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Response of broiler chicks to diets supplemented with *Moringa Oleifera* dry leaves and some antioxidants under tropical summer conditions

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The present study was designed to evaluate the beneficial effects of *Moringa oleifera* dry leaves (MOL) with some antioxidants to improve heat tolerance of broiler chicks under heat stress condition. A total number of 250 commercial broiler chicks were randomly assigned to five dietary treatments (commercial basal diet as control; commercial basal diet supplemented with 0.2% MOL; 0.2% MOL+200 mg/kg vitamin E; 0.2% MOL+200 mg/kg vitamin C and 0.2% MOL+0.35 g/kg organic zinc). Results showed that adding MOL with vitamin E, vitamin C or zinc significantly ($p<0.05$) increased body weight gain as compared with the control and MOL. Feed conversion ratio was significantly improved with all treatment groups compared with the control. All dietary supplements significantly improved hemoglobin concentration, heterophils to lymphocytes ratio, plasma total protein, albumin and globulin levels. Aspartate aminotransferase decreased significantly while, alanine aminotransferase did not affected by dietary supplements. Thyroid hormone T_3 was significantly increased with MOL+zinc and T4 significantly increased with all dietary supplements, while the T_3/T_4 ratio not changed. Moreover, it has been noticed a significant increase occurrence in values of glutathione peroxidase and super-oxide dismutase. Malondialdehyde level was significantly decreased. The results of the present study indicated that using MOL in broiler diets with other antioxidant (vitamin E, vitamin C or organic zinc) had a beneficial effect on performance and overcome deleterious impacts of heat stress.

Keywords: ***Moringa oleifera,*** **broiler, heat stress, vitamin, zinc.**

INTRODUCTION

The relatively high environmental temperature is considered a major constraint to poultry production in tropical countries. Published research work showed that high environmental temperature that exceeds those of safe thermal zone resulted in low growth, poor feed conversion and consequently low live body weight (Estevez, 2007). Antioxidants are known to be helpful agents that can combat the effect of heat stress. Amongst the most popular antioxidants is vitamin C, which is a natural component of different plants. Khan et al., (2012) showed that vitamin C supplementation to the diets of birds removed the oxidative injuries of chicks raised under heat stress conditions. Luqman et al., (2012) and William et al., (2014) showed that *Moringa* leaves are a rich source of vitamins C and E and polyphenolic compounds which are considered important agents in combating the free radicals. Vitamin E level in the diet is also essential for the performance of birds as it affects the nervous system and the immune system (Habibian et al., 2014). Vitamin E supplementation to broiler diets at higher levels during heat stress has resulted in positive effects on growth performance (Sahin et al., 2002). Zinc (Zn) is used in poultry diets because of its anti-stress effects and plays a crucial role in enhancing immune function and maintaining cell integrity by avoiding oxidative damage (Sahin et al., 2009). Moreover, it is evident from different research work that the requirement of Zn is increased and its retention decreases during stress that indicates the importance of supplementation of Zn during stress (Imtiaz et al., 2014). This study was designed to evaluate the beneficial effects of adding *Moringa oleifera* dry leaves as antioxidant agent with other antioxidants (vitamin E, vitamin C or organic zinc) to broiler diets raised under heat stress conditions.

MATERIALS AND METHODS

Experimental chicks

A total number of two hundred and fifty, one-day old commercial broiler chicks (Cobb) were assigned randomly to five groups (10 replicates of 5 birds each). Replicates were randomly allocated in batteries of three tire system that has 50 compartments. The broilers received a commercial starter diet (1- 21 days) containing 22% of crude protein (CP) and 3100 kcal/kg, while, grower diet (21-37 days) containing 20% CP and 3000 kcal/kg diet. The average initial live body weight of all replicates was nearly similar (44 gram). The five dietary treatments involved (commercial basal diet as control; commercial basal diet supplemented with 0.2% MOL; 0.2% MOL+200 mg/kg vitamin E; 0.2% MOL+200 mg/kg vitamin C and 0.2% MOL+0.35 g/kg organic zinc). All groups were maintained under the same environmental and managerial conditions, water and feed were provided *ad-libitum* 24 h /day during the experimental period and the range of environmental maximum temperature was 32-38°C.

