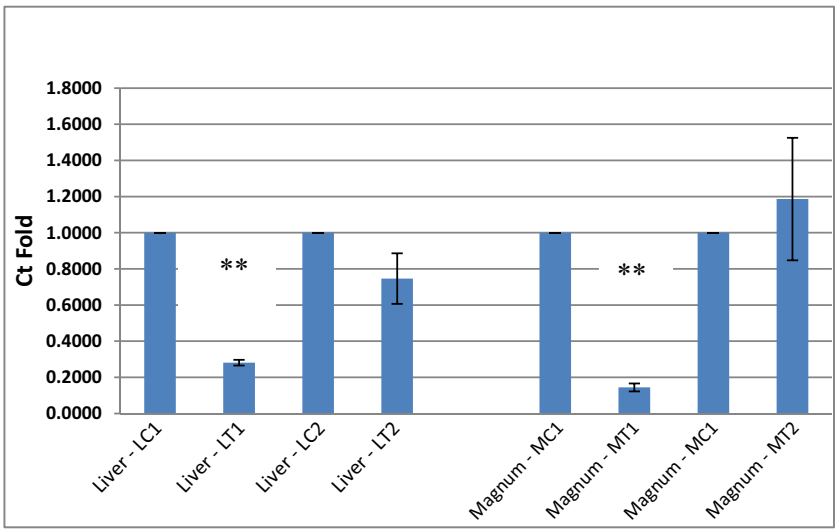


Figure 3. Relative gene expression of MMP3 gene with respect to actin housekeeping gene in liver and magnum tissues of PD3 line chicken. C1 and C2–Control for Treatment 1 and 2 respectively. MMP3-Matrix metalloproteinase 3. $^{**}P \leq 0.001$, N = 5.

cholesterol level was significantly higher in the control group when compared to the treatment groups. However, higher dose of FYC significantly decreased ($P < 0.01$) the expression of SCD gene.

MMPs are a large family of zinc-endopeptidases which play an essential role in maintaining multiple physiological and pathological processes. The MMP-3 enzyme degrades different collagen types, proteoglycans, fibronectin, etc. In addition, it also activates other MMPs; thus MMP-3 plays a crucial role in connective tissue remodeling. Multiple hormones, cytokines and growth factors can also induce MMP expression. The proteolytic activities of MMPs influence essential cellular processes like cell proliferation, migration, and adhesion, as well as many key physiological events involving tissue remodeling [44]. In the present study, higher gene expression of liver MMP3 in the control group (Figure 3) may indicate greater proteolytic activity in the liver and magnum tissues in the control group during summer season. In case of MMP-3 expression, the lower dose had greater efficacy in reducing the expression of MMP-3 gene in the magnum ($P < 0.001$) as well as in liver ($P < 0.001$) (Figure 3). We also observed that the response in the magnum was higher when compared to the liver tissue of treatment group. MMP-3 is up regulated under inflammatory circumstances [45]. Another study on cows [46] has reported that during early postpartum period, the plasma level of GH was more under negative energy balance conditions, and the expression of MMP-3 was up regulated.

During summer, the higher concentration of GH in the control group was positively related with higher expression of lipogenic and MMP 3 genes. The significant decrease in the concentration of GH upon supplementation of FYC was positively related with decrease in the expression of SCD, FAS, and MMP 3 genes.

Hence in the present experiment, expression of the SCD, FAS, and MMP3 genes was higher in the control group, which was exposed to high temperature during summer, without supplementation of FYC. This indicated higher lipogenesis and degrading activity in the control group.

The effect of supplementation of FYC during and post-summer was evaluated based on production performance in the post-summer period only, which coincided with the laying period. The mean ± SE feed intake during post-summer period, was, however, significantly less ($P < 0.05$) for the T1 and T2 groups, when compared to the control at 30 and 32 wk of age (Table 4). The

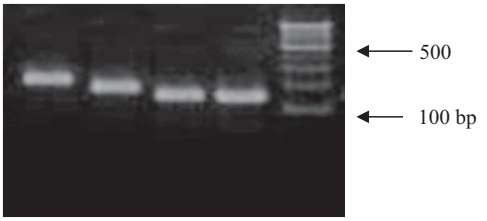


Figure 4. PCR products loaded on a 2% Agarose gel.

Table 4. Feed Intake (g/bird/d) of Birds During Post-Summer Season.

Weeks[1]	Control	T1	T2
28	78.36[a] ± 0.634	76.32[a] ± 0.610	74.31[b] ± 602
30	109.20[a] ± 0.734	100.01[b] ± 0.734	76.21[c] ± 0.734
32	114.23[a] ± 1.10	100.23[b] ± 1.10	80.23[c] ± 1.10

[1]Weeks represent the age of the birds during post-summer period.

