



The effect of ginger powder and Chinese herbal medicine on production performance, serum metabolites and antioxidant status of laying hens under heat-stress condition



Fahar Ibtisham^{a,b,1}, Aamir Nawab^{a,1}, Yanfeng Niu^{a,1}, Zhi Wang^a, Jiang Wu^a, Mei Xiao^a, Lilong An^{a,*}

^a Animal Science Department, Agriculture College, Guangdong Ocean University, Haida Road, Mazhang District, Zhanjiang 524088, Guangdong, China

^b Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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ABSTRACT

This study was done to evaluate the effects of Chinese herbal medicine (CHM) and ginger powder on layers-production performance, serum metabolites and antioxidant status under heat stress condition. Two hundred and fifty Lohmann layers were randomly divided into 5 different, including two controls and three experimental groups (H1, H2, and H3). Control groups were fed the basic diet without supplementation, while, the feed of three experimental groups was supplemented with 3.32 g CHM, 10 g ginger powder, and 10 g ginger powder + 3.32 g CHM per kg of diet, respectively. Results showed that feed consumption and production rate were decreased in the HC group, while, feed intake and production significantly improved when birds were given supplemented diet. The production rate and feed intake of the H3 group were even significantly higher than the NC group. The birds that received supplemented diet had higher glucose level compared to HC. Triglycerides and serum cholesterol had significantly decreased level in supplemented groups compared to HC. Fungal catalase (CAT) level was significantly improved in H2 and H3 groups. Nitric oxide (NO), glutathione peroxidase (GSH-PX) and Total Protein (T-AOC) level were significantly improved in supplemented groups. Findings showed that ginger powder and CHM could be a viable alternative to the synthetic antibiotic in poultry feed.

1. Introduction

Among various environmental conditions, heat stress is known as one of the most lethal stressors, which severely affects feed intake, body weight gain and feed efficiency of laying hens (Jang et al., 2014). Moreover, it also decreases the egg production, egg quality, egg mass and eggshell quality (Nawab et al., 2018). Therefore, averting and improving the harmful effects of heat stress is becoming progressively significant in the poultry industry.

Reactive oxygen species (ROS) are constantly generated and eliminated in the biological system; however, an imbalance between formation and removal of ROS can lead to a pathological condition called as oxidative stress (Ibtisham et al., 2018a). Heat stress also induces the oxidative stress (Tan et al., 2010), which in turn, causes lipid peroxidation and oxidative damage to cellular membranes (Mujahid et al., 2007).

Besides improving environmental management, nutritional

strategies have been developed to partially alleviate the negative impacts of heat stress in birds, including vitamin-like substances, herbs, plant extracts, and probiotics. Ginger is the rhizome of the plant *Zingiber officinale*, and it is widely used as a spice and in practice of traditional Chinese herbal medicine. Ginger has shown various pharmacological effects including antiapoptotic, immunomodulatory, antitumorigenic, antiapoptotic, antiinflammatory, antihyperglycemic, antiapoptotic, antilipidemic, and antiemetic effects (Zhao et al., 2011). Ginger can also stimulate the secretion of gastrointestinal fluid and promote feed digestion (Amerah and Ravindran, 2015) and preliminary research indicates that many compounds found in ginger can bind to serotonin receptors which in turn may influence gastrointestinal function. Therefore, ginger can be an alternative to synthetic growth promoter (Ashayerizadeh et al., 2009). Furthermore, ginger contains several compounds (e.g., gingerol, gingerdione, and shogaols) that possess antioxidant activity.

Natural medicine products have been widely used in the animal's

* Corresponding author.

E-mail address: anlilong@126.com (L. An).

¹ Equally contributed.

diet due to their antimicrobial and antioxidant properties (Ibtisham et al., 2018b). Chinese Herbal Medicines are full of vitamins, lipids, amino acids proteins, trace elements (Fan et al., 2017). Traditional CHM has increasingly attracted the attention of researchers as they have been reported as an immune booster (Kong et al., 2004). Many CHM also has demonstrated valuable effects when applied to treat heat stress syndrome (Liu et al., 2010). However, information is lacking on the effect of ginger and CHM as a feed additive on laying performance and antioxidant status of laying hens under heat stress condition. The objectives of this study were to investigate the effects of diet supplementation by CHM and ginger powder, singly or in combined forms, on production performance, serum metabolites and antioxidant status of laying hens under heat stress condition.

