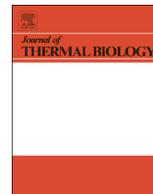




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Dietary *Moringa oleifera* improves growth performance, oxidative status, and immune related gene expression in broilers under normal and high temperature conditions

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ABSTRACT

The aim of this study is to evaluate the effects of *Moringa oleifera* (MO) on the performance, antioxidative status, and immune related gene expression in broilers raised under normal or heat stress conditions. Broiler chickens were distributed into 4 groups and fed diets with dietary MO at 0% or 5% (MO0 or MO5) and raised under ambient temperature $22 \pm 1^\circ\text{C}$ (N) or $35 \pm 1^\circ\text{C}$ (HS). HS conditions negatively affected the weight gain and FCR, while feeding MO exhibited beneficial effects especially under HS conditions. Triglycerides, total cholesterol and low-density lipoprotein cholesterol (LDL) levels were significantly ($P < 0.05$) higher in chickens raised in HS conditions and fed the basal diet than those in normal condition and fed with or without MO, while MO decreased triglycerides and total cholesterol levels in normal and HS conditions. Blood high-density lipoprotein cholesterol (HDL) was significantly decreased in broilers raised in HS conditions and fed diets without MO, while MO increased HDL level. Blood glutathione peroxidase (GSH-Px) was significantly ($P < 0.05$) decreased in broilers raised in HS conditions and fed the basal diet without MO. mRNA expression of GSH-Px was significantly ($P < 0.05$) downregulated in broilers raised in HS conditions and fed diets without MO. Broilers under normal or HS conditions and fed the basal diet exhibited significantly ($P < 0.05$) downregulated mRNA expressions of superoxide dismutase (SOD) and catalase (CAT) compared to chickens under normal conditions and fed MO. Liver and muscle thiobarbituric acid reactive substance (TBARS) were significantly ($P < 0.05$) increased in broilers under HS conditions and fed diet without MO. The expressions of interleukins (IL2 and IL6) were significantly ($P < 0.05$) downregulated in broilers under normal or HS conditions and fed diets without MO. To sum up, HS conditions depressed the performance, antioxidative status, and immune related gene expression in broilers, while MO obviously alleviated these negative effects in broilers.

1. Introduction

Broilers are usually suffering from heat stress (HS) due to its high metabolic and growth rates (Nawab et al., 2018). Severe HS depress the growth performance, physiological metabolism, immune function, and induce oxidative damage in organs of birds (Huang, 2017; Wan et al., 2018). During HS, the chicken's immune system is also suppressed and lose its ability to defend the bird's body from the infectious diseases (Nawab et al., 2018). HS decreased the ratio of immunoglobulins (IgG and IgM) and systemic humoral responses (Alagawany et al., 2017; Lara and Rostagno, 2013). Furthermore, the phagocytic and oxidative burst activities are reduced during HS in broilers (Gomes et al., 2014). HS can

change the ratio of circulating cells and increase the ratio of heterophil to lymphocyte, due to lower lymphocytes and higher number of heterophils (Lara and Rostagno, 2013; Prieto and Campo, 2010).

Boosting birds' immunity during HS is an essential focus for broiler farmers to control the harmful effects of HS. In this regard, different strategies have been applied, such as the inclusion of feed additives. The supplementation of functional or natural extracts rich in antioxidants may influence the metabolic homeostasis as well as the immunity of animals, birds and fish (Adel et al., 2016, 2017; Dawood et al., 2018, Dawood and Koshio, 2018; Cheng et al., 2018; Sudha et al., 2010; Sebola et al., 2015; Qwele et al., 2013; Sreelatha and Padma, 2009). *Moringa oleifera* (MO) is a highly valued plant in tropic and

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subtropical countries where it is mostly cultivated as a medicinal plant (Moyo et al., 2012; Sreelatha and Padma, 2009; Verma et al., 2009). *M. oleifera* leaves are rich of carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics which provide it with the antioxidative and immunostimulant potential (Abdel-Daim et al., 2017; Moyo et al., 2012; Verma et al., 2009). MO contains a lot of biological compounds which has antifungal, anti-inflammatory, anti-tumor and antioxidant properties (Falowo et al., 2018; Verma et al., 2009). Several studies concluded that, the inclusion of MO in chicken diets resulted in better growth performance, immune response and antioxidative status (Khan et al., 2017; Ogheneborhie and Oghenesuwue, 2016; Nkukwana et al., 2014a,b; Yeung et al., 2019). Cui et al. (2018) reported that dietary MO supplementation can act as ROS scavenger to improve the antioxidant capacity by activating the antioxidant enzymes and reducing the level of oxidative enzymes in broilers. Furthermore, Mousa et al. (2017) have reported that the inclusion of dietary MO could improve the immune response of chickens.

