Effect of dietary zinc-methionine supplementation on growth performance, nutrient utilization, antioxidative properties and immune response in broiler chickens under high ambient temperature

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ABSTRACT
This study was conducted to evaluate the effect of zinc-methionine (ZnM) on growth performance, nutrient utilization, antioxidant status and immune response in broiler chickens reared at high ambient temperature. A total of 480 one-day-old chicks were randomly distributed into 24 floor pens (20 chicks/pen) and were given either a control diet, 0 ZnM (G0) or 25, 50 and 100 mg/kg ZnM (G1, G2 and G3, respectively). The growth performance was significantly affected by the treatments. ZnM supplementation increased body weight gain and improved feed conversion (p < .05) in broilers. Protein utilization was improved by feeding ZnM (p < .05). Plasma total cholesterol was decreased, while plasma HDL-cholesterol was tending to be increased. Interestingly, an increase in ZnM supplementation enhanced Zn concentrations (p < .05) in breast muscle along with a reduction in malondialdehyde concentration and saturated fatty acids (p < .05) and an augmentation in unsaturated fatty acids (p < .01). Dietary ZnM supplementation resulted in a significant increase in serum glutathione peroxidase concentration which accompanied with an improving in humoral immune response. It could be concluded that dietary organic Zn supplementation improved growth performance, nutrient digestibility, Zn content in raw meat, antioxidative properties and humoral immunity and reduced meat lipid peroxidation in broilers under high ambient temperature.

1. Introduction
In majority of tropical countries, heat stress is one of the most important stressors negatively affecting poultry industry leading to a great economic loss each year. Higher ambient temperature is detrimental to live weight gain, feed intake (FI), feed efficiency, nutrient utilization, protein digestibility, total mineral retention and immune response of chicken broilers (Sahin and Kucuk 2003; Sahin et al. 2009; Saleh, Hayashi, et al. 2013, 2014). Moreover, heat stress can potentially promote formation of excess quantities of reactive oxygen species (ROS), which damages cell phospholipid membranes and other vital macromolecules causing lipid peroxidation, that consequently associated with disorders, such as apoptosis, various diseases and impairing muscle membrane integrity (Ebeid et al. 2013; Saleh 2014; El-Deep et al. 2016; Chand et al. 2017). Therefore, a balance between ROS production and the antioxidant system must be established to maintain immune function, health and productivity (Suraí 2002; Ebeid et al. 2011; Chand et al. 2016; Saleh et al. 2017).

Several methods are available to alleviate the negative effects of high ambient temperature on broiler performance. Because it is expensive to cool poultry buildings, such methods are mostly focused on dietary manipulation. In terms of reducing the negative effects of environmental stress, trace minerals and vitamins are used in the poultry diets to ameliorate the negative effects of stress. Dietary zinc (Zn) supplementation has also been reported to have a positive effect on the growth rate and feed efficiency of growing poultry under stress conditions (Sahin et al. 2005; Rao et al. 2016). Zinc is a fundamental element required for normal growth performance, bone development, feathering, skin quality, immunity, appetite regulation and structure and function of more than 300 enzymes associated with carbohydrate and energy metabolism, protein degradation and synthesis, nucleic acid synthesis, carbon dioxide transport and many other reactions (Prasad and Carbonaro 2003; Prasad and Kucuk 2002; Sahin et al. 2009; Saleh et al. 2011).

The NRC (1994) specified 40 mg/kg of feed as Zn requirement of broilers. Zinc could be added to broiler’s diet as inorganic Zn or organic Zn (complexes with amino acids, proteins or carbohydrate). In recent years, organic Zn sources have been used progressively due to their potentially higher Zn bioavailability (Salim et al. 2011). Zinc-methionine (ZnM) is an organic Zn which is devoid of free divalent cations for chelation in the intestinal lumen with phytic acid. Therefore, it is metabolized in different methods which facilitate enhanced absorption of Zn (Burrell et al. 2004). In this context, ZnM could be...
advantageously incorporated in broiler's diet at lower levels as compared to inorganic Zn for apprehending higher Zn bioavailability and lowering excretion of Zn to the environment (Sunder et al. 2013). It could, therefore, be hypothesized that heat stress increases Zn requirements of broilers and dietary organic Zn might enhance immune response by maintaining the oxidative balance. The objective of the present study was to inspect the effects of ZnM on growth performance, nutrient utilization, antioxidant status and immune response in broilers reared at high ambient temperature.