[a-c]Mean± SE values with different superscripts are significantly different ($P \leq 0.05$) from each other in a row. [2]Number of birds in each group was forty.

Table 5. Body Weight (g) of Birds During Post-Summer Season.

Weeks[1]	Control	T1	T2
28	1488 ± 4.26	1490 ± 3.28	1507 ± 3.28
30	1482 ± 4.25	1500 ± 4.10	1525 ± 4.26
32	1500[a] ± 3.44	1515[a] ± 3.44	1535[b] ± 3.44

[1]Weeks represent the age of the birds in the post-summer period.

[a-b]Mean± SE values with different superscripts are significantly different($P \leq 0.05$) from each other in a row. [2]Number of birds in each group was 40.

decrease in feed intake did not decrease body weight or egg production.

On the contrary, we observed an average egg production (in percentage) higher in the treatment groups when compared to the control for the period between 26–32 wk of age 60% (C) vs. 62% (T1) vs. 64% (T2, Table not given). Body weight was observed to be greater in the T2 group in the post summer period only; the difference was significant only at 32 wk (Table 5). Based on that, we concluded that supplementation of FYC decreased heat stress markers like plasma cholesterol, MDA, expression of lipogenic, and MMP3 genes. We also noticed a decrease in feed intake that did not result in any adverse effect on body weight and egg production potential during post summer period. Hence, FYC supplementation during and post summer benefitted PD3 (Delham Red) hens/pullets.

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) test using general linear model procedure evaluated on SPSS 10 software for Windows [24]. LSD was utilized for pair wise comparison of the least squares mean. For the gene expression analysis, comparison between the control and treatment groups was performed by t-test.

Detailed Laboratory and Bird Management Procedures

The birds were procured from ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad, India. Each hen was placed in a single cage, feed and water were supplied *ad libitum* (Table A). CP and ME of the grower's feed was 18% and 2800 kcal/kg whereas for layer's feed it was 15% and 2500 kcal/kg, respectively. Body weight and feed intake were recorded at fortnight interval. In the laying period, during the post summer period eggs were collected daily, and weight was recorded. A 3 ml of blood from ten randomly assigned birds of each group was collected from brachial vein at biweekly interval during 8 wk of summer season only. During laying period, blood was not collected from birds, as it stressed the birds. The collected blood was immediately transported on ice to lab. The blood samples were centrifuged at 3000 rpm for 15 min, and plasma was separated and stored at −20 °C for assay of parameters, like GH, cholesterol, MDA. From 24 till 32 wk of age, studies were restricted to egg production, feed, and body weight parameters. Supplementation of FYC continued during this tenure also.

Source of Stick, Equipment, and Materials

[a]Chromous Co. Biotech Pvt. Ltd, Bangalore, India.

[b]BioGene Biotech, Shanghai, China.

[c]BioAssay Systems, CA 94,545, USA.

[d]Hi Media Co. Pvt., Ltd., Mumbai, India.

CONCLUSIONS AND APPLICATIONS

1. Supplementation of FYC at the concentration of 1.25 mg/kg to the pullets/chickens improved production parameters through modulation of levels of plasma hormones.
2. FYC supplementation also decreased the adverse effects of heat stress experienced during the summer season on GH, metabolites, expression of MMP3 in liver and magnum.
3. FYC also increased the egg production in PD3 layers.
4. FYC can be used in layers for increase in production performance.

REFERENCES AND NOTES

1. Sams, A. 1997. The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. Poult. Sci. 76:1616–1620.

2. Mashaly, M. M., G. L. Hendricks, M. A. Kalama, A. E. Gehad, A. O. Abbas, and P. H. Patterson. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83:889–894.

3. Babinszky, L., V. Halas, and M. W. Verstegen. 2011. Impacts of climate change on animal production and quality of animal food products. Pages 165–190 in Climate Change Socioeconomic Effects. Blanco, J., and H. Kheradmand, ed. InTech, Rijeka.

4. Fellenberg, M. A., and H. Speisky. 2006. Antioxidants: Their effects on broiler oxidative stress and its meat oxidative stability. Worlds Poult. Sci. J. 62:53–70.

5. Hrabia, A., H. E. Paczoska-Eliasiewicz, and L. R. Berghman et al. 2008. Expression and localization of growth hormone and its receptors in the chicken ovary during sexual maturation. Cell Tissue Res. 332:317–328.