So far, there was no data reported on the effect of HS on the antioxidant and immune responses of broilers fed MO additives. Thus, we hypothesized that MO addition could exert beneficial effects on broilers performance under HS. The current study was conducted to evaluate the effects of dietary MO supplementation on growth performance, blood health, the antioxidant ability and immune related gene expressions in broilers subjected to HS.

2. Materials and methods

2.1. Ethical approval

All handling of birds was conducted in accordance with the guidelines for the care and use of animals for scientific purposes established by the “Ethics Committee of the Kagoshima University, Japan”.

2.2. Birds and management

Broiler chicks (Chunky strain) were supplied by a commercial hatchery (Kumiai Hina Center, Kagoshima, Japan). The chicks were housed in an electrically heated battery brooder and provided with water and a commercial starter diet (22% crude protein and 3000 kcal/kg; Nichiwa Sangyou Company) until 12 d of age. On day 12, twenty-four birds were selected with similar body weight and were housed individually in wire-bottomed aluminium cages (Mahmoud H et al., 2016). The selected chickens were distributed into 4 groups where each group has 6 birds where each bird was considered as a replicate ($n = 6$) from 15 to 30 d of age. The birds were preconditioned for 3 d before the treatment and fed on a basal diet.

The experimental diets were formulated using mainly ground yellow maize and a soybean meal, as shown in Table 1. *M. oleifera* leaves (MO) were supplemented at 0% or 5% (MO0 or MO5) and fed to birds which maintained at $22 \pm 1^\circ\text{C}$ (normal, N) or $35 \pm 1^\circ\text{C}$ (heat stress, HS) to be (N/MO0, N/MO5, HS/MO0 or HS/MO5 groups). The chemical composition of the basal diet was analyzed according to AOAC (2005) (Table 1). Diets were burned in muffle at 600°C then, ash, Ca, and P were analyzed according to the AOAC (method 942.05; 927.02; 965.17; respectively) AOAC (2005). The sodium content in diets was analyzed by atomic absorption spectrometry (ISO 6869, 2000). The experiment was conducted in a temperature-controlled room with a 24 h of light and the birds were given the test diets from 15 to 30 d of age. The birds were kept at moderate temperature ($22 \pm 1^\circ\text{C}$), while another group of birds were subjected to acute HS ($35 \pm 1^\circ\text{C}$ for 9 h) with a relative humidity from 50 to 70% throughout the experiment. Temperature and relative humidity were inspected 3 times a day using digital thermometer to ensure no fluctuations in the temperature and ventilation. The accuracy of the thermometers was tested twice a day using manual temperature thermometer and a hygrometer, which were placed at the center of the house and beside the digital thermometers, to

Table 1
Formulation and composition of the basal diet.

Ingredients	%
Corn meal	55.10
Alfalfa meal	2.90
Soy bean meal	33.50
Corn oil	4.70
DL-Methionine	0.14
CaHPO ₄	2.00
CaCO ₃	0.66
NaCl	0.50
Mineral and vitamin primex ^a	0.50
Composition	
Crude protein (%)	20.0
Metabolizable energy (Mcal/kg)	3100
Ca (%)	1.00
Available P (%)	0.62
Na (%)	0.21

^a Content per kg of the vitamin and mineral premix: vitamin A90 mg; vitamin D3 1 mg; DL-alpha-tocopherol acetate 2000 mg; vitamin K3 229 mg; thiamin nitrate 444 mg; riboflavin 720 mg; calcium d-pantothenate 2174 mg; nicotinamide 7000 mg; pyridoxine hydrochloride 700 mg; biotin 30 mg; folic acid 110 mg; cyanocobalamine 2 mg; calcium iodinate 108 mg; MgO 198,991 mg; MnSO₄ 32,985 mg; ZnSO₄ 19,753 mg; FeSO₄ 43,523 mg; CuSO₄ 4019 mg and choline chloride 299,608 mg. CRWW, concentrated rice-washing waster.

make sure the readings match. No clinical signs were observed in broilers raised in neutral temperature while those under HS condition were suffering from labored breathing, panting, pale combs/wattles and lifting wings away from body during the HS period (9 h).

Body weight was recorded every 6 d, and feed intake was recorded daily during the experimental period, and feed conversion ratio (FCR, feed/gain) was calculated using the following formulae:

$$\text{WG} = \text{FBW} - \text{IBW}; \text{FCR} = \text{FI} / \text{WG}$$

Where FBW = body weight final (g), IBW = body weight initial (g), WG = weight gain (g).