2. Methods and materials

2.1. Ethics statement

The experiment was conducted in Guangdong Ocean University, Zhanjiang, Guangdong, China. The Animal Care Committee of Guangdong Ocean University (Guangzhou, People's Republic of China) approved this study. The animals involved in this study were humanely sacrificed as necessary to ameliorate suffering.

2.2. Materials

The ginger powder was bought from the agricultural vegetable limited company, Jiangshu and Chinese herbal medicine were purchased from Yisheng pharmacy, Zhanjiang, Guangdong, China.

2.3. Layers and environment

Two hundred and fifty Lohmann layers of 25 weeks old were randomly divided into 5 different groups with five replications, thermo-neutral group (NC) at room (22–28 °C) temperature, heat-stressed control group (HC) at high (32–38 °C) temperature, high-temperature treatment group 1,2 and 3 (H1, H2 and H3–32 ~ 38 °C). All the birds were raised in 3-tier caged layer houses (2 hens per cage). A photoperiod of 16 h/day was maintained with the intensity of 10–15 Lx and humidity was maintained at 60–80%.

2.4. Feeding management

Birds were allotted into five groups including two control groups which were fed the basal diet without supplements and the other 3 groups were fed the basal diet including experimental supplementation (Table 1). The ingredients and nutrient composition of the basal diet are presented in Table 2, and water was given ad libitum.

2.5. Production performance

Egg production and laid egg weight of per replicate were recorded daily, while the total number of egg per bird and average egg mass per bird was calculated at the end of each three experimental weeks. On daily basis, abnormal eggs (cracked, broken, soft shell) were also

Table 1
Diet plan of layer groups.

Group	Diets
NC	Basal diet
HC	Basal diet
H1	Basal diet + 10 g/kg Ginger powder
H2	Basal diet + 3.32 g/kg Chinese Herbal Medicine
H3	Basal diet + 10 g/kg Ginger powder + 3.32 g/kg Chinese Herbal Medicine

Table 2

Ingredient and chemical composition of the basal diet (air-dry basis, %).

Ingredients	Present (%)	Nutrients	Content
Corn	61.0	ME/(MJ/kg) 2	11.42
Soybean meal	22.0	CP	18.17
Wheat bran	2.0	Ca	3.73
Fish meal	4.5	TP	0.64
Limestone	9.0	Met	0.31
CaHPO ₄	1.0	Cys	0.27
NaCl	0.2	Lys	0.97
Premix ¹	0.3		

Note: 1. The premix provided per kg of diet: VA 9000 IU, VD 2500 IU, VE 20 IU, VB 1212 µg, VK 2.4 mg; Mn100 mg, Zn 60 mg, Fe 25 mg, Cu 5 mg, Co 0.1 mg (Mn, Zn, Fe, Cu, Co were provided in the form of sulPhate), Se (N₂SeO₃·5H₂O) 0.2 mg, I(KI) 0.5 mg; 2.

recorded. At the end of each week, feed intake was measured. Feed conversion ratio (g feed/g egg) and egg volume (g egg/hen/day) were calculated from feed intake, egg production, and egg weight. Body weights of birds were measured at the start and end of the study to determine the body weight change.

2.6. Blood sampling and analysis

Blood samples were taken via the wing vein of six randomly selected hens from each group, during the 3rd, 6th and 9th week respectively, of the experiment. Supplementation was stopped before 12 h of blood sample collection. After collection, samples were immediately transferred to the lab where samples were centrifuged at 3500g at 4 °C for 5 min to separate the serum and plasma. The heparinized plasma samples were frozen at –20 °C and serum samples were frozen at –80 °C until analysis.

2.7. Examine of antioxidant enzymes in serum

To check the antioxidant enzyme activity, serum was analyzed for Superoxide Dismutase (SOD), glutathione peroxidase (GSH-PX), total antioxidant capacity (T-AOC) and content of malondialdehyde (MDA). Enzymatic activity was measured by using assay kit obtained from Nanjing Jiancheng Bioengineering Institute (strictly according to kit manufacturer protocol).