2. Materials and methods

2.1. Experimental design and dietary treatments

The experiment was conducted in accordance with the guidelines of the Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Egypt. All procedures were approved by the Animal Care and Welfare Committee of the Institute. A total of 480 one-day-old male broiler chicks (Cobb 500) were randomly assigned to 24 floor pens with 20 birds per pen (10 bird/m²). This study was conducted under hot climate conditions in Egypt (July and August). During the experimental period, the average daily temperature and relative humidity inside the house ranged from 33°C to 36°C and from 60% to 70%, respectively. Typical iso-energetic and iso-nitrogenous starter (0–2 week), grower (2–5 week) and finisher (5–6 week) diets, based on maize-soybean meal were formulated in mash form and offered ad libitum (Table 1). There is no Zn in the premix which used in all the diets. At one day, chicks received one of four dietary treatments: (i) G0 = control (0 ZnM), (ii) G2 = control + 25 mg/kg ZnM, (iii) G3 = control + 50 mg/kg ZnM and (iv) G4 = control + 100 mg/kg ZnM. Broiler chicks were reared under similar managerial and hygienic conditions with a 24-h light programme. ZnM complex was obtained from Ibex Company, Egypt and the purity of ZnM complex was 98%.

2.2. Growth performance and carcass parts

Feed intake (FI) and body weight (BW) were recorded weekly by pen, the average daily gain (ADG) and feed conversion ratio (FCR) were computed. Mortality was checked daily and weights of dead birds were used to adjust FCR. At the conclusion of the trial, birds were selected. After euthanasia, feathers, heads, necks and shanks were removed, and the remaining carcasses were dissected. The yield percentage of breast, liver and abdominal fat were calculated based on dressed weight.

2.3. Chemical analysis

At 40 d, excreta were collected from 10 birds per group. Excreta samples were weighed, dried in an oven at 60°C for 24 h, homogenized and finely ground to determine dry matter and crude protein digestibilities according to AOAC (2000). Crude protein content in diet and excreta was measured to determine nitrogen retention using the Kjeldahl method. The calculations were as follows;

Nitrogen retention (%) = (total nitrogen intake – total nitrogen excreted)/ total Nitrogen intake × 100.

2.4. Blood samples and plasma biochemical analysis

At the end of the experimental period (42 d), blood samples were collected in heparinized test tubes and then centrifuged (3000 rpm for 20 min) to separate the plasma. Plasma and meat samples were stored at −30°C and −10°C, respectively, until further analysis. Total concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, total protein, albumin and globulin were measured calorimetrically using commercial kits (Diamond Diagnostics, Egypt) according to the procedures outlined by the manufacturer. Serum glutathione peroxidase (GSH-Px) activity was determined by the method of Levander et al. (1983) and glutamic oxalacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were determined by the method of Reitman and Frankel (1957). Concentration of muscle malondialdehyde (MDA) was measured by the method of Okhawa et al. (1979).

2.5. Assay of serum antibody titres

Serum antibody titres against Newcastle disease (ND) and Avian Influenza (H9N1) were determined by means of hemagglutination inhibition (HI) test using standard methods described in OIE (2009). Antibody titre to infectious bursal disease virus was determined by commercial ELISA kits (Symbiotics Laboratories, USA), according to manufacturer's instructions.

2.6. Meat analysis

Breast muscle tissue was used to measure muscle Zn concentration and fatty acids profile according to the method described by Saleh (2013). Lipids were extracted from muscle

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Table 1. Ingredients and nutrient composition of the basal diet.

| Ingredients | Starter 0–2 week | Grower 2–5 week | Finisher 5–6 week |
|-------------|------------------|-----------------|------------------|
| Yellow corn | 54.9             | 64.705          | 67.545           |
| Soybean meal, 44% | 35.8             | 25              | 20               |
| Corn gluten meal, 62% | 3.495           | 5               | 6.35             |
| Vitamin-mineral premix | 0.3            | 0.3             | 0.3              |
| Soybean oil | 2.3              | 1.5             | 2.5              |
| Dicalcium phosphate | 1.3            | 1.4             | 1.4              |
| Limestone | 1.1              | 1               | 1                |
| Salt | 0.35             | 0.35            | 0.35             |
| L-Lysine | 0.17             | 0.31            | 0.3              |
| DL-Methionine | 0.25           | 0.23            | 0.15             |
| L-Threonine | 0.03            | 0.1             | 0.1              |
| Phytase enzyme | 0.005        | 0.005           | 0.005            |
| Total | 100              | 100             | 100              |
| Calculated composition |               |                 |                  |
| Crude Protein (%) | 22.5            | 19.5            | 18.3             |
| ME (kcal/kg) | 3035            | 3080            | 3180             |
| Crude fibre (%) | 3.94            | 3.38            | 3.1              |
| Ether extract (%) | 4.88            | 4.31            | 5.3              |
| Calcium (%) | 0.84             | 0.82            | 0.77             |
| Available phosphorus (%) | 0.45          | 0.43            | 0.41             |