Performance measurements

Birds were weighted and feed consumption was recorded per replicate weekly after fasting overnight. Body weight gain and feed conversion ratio were then calculated.

Physiological and biochemical measurements

At the end of the experiment (37 days) blood samples were collected from ten birds for each treatment in heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min. and plasma obtained was stored at -20 ^{o}C in eppendorf tubes until analysis. Few drops of fresh blood samples were taken to determine blood hemoglobin (Hb) and packed cell volume (hematocrit, Ht). Blood smears were also done, stained with Wright's stain procedure and used to calculate the number of lymphocytes (L) and heterophils (H) in 100 white blood cells then the H/L ratio was calculated. Total proteins were determined according to the method described by Henry (1974). Albumin was determined according to Doumas et al., (1971). Globulin was calculated by subtraction of albumin from total protein. Enzyme activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method of Moussa et al., (2016)**.** Plasma triiodothyronine (T_3) and thyroxin (T_4) were determined according to Akiba et al. (1982). Glutathione peroxidase enzyme (GPX) activity was determined according to Bell et al., (1986), superoxide dismutase (SOD) activity was measured by the method of Marklund and Marklund (1974) and malondialdehyde (MDA) was determined by the method described by Placer et al., (1966).

Statistical analysis

The obtained data were statistically analyzed using the general linear model procedure described in SAS User's Guid (SAS, 2001). Differences among means were tested using Duncan's multiple range tests (Duncan's, 1955).

RESULTS

Growth performance

Performance of chicks fed the different experimental diets during the two feeding intervals (1-21 and 22-37 days of age) and the entire period (1-37 days of age) is summarized in Table (1). The results showed that body weight (BW) and body weight gain (BWG) was significantly ($p<0.05$) increased in birds fed *Moringa olefiera* Leaves (MOL) supplemented diets compared to the control. However adding vitamin E, vitamin C or zinc with MOL gave significantly ($p<0.05$) more increased in BW and BWG. During the intervals periods there were no significant differences in BWG among birds fed diets supplemented with MOL+vitamin E, MOL+vitamin C or MOL+zinc while, the entire period the highest BWG was resulted with birds fed MOL+zinc followed by those fed MOL + vitamin C then those fed MOL+vitamin E with a significant differences among them ($p<0.05$).

Table 1. Effect of treatments on growth performance of broiler chicks.

Item	Control	MOL	MOL+ Vit E	MOL+ Vit C	MOL+ Zn
BW (21 d)	483.7[c]	514.1[b]	541.7[a]	543.6[a]	546.9[a]
BW (37 d)	1391.2[e]	1571.5[d]	1671.2[c]	1677.9[b]	1683.5[a]
BWG (1-21 d)	439.1[c]	470.4[b]	497.5[a]	499.1[a]	502.6[a]
BWG (21-37 d)	907.5[c]	1057.4[b]	1129.5[a]	1134.3[a]	1136.6[a]
BWG (1-37 d)	1346.6[e]	1527.7[d]	1626.9[c]	1633.4[b]	1639.2[a]
FI (1-21 d)	807.8[c]	862.6[b]	906.4[a]	908.2[a]	913.6[a]
FI (21-37 d)	2472.6[a]	2402.1[b]	2348.9[c]	2356.1[c]	2358.1[c]
FI (1-37 d)	3280.3[a]	3264.6[b]	3255.3[c]	3264.3[b]	3271.7[b]
FCR (1-21 d)	1.839[a]	1.833[a]	1.822[b]	1.820[b]	1.817[b]
FCR (21-37 d)	2.725[a]	2.272[b]	2.079[c]	2.077[c]	2.075[c]
FCR (1-37 d)	2.436[a]	2.137[b]	2.001[c]	1.998[c]	1.996[c]

[a, b , c] Means within a row with different superscripts are significantly different ($P \leq 0.05$).

Table 2. Effect of treatments on some hematological parameters of broiler chicks.