2.8. Antioxidant traits measurement

To measure the antioxidant parameters, heart, liver and kidneys samples were collected of randomly selected three chickens per replicate at the end of the trail. Tissue antioxidant index kit (Nanjing Jiancheng, China) was used strictly according to instructions of the manufacturer for analysis and samples were frozen at –20 °C until analysis.

2.9. Statistical analysis

We used SPSS (version 20.0) software and to determine the difference between groups Duncan's multiple range test was applied. $P < 0.05$ was for significant difference. All the results were expressed as means ± standard error formula for relative.

3. Results

3.1. Production performance and feed consumption

The effects of dietary supplements on the performance of laying hens are shown in Table 3. HC group had decreased feed consumption and production rate. Compared to HC the feed intake significantly

Table 3
Effect of ginger powder, Chinese herbal medicine on production performance of laying hens.

Parameters	Time/ week	NC	HC	H1	H2	H3
Daily feed intake	1–3 w	89.69 ± 6.29 ^{ab}	83.21 ± 4.00 ^a	85.05 ± 7.09 ^a	94.67 ± 5.42 ^b	103.31 ± 8.09 ^c
	4–6 w	95.41 ± 4.08 ^a	77.35 ± 9.84 ^b	88.74 ± 5.95 ^a	90.19 ± 5.72 ^a	110.32 ± 7.27 ^c
	7–9 w	107.94 ± 3.76 ^a	90.41 ± 3.27 ^b	94.84 ± 2.24 ^b	100.76 ± 5.88 ^c	113.34 ± 4.61 ^a
Laying rate (%)	1–9 w	97.68 ± 9.08 ^b	83.66 ± 8.15 ^d	89.54 ± 6.63 ^c	95.21 ± 6.95 ^b	104.96 ± 8.57 ^a
	1–3 w	75.97 ± 2.24 ^a	66.45 ± 5.95 ^b	69.05 ± 6.84 ^b	78.87 ± 3.10 ^{ac}	82.29 ± 2.81 ^c
	4–6 w	74.35 ± 1.24 ^a	65.73 ± 2.38 ^b	70.63 ± 2.38 ^c	79.62 ± 3.33 ^d	82.36 ± 5.19 ^d
Feed/Egg	7–9 w	73.65 ± 9.54 ^{ac}	65.62 ± 1.75 ^b	70.03 ± 1.78 ^{ab}	77.85 ± 8.07 ^c	81.18 ± 4.81 ^c
	1–9 w	74.66 ± 5.26 ^a	65.93 ± 3.62 ^b	69.90 ± 4.30 ^c	78.78 ± 5.08 ^d	82.02 ± 3.73 ^d
	1–3 w	2.44 ± 0.24	2.51 ± 0.37	2.35 ± 0.33	2.19 ± 0.21	2.45 ± 0.29
Average egg weight (g)	4–6 w	2.36 ± 0.06	2.27 ± 0.28	2.49 ± 0.58	2.13 ± 0.38	2.33 ± 0.19
	7–9 w	2.60 ± 0.28	2.51 ± 0.21	2.51 ± 0.30	2.32 ± 0.45	2.68 ± 0.44
	1–9 w	2.38 ± 0.26 ^{ab}	2.43 ± 0.30 ^{ab}	2.45 ± 0.41 ^{ab}	2.21 ± 0.35 ^a	2.49 ± 0.34 ^b
Egg culling rate (%)	1–3 w	54.66 ± 1.55 ^a	50.75 ± 1.79 ^c	52.43 ± 2.33 ^{bc}	54.02 ± 1.23 ^{ab}	53.82 ± 0.56 ^{ab}
	4–6 w	54.81 ± 2.27 ^{ac}	50.49 ± 2.29 ^{ab}	49.94 ± 0.75 ^b	53.24 ± 2.47 ^{ab}	57.80 ± 5.77 ^c
	7–9 w	55.41 ± 1.94 ^a	53.85 ± 2.23 ^{ab}	53.16 ± 1.26 ^b	54.83 ± 4.2 ^{abc}	57.39 ± 1.15 ^c
Egg culling rate (%)	1–9 w	56.35 ± 2.77	51.70 ± 2.53 ^b	51.84 ± 2.06 ^b	54.03 ± 2.83 ^a	56.34 ± 2.47 ^a
	1–3 w	0.84 ± 1.69 ^a	4.25 ± 2.92 ^b	2.22 ± 2.33 ^{ab}	3.01 ± 1.16 ^{ab}	1.66 ± 1.55 ^{ab}
	4–6 w	2.33 ± 2.57	1.80 ± 1.49	3.89 ± 2.75	5.68 ± 3.60	3.92 ± 2.48
Egg culling rate (%)	7–9 w	0.43 ± 0.87	0.37 ± 0.74	1.56 ± 2.97	1.66 ± 2.30	0.95 ± 1.47
	1–9 w	1.20 ± 1.94 ^a	3.68 ± 3.45 ^b	2.56 ± 2.73 ^{ab}	3.56 ± 3.06 ^b	2.18 ± 2.21 ^a