At the end of the experimental period, the birds were slaughtered, and liver and skeletal muscle were dissected out and stored at -80°C until further analysis ($n = 6$). Then the blood samples were collected into heparinized test-tubes, quickly centrifuged at 5900 g for 10 min at 4°C to separate plasma and stored at -30°C until analysis.

2.3. Biochemical analysis

Total cholesterol levels, triglycerides, high and low-density lipoprotein cholesterol (HDL and LDL) in plasma were measured by “an automated Fuji DRY-CHEM 3500 (Fuji Medical Systems)”, according to the manufacturer’s instructions.

The activity of the glutathione peroxidase (GSH-Px) of blood serum was measured according to Paglia and Valentine (1967). To evaluate lipid peroxidation levels in liver and skeletal muscle of chickens, malondialdehyde content was determined calorimetrically as 2-thiobarbituric acid reactive substances (TBARS) according to the method described by Azada et al. (2010).

2.4. RNA extraction and real-time PCR

Total RNA was extracted from a piece of liver (about 50 mg) using an ISOGEN II Kit, according to the manufacturer’s protocol ($n = 6$). RNA concentration and purity were determined by NANODROP LITE Spectrophotometer, Thermo scientific, S17NPN0027, USA. Complementary DNA was synthesized at 40 ng RNA per 10 μl of the reaction solution with the PrimeScript™ RT Master Mix Kit (Perfect Real Time; Takara) using the Program Temp Control System PC320 (Asterc)

Table 2

List of primers sequences used for qualitative real time polymerase chain reaction in chickens.

Gene		Sequence (5'-3')	Size (BP)	Accession no.
GSH-Px	Forward	TTGTAACATCAGGGGCAAA	140	NM_001163245.1
	Reverse	TGGGCCAAGATCTTTCTGTAA		
SOD	Forward	AGGGGGTCATCCACTTCC	122	NM_205064.1
	Reverse	CCCATTGTGTGTCTCCAA		
CAT	Forward	GGGGAGCTGTTACTGCAAG	138	AJ719360.1
	Reverse	CTTCCATTGGCTATGGCATT		
Interleukin-2	Forward	TGCAGTGTACCTGGGAGAA	148	GU119890.1
	Reverse	CTTGCATTCACTTCGGGTGT		
Interleukin-6	Forward	GACTCGTCCGGAGGAGGTTG	138	HM179640.1
	Reverse	CGCACACGGTGAACCTTCTT		
18s ribosomal RNA	Forward	AAACGGCTACCCACATCCAAG	154	KC433410.1
	Reverse	CCTCCAATGGATCCTCGTTA		

with the following protocol: reverse transcription at 37 °C for 15 min; inactivation of RT at 85 °C for 5 s; refrigeration at 4 °C for 5 min. The primers used in this study are listed in Table 2. Gene expression was measured by real-time PCR using the 7300 Real Time PCR system (Applied Biosystems) with the SYBR[®] Select Master Mix. The thermal cycle was as follows: one cycle at 50 °C for 2 min and 95 °C for 2 min and sixty cycles at 95 °C for 15 s, 60 °C for 15 s and at 72 °C for 1 min. The expression of 18s ribosomal RNA was used as an internal standard and was not significantly different between the experimental groups. Results of gene expression are expressed as the percentage of the control value.

2.5. Statistical analysis

Shapiro-Wilk and Levene tests confirmed normal distribution and homogeneity of variance. All significant differences (for growth performance, blood parameters and qRT-PCR data) were assessed by one-way ANOVA (SPSS version 22, SPSS Inc., IL, USA) with Duncan's post hoc tests where differences in experimental groups occurred. The level of significance was defined as $P < 0.05$. All data are presented as the mean \pm standard error (SE).

3. Results

3.1. Growth performance

The growth performance of broilers raised under normal and HS conditions and fed diets supplemented with or without MO are displayed in Table 3. The final body weight was not affected significantly ($P > 0.05$) by MO supplementation. However, the body weight gain was significantly ($P < 0.05$) decreased in broilers raised in HS conditions compared to chickens raised in normal conditions without no clear effect for MO supplementation in case of normal or HS conditions. Feed conversion ratio followed the same trend and decreased ($P < 0.05$) in broiler chickens raised in HS conditions and fed the basal diet without MO supplementation compared to the normal conditions without no difference with the other groups.

Table 3

Effect of dietary MO on growth performance in broiler chickens under normal or HS conditions.