Item	Control	MOL	MOL+ Vit E	MOL+ Vit C	MOL+ Zn
Hb (mg/100ml)	8.83[e]	9.37[d]	9.63[c]	9.83[b]	10.03[a]
Ht	0.37[a]	0.35[a]	0.32[a]	0.34[a]	0.35[a]
H/L ratio	0.66[a]	0.59[b]	0.59[b]	0.56[c]	0.54[c]

[a-b] Means within a column with different superscripts are significantly different ($P \leq 0.05$).

Feed intake was significantly ($p<0.05$) decreased by treatment groups compared with control group during the entire period. These results ultimately lead to better feed conversion ratio (FCR) with treatment groups compared with control group. Feed conversion ratio was significantly ($p<0.05$) improved with dietary treatments supplementation compared to the control. FCR increased significantly with birds fed MOL+zinc, and without significant differences with birds received MOL+vitamin E or MOL+vitamin C, respectively.

Hematological parameters

Data presented in Table (2) clarify the effect of dietary supplementation of MOL and MOL plus vitamin E, vitamin C or organic zinc on blood hemoglobin (Hb) concentration, hematocrit (Ht) and heterophils to lymphocytes ratios (H/L ratio). Present data showed significant ($p<0.05$) increase on Hb as affected by all dietary supplements compared to the control, the best value were recorded for MOL+Zn followed by MOL+vitamin C and MOL+vitamin E, receptively. But values recorded for Ht did not affect significantly with treatments. The H/L ratio was significantly ($p<0.05$) higher in the control group as compared with the other treatment groups.

Biochemical parameters

Results of protein fractions, AST and ALT activities as influenced by dietary supplementation of treatments are shown in Table (3). Total protein; albumin levels and globulin levels was significantly ($p<0.05$) increased in MOL+Zn and MOL+vitamin C followed by MOL+vitamin E and MOL as compared with the control group. The results showed that dietary supplementation significantly decreased AST and the more effect was observed with MOL+other antioxidants. ALT did not significantly affected by dietary treatments.

Thyroid hormones (T_3 and T_4) concentration

Effect of dietary treatments supplementation on Thyroid hormones (T_3 and T_4) concentration and T_3/T_4 ratio of broiler chicks were presented in Table (4). Dietary supplementation significantly ($p<0.05$) affected on T_3 and T_4, while, the ratio between T_3 and T_4 was not significant. The highest values of T_3 and T_4 recorded for the fifth group which fed diets supplemented with MOL plus organic zinc.

Table 3. Effect of treatments on plasma protein fractions and plasma aminotranseferase activity of broiler chicks.

Item	Control	MOL	MOL+ Vit E	MOL+ Vit C	MOL+ Zn
TP (g/dl)	3.82^c	4.37^b	4.49^b	4.89^a	4.95^a
Alb (g/dl)	1.75^b	1.76^b	1.82^{ab}	1.90^a	1.93^a
Glo (g/dl)	2.07^c	2.61^b	2.67^b	2.99^a	3.02^a
AST (U/L)	199.0^a	146.0^b	137.0^c	135.7^c	132.3^c
ALT (U/L)	11.7^a	10.3^a	9.0^a	8.3^a	7.7^a

$^{a-b}$ Means within a column with different superscripts are significantly different (P≤0.05).

Table 4. Effect of treatments on Thyroid hormones (T3, T4) concentration and T3/T4 ratio of broiler chicks.

Item	Control	MOL	MOL+ Vit E	MOL+ Vit C	MOL+ Zn
T_3 (ng/dl)	3.82^b	4.38^{ab}	4.51^{ab}	4.57^{ab}	5.03^a
T_4 (ng/dl)	18.79^b	24.19^a	22.74^a	23.42^a	24.23^a
T_3/T_4 ratio	0.204^a	0.182^a	0.198^a	0.195^a	0.207^a

$^{a-b}$ Means within a column with different superscripts are significantly different (P≤0.05).

Table5. Effect of treatments on antioxidant Status of broiler chicks.

Item	Control	MOL	MOL+ Vit E	MOL+ Vit C	MOL+ Zn
GPX (U/ml)	6.21^c	6.73^c	8.83^b	9.45^{ab}	11.09^a
S OD (U/ml)	1.35^c	2.34^b	2.51^a	2.88^a	2.89^a
MDA (nmol/ml)	6.01^a	4.47^{ab}	4.54^{ab}	4.84^{ab}	4.31^b

$^{a-b}$ Means within a column with different superscripts are significantly different (P≤0.05).