Note: Values with different lowercase letters significant difference indicate significant difference (P < 0.05).

improved when birds were given supplemented diet, while, H3 group had significantly higher levels of intake compared to the NC group.

The HC group had significantly decreased the egg production and weight. In contrast, the supplemented groups had a significantly higher production rate than the HC group, while, the production rate of the H3 group was even significantly higher than the NC group. The culling rate was significantly higher in HC group compared to others. The H3 group had significantly lower egg culling rate compared to HC group.

3.2. Serum metabolic parameters

The effects of ginger powder and Chinese herbal compounds on serum metabolic parameters in laying hens are shown in Table 4. Compared with the HC group, the diet supplemented groups had increased glucose level. The H1 group had significantly higher (P < 0.05) glucose level compared to the HC group. Serum cholesterol level was increased (P > 0.05) in HC group compared to NC, but supplemented group had decreased level (P > 0.05) compared to control group.

Triglycerides had significantly decreased levels in supplemented groups compared to HC, almost the same level as the NC. The HC group also had significant (P < 0.05) reduced levels of total protein. Feed additive increased the level of total protein, but the difference was not significant (P > 0.05).

Table 4
Effects of ginger powder and Chinese herbal medicine on Serum Metabolic Parameters of laying hens.

Parameters	Time/Week	NC	NH	H1	H2	H3
GLU (mmol/L)	3 w	10.66 ± 0.35ab	10.03 ± 0.55a	12.47 ± 0.87c	11.60 ± 0.8 BCE	11.34 ± 0.8abc
	6 w	12.51 ± 0.78	11.97 ± 0.47	12.72 ± 1.27	12.04 ± 0.82	12.56 ± 0.69
	9 w	12.08 ± 0.18ab	10.86 ± 0.22b	13.00 ± 1.26a	10.99 ± 0.82b	10.92 ± 0.30b
CHOL (mmol/L)	3 w	1.59 ± 0.16	1.76 ± 0.14	1.50 ± 0.24	1.84 ± 0.31	1.67 ± 0.24
	6 w	1.88 ± 0.35a	1.84 ± 0.16ab	1.57 ± 0.18ab	1.36 ± 0.32b	1.41 ± 0.16ab
	9 w	1.69 ± 0.50	2.06 ± 0.37	1.93 ± 0.60	1.71 ± 0.63	1.80 ± 0.17
TG (mmol/L)	3 w	5.18 ± 0.28a	5.84 ± 0.17b	4.99 ± 0.36a	5.28 ± 0.06a	5.01 ± 0.32a
	6 w	5.16 ± 0.69ab	5.64 ± 0.17c	4.74 ± 0.40b	4.79 ± 0.23b	4.97 ± 0.35ab
	9 w	5.24 ± 0.39ab	5.95 ± 0.60c	5.25 ± 0.17ab	4.77 ± 0.73b	4.86 ± 0.41b
TP (g/L)	3 w	35.20 ± 0.26ab	32.37 ± 3.06a	39.37 ± 2.87b	37.43 ± 0.65b	37.30 ± 2.22b
	6 w	35.43 ± 0.85a	32.13 ± 1.02b	38.10 ± 2.50a	37.50 ± 2.25a	38.00 ± 1.04a
	9 w	35.20 ± 0.69ab	31.73 ± 1.46a	38.97 ± 2.75b	38.47 ± 5.20b	39.67 ± 3.94b

Note: Values with different lowercase letters significant difference indicate significant difference (P < 0.05).