	N/MO0	N/MO5	HS/MO0	HS/MO5
Final body weight (g)	1341.5 \pm 52.8	1188.8 \pm 19.4	1207.6 \pm 91	1214.8 \pm 66.5
Body weight gain (g)	810.5 \pm 36.6 ^a	667.76 \pm 24.2 ^{ab}	601.7 \pm 84.6 ^b	632.6 \pm 44.9 ^{ab}
Feed intake (g/15 days)	1323.8 \pm 72.1	1110 \pm 24.5	1073.04 \pm 131.7	1120.9 \pm 70.5
Feed conversion ratio	1.63 \pm 0.001 ^a	1.66 \pm 0.001 ^{ab}	1.52 \pm 0.01 ^b	1.63 \pm 0.03 ^{ab}

* Values are means \pm SE ($n = 6$). Different letters indicate significant differences ($P < 0.05$).

3.2. Blood biomarkers

Triglycerides, total cholesterol and LDL levels were significantly ($P < 0.05$) higher in chickens raised in HS conditions and fed the basal diet than those raised in normal condition and fed with or without MO, while MO supplementation significantly ($P < 0.05$) decreased triglycerides and total cholesterol levels in normal and HS conditions (Table 4). HDL was significantly decreased in broilers raised in HS conditions and fed diets without MO compared with the other groups, while MO supplementation significantly ($P < 0.05$) increased HDL level compared to chickens raised in HS conditions (Table 4).

Blood GSH-Px was significantly ($P < 0.05$) decreased in broilers raised in HS conditions and fed the basal diet without MO compared to the other groups, while broiler chickens raised in HS conditions and fed MO exhibited higher blood GSH-Px than those fed without MO in case of HS conditions but lower than broilers raised in normal conditions (Fig. 1).

3.3. Antioxidant status

mRNA expression of GSH-Px was significantly ($P < 0.05$) down-regulated in broilers raised in HS conditions and fed diets without MO without no difference with those under the normal conditions and fed the basal diet (Fig. 2A). Broilers under normal or HS conditions and fed the basal diet only exhibited significantly ($P < 0.05$) downregulated mRNA expression of SOD compared to chickens under normal conditions and fed MO (Fig. 2B). mRNA expression of CAT was significantly ($P < 0.05$) downregulated also in broilers under HS conditions and fed the basal diet compared to those under the normal conditions and fed MO without no differences with the other groups (Fig. 2C).

Liver TBARS was significantly ($P < 0.05$) increased in broilers under HS conditions and fed diet without MO compared to the other groups (Fig. 3A). The lowest level of liver TBARS was observed in case of broilers under normal conditions and fed MO. Also, muscle TBARS was significantly ($P < 0.05$) increased in broilers under HS conditions and fed diet without MO compared to the other groups without no difference with those raised under HS and fed MO (Fig. 3B).

Table 4
Effect of dietary MO on growth performance and relative tissue weights in broiler chickens under normal or HS conditions.

	N/MO0	N/MO5	HS/MO0	HS/MO5
Triglycerides (mg/dl)	34.83 ± 0.79 ^c	30.33 ± 0.76 ^d	57.83 ± 11.79 ^a	43.5 ± 1.38 ^b
Total cholesterol (mg/dl)	179.83 ± 1.92 ^c	177.5 ± 5.27 ^{cd}	276.5 ± 2.36 ^a	246.17 ± 13.36 ^b
HDL (mg/dl)	57.33 ± 1.93 ^a	56.17 ± 1.62 ^a	35.33 ± 1.93 ^c	41.67 ± 1.61 ^b
LDL (mg/dl)	137 ± 2.21 ^c	131.33 ± 1.17 ^c	247.83 ± 3.68 ^a	202.17 ± 1.83 ^b

* Values are means ± SE (n = 6). Different letters indicate significant differences (P < 0.05).

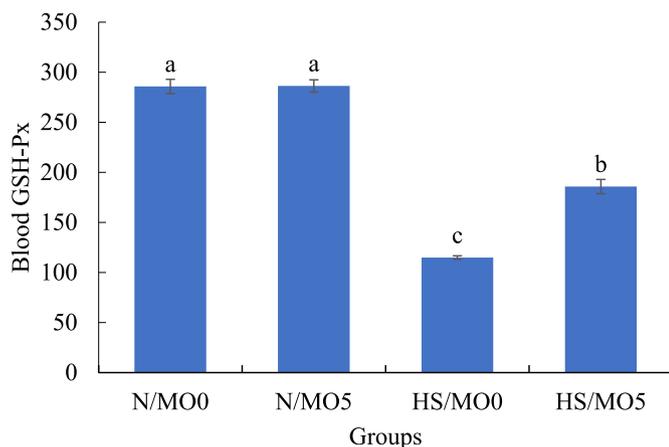


Fig. 1. Blood glutathione peroxidase (GSH-Px) (U/L) of broiler chickens fed diets with or without moringa in normal and heat stress conditions. Results are expressed as ratios relative to the expression of genes in control group, whose expression levels were equal to 100%, and reflect the means ± SE (n = 6). Means within columns carrying different superscript letters are significantly different from control (P ≤ 0.05).