Antioxidant Status

Results in the Table (5) showed the impact of different treatments on the enzyme activities of GPX, SOD and MDA. There was a significant effect of treatments on GPX and SOD activity with higher levels being recorded for groups fed diets supplemented with MOL plus organic zinc, MOL plus vitamin C or MOL plus vitamin E as compared with the MOL and control groups, respectively. Malondialdehyde level was higher in the control, while supplementation of MOL or MOL+other antioxidant induced a decrease in MDA level. Supplementation of MOL+Zn significantly decreased MDA compared to the control.

DISCUSSION

In this study the higher body weight in birds fed diet supplemented with MOL may be attributed to the increased digestibility and absorption of nutrient from gut. It is speculated that the main site of activity is within the gastro-intestinal tract is by modification of gut microflora, maximize both feeding ability and growth rate as reported by Jamroz et al., (2005). MOL is reported to contain antioxidant which increases blood total protein by increasing corticosterone secretion which could limit protein catabolism under heat stress condition. The present findings were in agreement with (Avijit and Sarathi, 2013). Nkukwana et al., (2014) found that supplementation of MOL significantly improve feed utilization efficiency and tissue accretion in broiler chicks. Hassan et al. (2016) found that MOL had a positive effect on productive performance, physiological responses and enhances the ability of broilers to resist the heat stress conditions and the best level of MOL was 0.2%. Adding zinc to MOL diet significantly improved live weight gain and feed efficiency, this findings are in agreement with Ahmed et al. (2018). Osman et al., (2003) reported that heat stress causes of accumulate zinc in the liver, resulting in a decrease in plasma zinc concentration and, thus, probably exacerbating a marginal zinc deficiency or an increased zinc requirement. Also zinc has an important role in numerous biological processes in poultry, for instance, Zn is an essential component of many enzymes, and it has both structural and catalytic functions in metalloenzymes (Sahin et al., 2009). Moreover, heat stress reduces the chances of absorption of vitamin E and C and therefore has to be to increase the nutritional needs of these vitamins under heat stress (Khan et al., 2012). Therefore, adding vitamin E, vitamin C or zinc to MOL diets in heat stress broilers chicks lead to maximize the beneficial effect of MOL. All treatments significantly increased Hb, Ogbe and Affiku (2012) explained that the improvement of Hb related to the high content of MOL from iron. Also, results showed that adding one of antioxidants to MOL diet lead to a significant improvement in Hb and H/L ratio as compared with MOL diet. In this respect, Olugbemi et al., (2010) reported that red blood cells responsible for carrying oxygen and carbon dioxide to and from the blood, as well as the manufacture of hemoglobin, reflecting better health in birds. Vitamin E works to protect phospholipids of cellular membranes and sub-cellular, by preventing the oxidation of fatty acids with unsaturated bonds, this antioxidant effects of vitamin E in efficient high concentrations of oxygen and therefore is concentrated in red blood cells as well as manufacture of haemoglobin (Muller, 2010 and Gouda et al., 2015). According to Gross and Siegel (1983), they postulated that the H/L ratio could be used as a reliable indicator of stress in birds. All treatments groups significantly decreased H/L ratio compared to the control group. The improvement resulting from MOL on H/L ratio could be due to its high content from vitamin C which aid to increasing ability to alleviating the negative responses to heat stress condition on birds to became healthy (Osman et al., 2003; Onu and Aniebo, 2011 and Ebenebe et al., 2012). Naseem et al. (2005) reported that vitamin C improve absorption and utilization of nutrients which effect on improvement of Hb and immune response in birds under heat stress to became healthy which improvement H/L ratio, this action from vitamin C plus MOL increasing the improvement compared with additive MOL to dietary alone. Similarly, results are in agreement with those reported by El-Kaiaty et al., (2001) who observed that the number of H/L ratio was significantly decreased by using Zn as a feed additive. Increasing total protein, albumin and globulin may indicate that an enhancement of immunity occurred corresponding to feeding MOL alone or MOL with other antioxidant (vitamin E, vitamin C or organic zinc) as a result of absorption and utilization of nutrients and improving feed conversion. Vitamin C works to reduce the concentration of corticosterone