3.3. Serum antioxidant status

Table 5 lists the antioxidant activity of ginger powder and Chinese herbal in laying hens. SOD activity was downregulated in the HC group (P < 0.05). Supplemented groups had improved SOD activity compared to HC; however, H3 showed better results compared to H2 and H3. Fungal catalase (CAT) level difference between NC and HC group was not significant, but feed additive supplemented H2, and H3 groups had significantly improved CAT activity. Heat stress significantly decreased the T-AOC but in supplemented the T-AOC level (P < 0.05) was increased, and as a whole, H3 group had better results compared to H1 and H2. Nitric oxide (NO) and GSH-PX levels were decreased in the HC group, while, supplemented groups had increased level of NO and GSH-PX, however, only in H3, the difference was significant.

MDA level was significantly (P > 0.05) increased HC group, but H1, H2, and H3 group had a significant reduction in the content of serum MDA of layers under heat stress.

3.4. Tissue total antioxidant level

Fig. 1 shows the total antioxidant level in different organs. Heat stress significantly decreased the hepatic T-AOC, but, in the heart and kidney, the difference was not significant (P > 0.05). H3 group showed effective response compared to the H1 and H2 groups. H3 group had significantly increased T-AOC levels.

Table 5
Effects of ginger powder and Chinese herbal medicine on antioxidant status of laying hens.

Parameters	Time/ Week	NC	NH	H1	H2	H3
SOD (U/mL)	3 w	370.91 ± 32.73 ^a	260.38 ± 49.35 ^b	324.53 ± 34.58 ^{ab}	267.92 ± 75.38 ^{ab}	279.25 ± 62.35 ^{ab}
	6 w	330.91 ± 76.62 ^{ab}	256.61 ± 52.28 ^a	343.40 ± 71.89 ^{ab}	313.21 ± 17.29 ^{ab}	377.36 ± 23.57 ^b
	9 w	382.72 ± 44.66 ^a	269.09 ± 53.81 ^b	370.91 ± 43.64 ^a	370.91 ± 28.86 ^a	389.09 ± 27.46 ^a
CAT (U/mL)	3 w	7.36 ± 0.09 ^a	7.63 ± 0.32 ^{ab}	7.82 ± 0.30 ^{bc}	8.04 ± 0.14 ^c	8.11 ± 0.07 ^c
	6 w	7.43 ± 0.27 ^a	7.22 ± 0.39 ^a	8.18 ± 0.05 ^b	8.24 ± 0.10 ^b	8.46 ± 0.21 ^b
	9 w	7.16 ± 0.43 ^a	7.20 ± 0.46 ^a	8.16 ± 0.81 ^{ab}	8.85 ± 0.05 ^b	8.46 ± 0.56 ^b
T-AOC (U/mL)	3 w	4.69 ± 0.43 ^a	2.01 ± 0.50 ^b	2.88 ± 0.74 ^{bc}	2.96 ± 0.65 ^{bc}	3.74 ± 0.43 ^{ac}
	6 w	4.89 ± 0.53 ^a	3.00 ± 0.23 ^b	4.32 ± 0.83 ^a	4.77 ± 0.96 ^a	5.22 ± 0.22 ^a
	9 w	4.15 ± 0.87 ^{ab}	3.04 ± 0.43 ^a	4.36 ± 1.06 ^{ab}	4.36 ± 0.68 ^{ab}	5.55 ± 2.22 ^b
GSH-Px (U/mol)	3 w	478.84 ± 59.23 ^{ab}	423.78 ± 79.47 ^a	545.58 ± 11.91 ^b	537.66 ± 19.62 ^{ab}	528.29 ± 82.63 ^{ab}
	6 w	466.52 ± 11.39 ^{ab}	421.72 ± 17.91 ^a	560.00 ± 63.98 ^b	511.03 ± 76.55 ^{ab}	580.00 ± 87.98 ^b
	9 w	454.86 ± 59.74 ^{ab}	446.34 ± 45.03 ^a	539.52 ± 46.89 ^{ab}	503.94 ± 76.05 ^{ab}	569.52 ± 69.96 ^b
NO (umol/L)	3 w	63.08 ± 11.09 ^a	25.08 ± 8.80 ^b	40.32 ± 25.02 ^{ab}	43.49 ± 23.60 ^{ab}	37.14 ± 10.81 ^{ab}
	6 w	63.08 ± 3.08 ^a	30.48 ± 4.37 ^b	44.76 ± 3.30 ^c	44.28 ± 7.42 ^c	47.14 ± 3.78 ^c
	9 w	64.17 ± 17.06 ^a	30.88 ± 6.05 ^b	43.33 ± 4.38 ^{ab}	48.07 ± 11.97 ^{ab}	42.46 ± 10.80 ^b
MDA (nmol/mL)	3 w	12.67 ± 1.53	15.22 ± 1.07	13.56 ± 2.00	14.22 ± 2.83	12.00 ± 0.88
	6 w	12.00 ± 1.33 ^a	15.77 ± 0.19 ^b	13.23 ± 0.96 ^a	13.56 ± 1.83 ^a	11.67 ± 1.15 ^a
	9w	12.08 ± 1.53 ^a	16.22 ± 1.07 ^b	12.56 ± 0.38 ^a	12.00 ± 0.88 ^a	11.78 ± 1.02 ^a