3.4. Immunity

mRNA expression of IL2 and IL6 of broiler chickens fed diets with or without MO in normal or HS conditions are presented in Fig. 4A and B. The expressions of IL2 and IL6 were significantly (P < 0.05) down-regulated in broilers under normal or HS conditions and fed diets without MO compared to those fed MO. The highest levels of IL2 and IL6 were observed in case of chickens under normal conditions and fed MO at 5%.

4. Discussion

High temperature above 30 °C results in severe stress on broiler chickens (Seifi et al., 2018). This study investigated the potential of using dietary MO in preventing HS-induced changes on the growth performance, oxidative status and immunity of broilers. To our knowledge, no published data on the detailed effects of MO supplements on the performance of broilers under HS conditions. In this study, we revealed for the first time the mechanistic effects of MO on broilers growth, blood profile, antioxidative status and immune related gene expressions.

HS is well known of reducing the growth performance of broilers (Al-Harathi et al., 2002; Dagher, 2008). In this study, the final body weight was decreased in chickens raised in HS condition and followed by decreased feed intake and feed conversion ratio (FCR). When birds are grown in HS conditions, the chicken's appetite and feed consumption are decreased to lower the heat load which leads to low weight gain (Azada et al., 2010; Laganá et al., 2007; Habashy et al., 2017a). Also, HS can negatively change the expressions of mRNA of related nutrients transporters in broilers, and then decreasing the utilization of nutrients "e.g. oligopeptides, glucose, lipids, and amino acids" which accordingly reduce the feed utilization (Habashy et al., 2017a, 2017b).

Furthermore, during HS conditions blood flow shifts from the internal organs to the periphery for heat dissipation to the exterior which may reduce nutrient absorption in the intestine (Dagher, 2008). Hence, decreased nutrient absorption as well as increased energy expenditure for heat dissipation, lowers the FCR. The reduced feed intake is attached to the declined intestinal absorption which then reduces the growth and health status of birds under HS conditions.

A lot of studies have illustrated the positive effect of MO on broilers performance and health status (Khan et al., 2017; Nkukwana et al., 2014a,b; Ogheneborhie and Oghenesuvwe, 2016). The present study also confirmed that dietary inclusion of MO had positive effects on the growth performance of broilers under HS conditions. Sebola et al. (2015) and Nkukwana et al. (2014a,b) have demonstrated that dietary MO supplementation can improve the growth performance of broiler chickens. The enhanced weight gain and feed efficiency of broilers fed MO in the present study would be attributed to the enhanced FCR.

Blood biochemical markers can be used as physiological biomarkers to recognize probable alterations in the organism health raised in stressed conditions (Dawood et al., 2019a,b; Lu et al., 2016). In this study, blood cholesterol, triglycerides and LDL, all were increased in case of chickens raised in HS conditions, while HDL was decreased. Under HS conditions, MO decreased blood cholesterol, triglycerides and LDL with increased HDL level. The low levels of cholesterol, triglycerides and LDL in broilers consumed MO is in parallel to earlier findings by Balami et al. (2018). Recently Zanu et al. (2012) reported the effect of MO to decrease the cholesterol, triglycerides and LDL in broilers. MO contains high levels of "polyphenols, flavonoids, alkaloids and phenolic compounds" which represents hypocholesterolaemic effect (Verma et al., 2009). Also, contains high level of crude fiber which may be responsible for less absorption of triglycerides and cholesterol from the intestinal tract of the birds (Mandal et al., 2014). HDL can help in the passage of excess fatty acids and cholesterol from the body different tissues to the liver, then taking away from the body (Balami et al., 2018; Blake et al., 2002). The findings of this study revealed that, MO supplementation in the diet of broilers had beneficial influence on the lipid profile of broiler chickens. This agrees with the result of Olugbemi et al. (2010) and that of Ashong and Brown (2011) who reported that MO had positive impact on the lipid profile of broiler chickens.