in blood through inhibitory effect of vitamin C on glucocorticoid synthesis which improves performance of poultry results from increases in blood plasma protein fractions (Naseem et al., 2005 and El-Wardany et al., 2012). This may reduce the protein catabolism under heat stress. These confirm the previous reports and support the findings of different researches (Sahin et al., 2002 andSeyrek et al., 2004). Ebenebe et al. (2012) indicated that supplementation of MOL in the diet under heat stress could enhance protein profile of broiler due to its high content of vitamin C which aid to good health and increase the metabolism of protein in the chicken's organ (Melesse et al., 2013). Also, Sahin et al., (2002) and Rashidi et al., (2010) found that vitamin E supplementation increased plasma total protein, albumin and globulin concentration. It appears that, vitamin E as an antioxidant reduces free radical induced pathological changes during both normal metabolic states and inflammation (Packer and Suzuki, 1993). Vitamin E works an important role as an antioxidant to protect of fatty acid peroxidation (Benedich, 1990), fatty acids can act as immuno regulatory molecules that mediate cellular communication, this prevents have made a good indicator for increasing plasma total protein, albumin and globulin concentration (Leshchinsky and Klasing, 2001). The data showed significant decreased ($p<0.05$) in AST related to dietary supplements. This trend was also continued in ALT but this decrease was not significant these result are in agreement with El-Gendi et al., (2000). Thyroid hormones played an important role in the physiological adaptation to heat stress by regulating the metabolic process in birds, correspondingly as the ambient temperature increased the thyroid hormones output depressed Khan et al., (2012). The elevation in T_3 level of MOL groups may be due to the high content of vitamin C in *Moringa olifera* which helps to alleviate the adverse effect of heat stress Osman et al., (2003). Sahin et al. (2002) and Hemid et al., (2013) found that increase the level of T_3 and T_4 in the plasma under the influence of heat stress refer to add vitamin C to diets. In the present study, plasma concentrations improved with dietary vitamin E treatments, these results could be due to the importance of vitamin E as an antioxidant to avoid the negative effects of heat stress, and several researchers reported reduced concentrations of T_3 and T_4 in heat stressed chickens (Sahin et al., 2002; Khan et al., 2012 and Hemid et al., 2013). Concerning the improvement of T_3 and T_4 with adding zinc may be due to the mechanism of Zn action which plays an important role in thyroid hormone metabolism at the cellular level and it may be needed for thyroid hormone attachment to receptor (Ramirez et al., 1991). Thyroid hormone receptors require zinc ion, which facilitate folding into active shape (Sustrova and Strbak, 1994). Vitamin E functions as the primary chain-breaking antioxidant in the avian body, by scavenging free radicals and inhibiting the propagation of lipid oxidation (Surai, 2006). Vitamin E plays a supportive role to another antioxidants as a component of several glutathione peroxidases, which are responsible for the cellular removal of the precursors of free radicals (Onu and Aniebo, 2011). According to antioxidant theory lipid per-oxidation increases in the plasma and tissues by decreasing on antioxidant concentrations, which causing destruction of cell membranes (Klasing, 1993). Under the influence of heat stress birds tend to increase respiration and evaporation this in turn increases the metabolism and energy consumption and mobilization of lipids from stored fat takes place which increases lipid oxidation processes and increases the level of MDA (Ebenebe et al., 2012). The improvement of antioxidant status by adding zinc in broiler diets, because zinc is important biochemical processes element enters in many activities of some ant-oxidation enzymes in the body, such as carbonic anhydrase, liver alcohol dehydrogenase and alkaline phosphatase (Ertek1 et al., 2010).

CONCLUSION

Results of the current study indicated that dietary supplementation of vitamin E, vitamin C or organic zinc with 0.2 % MOL to broiler diets have significantly improved productive performances and the other physiological parameters, this improvement can lead to increasing broiler production under heat stress conditions in tropical and sub-tropical countries. However, more studies are needed to evaluate the effect of more antioxidants supplementation in different combination with MOL to broiler diets under different conditions.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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