Note: Values with different lowercase letters significant difference indicate significant difference (P < 0.05).

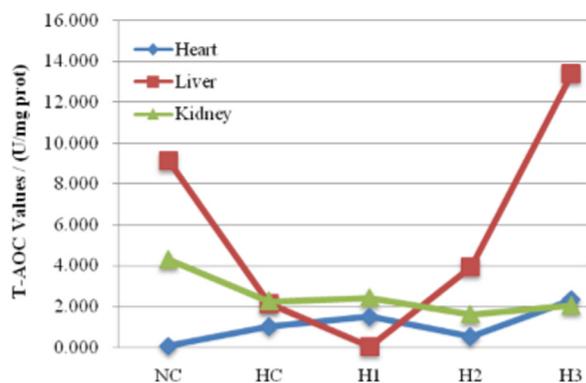


Fig. 1. Effects of ginger powder and Chinese herbal medicine on T-AOC values in different tissues of hens at high temperature. Heat stress significantly decreased the hepatic T-AOC, while the H3 group (supplemented with mixture of ginger powder and CHM) had significantly high level of T-AOC.

4. Discussion

In the present study, birds feed consumption and production decreased under heat stress condition. Production performance and feed consumption increased when birds were given the diet supplemented with CHM and ginger powder. The production performance and feed consumption rate of H3 group was better compared the H1 and H2 groups. Herbs are the efficient promoter of follicular development and immunity (Liu et al., 2010), therefore the supplemented groups had increased production rate. Moreover, gingers and herbs have positive effect on gastric secretion and digestive enzyme activities; therefore, experimental groups had increased feed consumption and production rate (Zhao et al., 2011). The other reported fact is that ginger improves the absorption of nutrient via stimulating lactic acid bacteria and decreasing pathogenic bacteria (Tekeli et al., 2011). In contrast to our findings, Krzysztof et al. (2018) did not find the positive effect of ginger powder on feed consumption and production rate. Heat stress had significantly increased the egg culling rate; most probably due to poor eggshell quality, because heat stress decreases the plasma calcium level (Ziaei et al., 2013). The feed supplemented groups had lower egg culling rate, however, the difference was only significant in H3 group compared to HC. These results are in general agreement with the previous studies which have reported that CHM (Xiang-Chun et al., 2014) and ginger (Karangiya et al., 2016) could improve the performance of birds. Interestingly, in this study, we found that the mixtures of CHM

and ginger had much better results on production performance compared to using them as a sole agent in layers diets.