HS could also induce severe effects on the biological function in broilers including, immunosuppression and impaired oxidative status which could weaken the general health (Habashy et al., 2017a,b; Han et al., 2010). These reductions further explain the reduction in the growth performance of broilers raised in HS conditions. HS is among the stressors which cause the production of ROS in broiler chickens' cells (Apel and Hirt, 2004). Among the antioxidant defence enzymes are SOD, CAT and GSH-Px (Abdel-Daim et al., 2018a,b; Huang et al., 2015). In this study, HS resulted in impaired antioxidant capacity in chickens when compared to those raised in neutral temperature. However, MO supplementation resulted in improved antioxidant capacity (SOD, CAT and GSH-Px enzymes). The improved activity of GSH-Px indicate the defensive role of MO in animal cells via "the maintenance of thiol-redox status and detoxification of exogenous and endogenous reactive molecules" (Mujahid et al., 2005). GSH plays a vital role against the damaging effects of bacteria, viruses, pollutants and free radicals. The obtained results are in agreement with the findings of Cui et al. (2018)

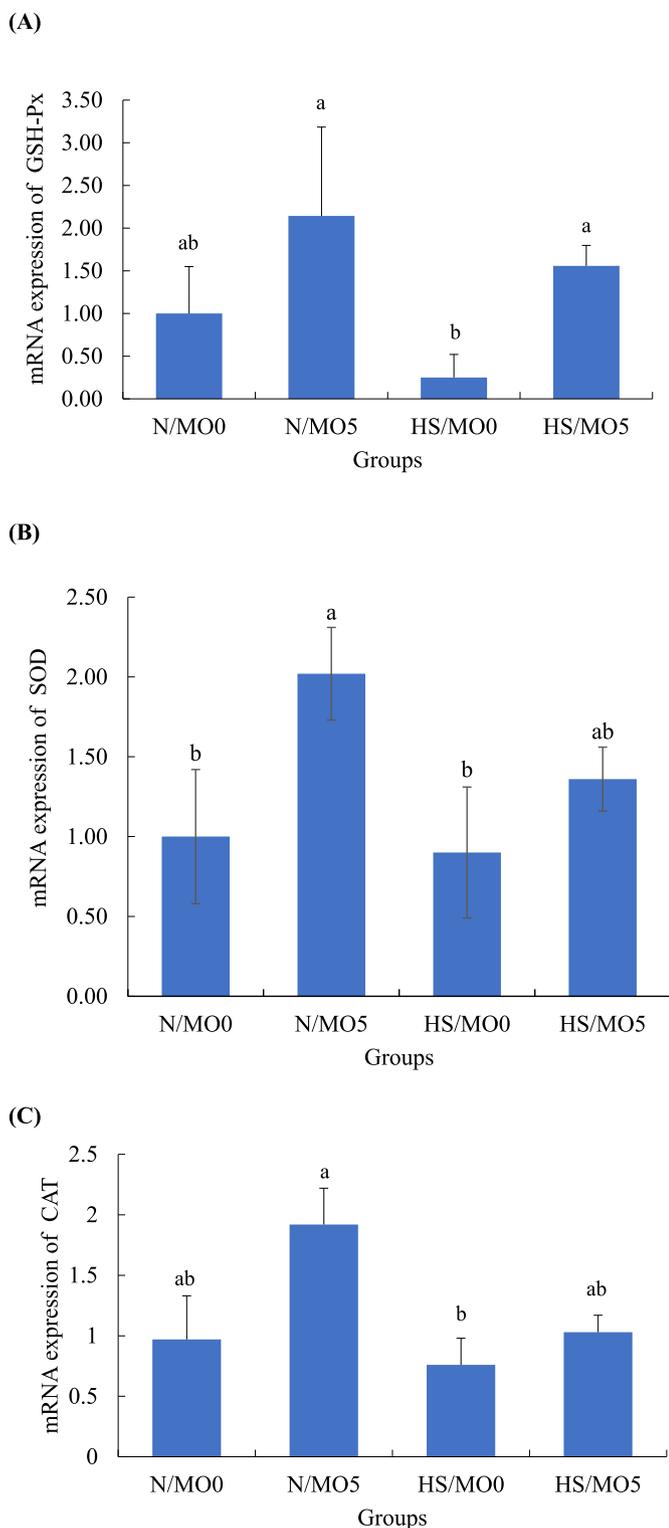


Fig. 2. mRNA expression of (A) glutathione peroxidase (GSH-Px), (B) superoxide dismutase (SOD) and (C) catalase (CAT) of broiler chickens fed diets with or without moringa in normal and heat stress conditions. Results are expressed as ratios relative to the expression of genes in control group, whose expression levels were equal to 100%, and reflect the means \pm SE ($n = 6$). Means within columns carrying different superscript letters are significantly different from control ($P \leq 0.05$).