6. Luna, M., C. G. Martínez-Moreno, M. S. Ahumada-Solórzano, S. Harvey, M. C. Marranza, and C. Arámburo. 2014. Extrapituitary growth hormone in the chicken reproductive system. Gen. Comp. Endocrinol. 203:60–68.

7. Waters, M. J., and A. J. Brooks. 2012. Growth hormone and cell growth. Endocr. Dev. 23:86–95.

8. Liu, Z., J. Cordoba-Chacon, R. D. Kineman, B. N. Cronstein, R. Muzumdar, Z. Gong, H. Werner, and S. Yakar. 2016. Growth hormone control of hepatic lipid metabolism. Diabetes 65:3598–3609.

9. Delitala, G., P. Tomasi, and R. Virdis. 1987. Prolactin, growth hormone and thyrotropin-thyroid hormone secretion during stress states in man. Baillieres Clin. Endocr. Metab. 1:391–414.

10. Xiao, Y., C. Wu, K. Li, G. Gui, G. Zhang, and H. Yang. 2017. Association of growth rate with hormone levels and myogenic gene expression profile in broilers. J. Anim. Sci. Technol. 8:43.

11. Del Vesco, A. P., E. Gasparino, V. Zancanela, D. O. Grieser, S. E. F. Guimarães, C. S. Nascimento, D. M. Voltolini, J. Constantin, and F. S. Gasparin. 2014. Acute heat stress and dietary methionine effects on IGF-I, GHR, and UCP mRNA expression in liver and muscle of quails. Genet. Mol. Res. 13:7294–7303.

12. Sehirli, O., A. Tozan, G. Z. Omurtag, S. Cetinel, G. Contuk, N. Gedik, and G. Sener. 2008. Protective effect of resveratrol against naphthalene-induced oxidative stress in mice. Ecotoxicol. Environ. Saf. 71:301–308. |

13. Ismail, I. B., K. A. Al-Busadah, and S. M. El-Bahr. 2013. Oxidative stress biomarkers and biochemical profile in broilers chicken fed zinc bacitracin and ascorbic acid under hot climate. Am. J. Biochem. Mol. Biol. 3:202–214.

14. Yardibi, H., and G. Turkay. 2008. The effects of vitamin E on the antioxidant system, egg production, and egg quality in heat stressed laying hens. Turk. J. Vet. Anim. Sci. 32:319–325.

15. Dridi, S., M. Taouis, A. Gertler, E. Decuypere, and J. Buyse. 2007. The regulation of Stearoyl-CoA Desaturase gene expression is tissue specific in chickens. J. Endocrinol. 192:229–236.

16. Overall, C. M. 2002. Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. MB 22:051–086.

17. Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

18. Nagase, H., R. Visse, and G. Murphy. 2006. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc. Res. 15:562–573.

19. Drozdowski, L. A., R. A. Reimer, F. Temelli, R. C. Bell, T. Vasanthan, and A. B. Thomson. 2010. Beta-glucan extracts inhibit the in vitro intestinal uptake of long-chain fatty acids and cholesterol and down-regulated genes involved in lipogenesis and lipid transport in rats. J. Nutr. Biochem. 21:695–701.

20. Kerckhoffs, D. A., G. Hornstra, and R. P. Mensink. 2003. Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. Am. J. Clin. Nutr.78:221–227.

21. Nidamanuri, A. L., S. Murugesan, R. K. Mahapatra, and P. Bhukya. 2017. Effect of supplementation of fermented yeast culture (Saccharomyces cerevisiae) during and post summer season on plasma hormones egg production potential feed efficiency of PD3 chicken line. IJABR. 7:456–464.

22. Panda, A. K., S. V. Rama Rao, M. V. L. N. Raju, M. Niranjan, and M. R. Reddy. 2012. Effect of nutrient density on production performance, egg quality and humoral immune response of brown laying (Dahlem Red) hens in the tropics. Trop Anim. Health Prod. 44:293–299.

23. Zlatkis, A., B. Zak, and A. J. Boyle. 1953. A new method for the direct determination of serum cholesterol. J. Laborat. Clin. Med. 41:486–492.

24. SPSS User Guide. SPSS Statistics for Windows, Version 10.0. SPSS Inc, Chicago.

25. Beerepoot, G. M. M., A. E. Freeman, and J. C. Detilleux. 1991. Effect of season, genetic line, and sire on growth concentrations of somatotropin in serum of Holstein cows in early lactation. J. Dairy Sci. 74:3202–3208.

26. Kataria, N., A. K. Kataria, and A. K. Gahlot. 2008. Ambient temperature associated variations in serum hormones and interrelated analytes of broiler chickens arid tract. Slov. Vet. Res. 45:127–134.