Heat stress had a negative impact on glucose level (Table 4), which indicates that the plasma insulin and glucagon production was inhibited since insulin and glucagon are the most important hormones that control glucose metabolism. Present study findings was in agreement of previous report Tang et al. (2013). It has also been reported that the decline in glucose level during heat stress is due to the decrease in concentration of thyroxin, which is strongly linked with energy metabolism during heat stress (Soleimani and Zulkifli, 2010). The decline in serum thyroxin is most likely to lower metabolic rate for thermoregulation and to avoid hyperthermia. Thyroid has an important role for sexual maturity and reproductive activity in birds, a changing of thyroid activity by heat stress would affect the reproductive performance of the hens (Lara and Rostagno, 2013). Although, all supplemented groups had improved glucose level compared to HC, H1 group showed a significant difference.

The HC group had an increased level of serum cholesterol, probably due to the increase in concentrations of corticosterone, by activation of the hypothalamic-pituitary-adrenal (Bishop-Williams et al., 2015). Ginger powder and CHM showed anti hypercholesterolemia activity in layer diet, which shows that these ingredients have the ability to decrease the stress condition. Result of present study for serum cholesterol level agree with Zhang et al. (2009), who reported that the levels of serum cholesterol level increased with ginger powder supplementation. Total protein level was significantly downregulated in HC group compared to NC, while, the feed supplemented groups had an increased level. Increasing serum level of total protein in supplemented groups is probably linked to the increase in synthesis and transport of sex hormones (Ranaweera and Wise, 1981). These findings were in agreement of the previous study reported by Xie et al. (2017), while, incongruence with the report of Habibi et al. (2014) who did not find a positive effect of diet supplementation on total protein level.

The antioxidative enzyme system is the first line of antioxidant defense and a little alteration in the activity of the antioxidative enzyme can change the balance between the production of ROS (e.g., OH[•], O₂^{•-}, and H₂O₂) and the antioxidant system. In the present study, MDA concentration was down regulated, while, SOD, GSH-Px activity was enhanced in supplemented group compared to HC. SOD and GSH-Px activity and MDA concentration are the main parameters to assess the oxidative status of birds (Wang et al., 2008). Therefore, well regulation of mentioned parameters shows that the antioxidant status of supplemented groups was improved. SOD catalyzes the dismutation of the superoxide anion (O₂^{•-}) into hydrogen peroxide and molecular oxygen.

Glutathione (GSH) is impotent for normal cell growth and proliferation; it reduces the oxides which could hinder the regulation of cell (Ibtisham et al., 2018b). GSH also prevent the DNA from oxidative damage, it is also known as body's master antioxidant. In antioxidation systems, GSH acts as a hydrogen donor, which neutralizes the free radicals. Since the GSH level increased in diet supplemented groups it appears that CHM and ginger were stimulating the action of glutathione to scavenge free radicals. CAT can remove the hydrogen peroxide radical (HOOH) by catalyzing two HOOH molecules to form two molecules of water and one molecule of O₂. Experimental results showed that diet supplementation raised the CAT activity compared to the control. This means that CHM and Ginger can increase scavenging of HOOH radicals. These findings were in agreement with the previous reports by Habibi et al. (2014), Zhao et al. (2011), and Kota et al. (2008). On tissue level, Results showed that heat stress had significantly decreased the hepatic T-AOC compared to the kidney and heart. However, diet supplemented group had increased T-AOC level.

5. Conclusion

Heat stress could significantly decrease the production performance and antioxidant status of layer chickens. Supplementation of ginger powder and CHM to diet increased the egg production, feed consumptions, serum glucose and total protein level of layers. Under high temperature, supplemented feed also improved the chicken antioxidant status by increasing their antioxidant enzymatic activities but the reduced concentration of MDA. The mixtures of CHM and ginger had much better results on production performance and antioxidant status, compared to using them as a sole agent in layers diet.

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Authors contribution

Ibtisham, Nawab and Niu has conceived the idea, collected the data and drafted the manuscript. Wang Participated in result analysis, Wu, Xiao and An have revised the manuscript.

Competing interests

The authors declare that they have no competing interests.

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