who reported a protective role of MO in broilers. SOD act significantly to protect the animal cells from the excessive production of ROS. SOD converted superoxide radical to H_2O_2 and molecular O_2 which in turn

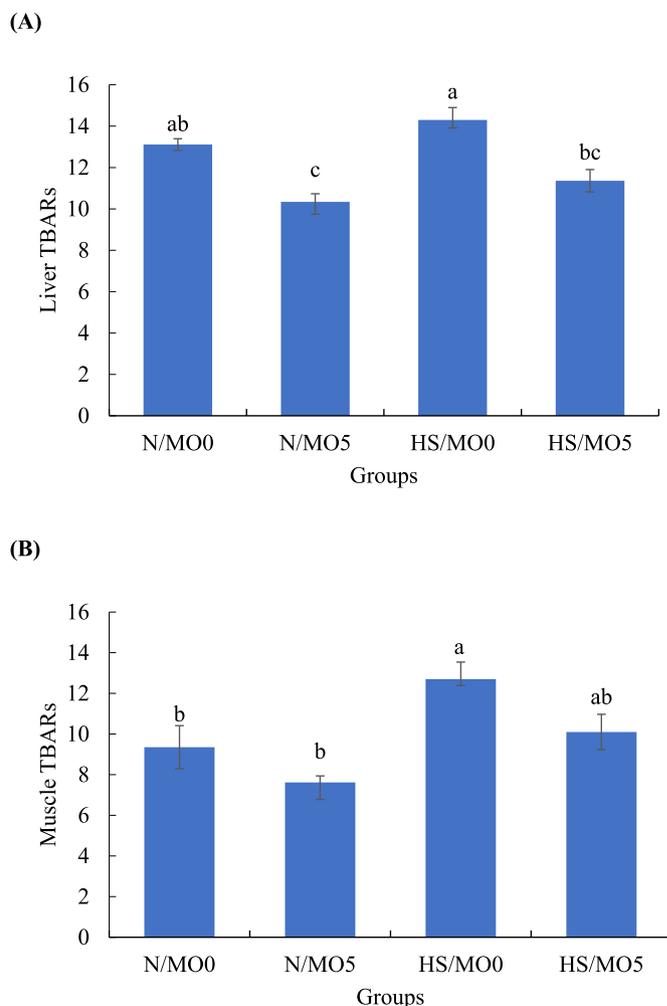


Fig. 3. Thiobarbituric acid reactive substance (TBARs) in (A) liver and (B) muscle of broiler chickens fed diets with or without moringa in normal and heat stress conditions. Means within columns carrying different superscript letters are significantly different from control ($P \leq 0.05$) ($n = 6$).

can be counteracted by catalase or GSH-Px reaction thereby reducing the cellular damage (Curtis et al., 1972). MO supplementation increased the activity of SOD in this study especially under HS conditions which indicated its ability to protect the chickens from oxidation. The effect of MO to decrease ROS is due to its content of phenolics and flavonoid (Robak and Gryglewski, 1988). The levels of CAT were increased in chickens fed MO indicating the ability of MO to improve the antioxidative status of broilers raised in HS conditions. CAT decomposes H_2O_2 and protect tissues from ROS (Sreelatha and Padma, 2009). This enzyme prevents the generation of hydroxyl radical and protects cellular constituents from oxidative damage in peroxisomes (Ashok Kumar and Pari, 2003). In this study, the differences among groups suggest the ability of MO on the antioxidant activity of chickens. TBARs is normally used as a measure of lipid oxidation in broiler liver and muscle (Nkukwana et al., 2014a,b). MO leaves as natural antioxidants are well known by its high oxidative stability and it contains flavonols quercetin and “kaempferol” in their “3'-O-glycoside” compounds with radical scavenging properties (Anwar et al., 2007; Joshi and Mehta, 2010; Mbikay, 2012; Park et al., 2011). Surprisingly, birds fed MO diets had the highest TBARs even those raised under HS conditions. Similarly, supplementation of MO was reported to exhibit the highest antioxidant capacity in broilers (Cui et al., 2018; Nkukwana et al., 2014a,b). The functionality of MO is due to its rich composition “e.g. carotenoids, vitamins, minerals, amino acids, sterols, glycosides,