27. Chaudhari, B. K. 2013. Plasma and milk hormones as biomarkers of stress during extreme summer season in Murrah buffaloes. Ph.D. Diss. National Dairy Research Institute. Haryana, India.

28. Ranabir, S., and K. Reetu. 2011. Stress and hormones. Indian J. Endocr. Metab. 15:18–22.

29. Lin, H., E. Decuypere, and J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. Comp. Biochem. Physiol. A Comp. Physiol. 144:11–17.

30. Abdelhady, D. H., M. A. El-Abasy, M. S. Atta, E. W. Ghazy, T. K. Abuzed, and A. M. El-Moslemany. 2017. Synergistic ameliorative effects of organic chromium and selenium against heat stress in japanese quails: performance, immunological, hematological, biochemical and antioxidant studies. alexandria. J. Vet. Sci. 55:113–123.

31. Zekovic, D. B., S. Kwiatkowski, M. M. Vrvic, D. Jakovljevic, and C. A. Moran. 2005. Natural and modified (1?3)-?-D-Glucans in health promotion and disease alleviation. Crit. Rev. Biotechnol. 25:205–230.

32. Imanpour-Jodey, S., S. Moghaddaszadeh-Ahrabi, and A. Rezapour. 2013. The effects of *Saccharomyces cervisiae* beta-glucan on blood lipids in broiler chickens. Ann. Biol. Res. 4:134–137.

33. Kusmiati,, and F. X. Dhewantara. 2016. Cholesterol-lowering effect of beta glucan extracted from Saccharomyces cerevisiae in rats. Sci. Pharm. 84:153–165.

34. Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res. 40:1549–1558.

35. Flees, J., H. Rajael-Sharlfabadl, E. Greene, L. Beer, B. M. Hargis, L. Ellestad, T. Porter, A. M. Donoghue, and S. Dridi. 2017. Effect of Morinda citrifolia (Noni)-enriched diet on hepatic heat shock protein and lipid metabolism-related genes in heat stressed broiler chickens. Front. Physiol. 8: 919.

36. Wang, P. H., Y. H. Ko, H. J. Chin, C. Hsu, S. T. Ding, and C. Y. Chen. 2009. The effect of feed restriction on expression of hepatic lipogenic genes in broiler chickens and the function of SREBP1. Comp. Biochem. Physiol. Part B. 153:327–331.

37. Drozdowski, L. A., R. A. Reimer, F. Temelli, R. C. Bell, T. Vasanthan, and A. B. R. Thomson. 2010. β-Glucan extracts inhibit the in vitro intestinal uptake of long-chain fatty acids and cholesterol and down-regulated genes involved in lipogenesis and lipid transport in rats. J. Nutr. Biochem. 21:695–701.

38. Gorinstein, S., H. Leontowicz, M. Leontowicz, J. Drzewiecki, K. Najman, E. Katrich, D. Barasch, K. Yamamoto, and S. Trakhtenberg. 2006. Raw and boiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. Life Sci. 78:655–663.

39. Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs1. J. Anim. Sci. 87:2614–2624.

40. Kucuk, O., N. Sahin, and K. Sahin. 2003. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. BTER 94:225–236.

41. Laxmi, Anand N., M. Shanmugam, M. R. Reddy, and R. K. Mahapatra. 2017. Improvement in Egg Production of PD 3 Chicken Line with histopathological conditions of the jejunum up on supplementation of fermented yeast culture during and post summer season. Int. J. Curr. Microbiol. App. Sci. 6:379–385.

42. Landau, J. M., A. Sekowski, and M. W. Hamm. 1997. Dietary cholesterol and the activity of stearoyl CoA desaturase in rats: evidence for an indirect regulatory effect Biochimica et Biophysica Acta (BBA). Lipids and Lipid Metab. 1345:349–357.

43. Ntambi, J. M., and M. Miyazaki. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 432:91–104.

44. Page-McCaw, A., J. Ewald, and Z. Werb. 2007. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 8:221–233.

45. Rosenberg, G. A. 2002. Matrix metalloproteinases in neuroinflammation. Glia 39:279–291.

46. Wathes, D. C., Z. Cheng, M. A. Fenwick, R. Fitzpatrick, and J. Patton. 2011.Influence of energy balance on the somatotrophic axis and matrix metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction. 141:269–281.

Acknowledgments

We acknowledge the ICAR-New Delhi, India for providing financial help to carry out this experiment and the support given by our Director, Dr. R.C. Chatterjee and colleagues from the Directorate of Poultry Research, Rajendranagar, Hyderabad, India, for providing facilities to carry out experiment.