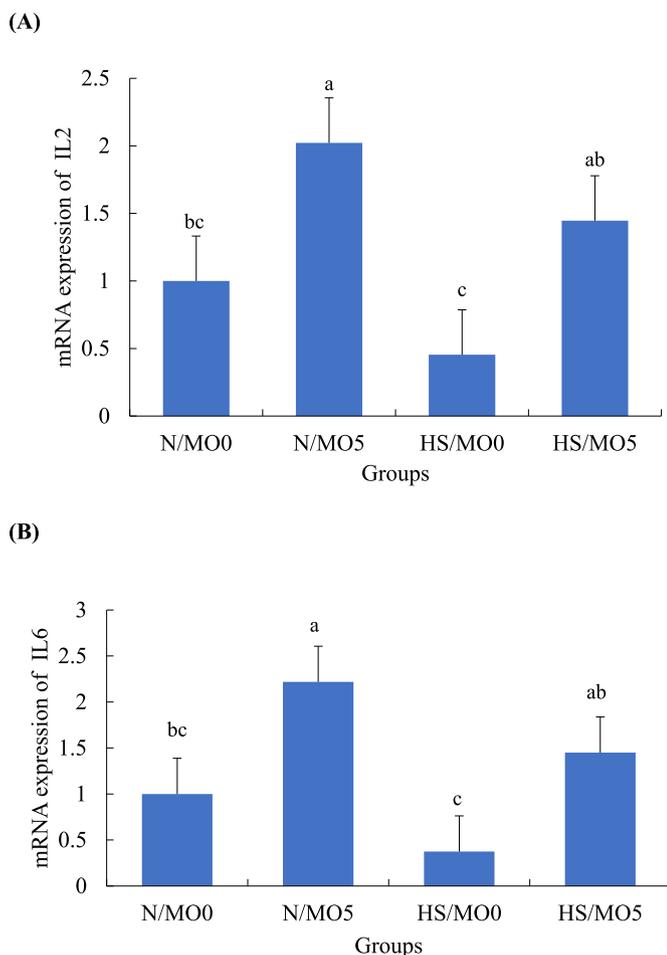


Fig. 4. mRNA expression of interleukins (A) IL2 and (C) IL6 of broiler chickens fed diets with or without moringa in normal and heat stress conditions. Results are expressed as ratios relative to the expression of genes in control group, whose expression levels were equal to 100%, and reflect the means \pm SE ($n = 6$). Means within columns carrying different superscript letters are significantly different from control ($P \leq 0.05$).

alkaloids, flavonoids and phenolics” which help to show the anti-oxidative activity of MO (Falowo et al., 2018; Verma et al., 2009).

Chickens raised in HS conditions also showed impaired immune response and, consequently, reduced resistance against diseases (Mahmoud H et al., 2016). It has been reported that MO feeding ameliorated the immune system of chickens (Mousa et al., 2017). In this study, MO supplementation upregulated the mRNA expression of IL-2 and IL-6 in chickens under HS condition. These results suggest that MO has an immunostimulatory effect in chickens, consequently alleviating the adverse effects of HS. Similar results were obtained by Kilany et al. (2018) when birds fed MO. IL-6 is secreted by T cells and macrophage to simulate immune response during infection or other tissue damage leading to inflammation. IL6's role as anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and activation of IL1 and IL10 (Kushner, 1993). It promotes differentiation of B cells into plasma cells, activates cytotoxic T cells, and regulates bone homeostasis (Arango Duque and Descoteaux, 2014).

Although, there are a small number of studies which have revealed the mechanism of action of the immunostimulatory compounds of herbal plants, but the exact molecular mechanisms of some herbs are not already known (Abdelkhalek et al., 2017; Hashemi and Davoodi, 2012). There are possible explanations for immunomodulation mechanisms of herbal plants and their derivatives that have been put forward. Our attempt here would be to look more closely at the herbal

plant's mechanisms involved, including from the immunomodulation point of view and relationships between structures and activities. Thus, further mechanistic studies are needed to reveal the role of MO in improving the immune response of chicken broilers.

5. Conclusion

From the obtained results, it can be concluded that MO supplementation could ameliorate the negative impacts of HS. MO supplementation of broiler's diet would also modulate the immune response by regulating mRNA expression levels of the innate immune response mediators, such as IL2 and IL6, and alleviating the degenerative changes that occurred in live tissue following HS. In addition, dietary MO supplementation improved the oxidative status by increasing the level of antioxidant enzyme activities (SOD, CAT and GSH-Px) and reducing the TBARS content in broilers under normal or HS conditions.

The limitation of this study is the use of limited number of birds in each group, and it is recommended to use a greater number of chicks to evaluate the effect of dietary MO in improving the growth performance, oxidative status, and immune related gene expression in broilers. Further knowledge of molecular mechanisms will be required to evaluate all possible mechanisms involved in feeding of MO on the oxidative status and immune response under normal and heat stress conditions.

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