*Each 3 kg of vitamin-mineral premix contain: 6,000,000 IU vit A, 900,000 IU vit D₃, 40,000 mg vit E, 2000 mg vit K, 2000 mg vit B₁₂, 2000 mg vit B₂, 2000 mg vit B₆, 10 mg vit B₁₂, 50,000 mg Niacin, 10,000 mg pantothenic acid, 50 mg Biotin, 3000 mg Folic acid, 250,000 mg choline, 8500 mg Mn, 50,000 mg Fe, 50,000 mg Cu, 200 mg I, 100 mg Se and 100 mg Co.*
by a mixture of chloroform-methanol (2:1) at the ratio of (1:5) in separator funnel and shaken carefully for 1 h. The extract was allowed to separate; the organic layer was taken, then passed through a glass funnel containing anhydrous sodium sulfate and finally evaporated to near dryness by a stream of nitrogen. A sample of total lipids (50 mg) was transferred into a screw-cap vial, and 2 ml benzene and 10 ml 1% H2SO4 in absolute methanol was added. The vial was covered under a stream of nitrogen before heating in an oven at 90°C for 90 min. Ten millilitres of distilled water were added to the cooled vial and the methyl esters in each vial were extracted. Ether extracts were combined and concentrated to its minimum volume by using a stream of nitrogen.

Analysis of fatty acids in meat was carried out by gas liquid chromatography using Shimadzu gas chromatograph GC-4 CM (PFE) equipped with flame ionization detector. A standard mixture of methyl esters was analysed under identical conditions prior to running the samples. The retention times of the unknown sample of methyl esters were compared with those of the standard. The concentration of methyl esters were calculated by the triangulation method.

2.7. Statistical analysis

Data were evaluated by analysis of variance (ANOVA) for a complete randomized block design using the general linear models procedure of SPSS Statistics 17.0 (SPSS 2008). When the ANOVA showed significant differences, Tukey’s multiple comparison test was applied. The overall level for statistical significance was set at \( p < 0.05 \). All values were expressed as means ± standard error of the mean.

3. Results

The effects of dietary ZnM supplementation on body weight gain (BWG), FI, FCR, breast muscle weight (BMW), liver and abdominal fat weights in broilers during heat stress condition are summarized in Table 2. Final BW was significantly increased \( (p < 0.05) \) in G2 compared with control group (G0); however, no significant differences were observed among G0, G1 and G3 \( (p > 0.05) \). Chicks received G2 and G3 had the highest significant ADG (51.45 and 51.24 g, respectively) as compared to those that had received G0 (47.60 g). Chicks received G1 had intermediate ADL (49.81 g) but it was not different from G0 \( (p > 0.05) \). FI and FCR were significantly decreased \( (p < 0.05) \) in birds received G2 and G3 as compared to those received G0. Breast muscle weight (pectoral superficial muscle) was significantly increased \( (p < 0.01) \) by the dietary supplementation of 50 and 100 mg ZnM/kg (G2 and G3, respectively) as compared to those which had received 0 or 25 mg ZnM/kg (G0 and G1, respectively). On the other hand, abdominal fat weight was decreased in groups G2 and G3 as compared to groups G0 or G1 while, liver weight was not influenced by treatment.

The dry matter digestibility and crude protein utilization results showed a significant differences because of treatment \( (p < 0.05, \text{Figure 1(A,B)}) \). Dry matter digestibility was high for birds that received G2 and G3, intermediate for birds that received G1 and low for the G0 group \( (p < 0.05) \). On the other hand, crude protein utilization was the best for chicks that received G2, while it was intermediate for G1 and G3 and it was the lowest for G0 \( (p < 0.05) \).

The effects of ZnM on fatty acids profile in breast muscle are summarized in Table 3. Palmitic acid, palmitoleic acid and stearic acid were decreased significantly by feeding ZnM \( (p < 0.05) \), while, oleic acid, vaccenic acid and linoleic acid were significantly increased \( (p < 0.01) \).

With respect to the effect of dietary ZnM supplementation on antioxidative properties including GSH-Px activity as well as lipid peroxidation index in blood plasma (Figure 2(A)), it could be observed that dietary 50 and 100 mg ZnM/kg significantly enhanced GSH-Px activity in comparison with control. Breast muscle Zn concentration was increased linearly as the level of ZnM increased in the diet \( (p < 0.05, \text{Figure 2(C)}) \). The influences of dietary ZnM supplementation on lipid oxidation in breast muscle of broilers reared in high ambient temperature are graphically presented in Figure 2(B). Using MDA as an index of lipid oxidation, it could be observed that dietary treatments decreased MDA values in meat \( (p < 0.05) \). In addition, the 100 mg ZnM/kg treatment was the most effective inhibitor of lipid oxidation, followed by 50 mg ZnM/kg compared with control.

Results of immune response, presented in Table 5, show that different levels of dietary organic Zn had a positive effect on humoral immunity as measured by antibody titres against ND when compared with the control diet \( (p < 0.05) \) under heat stress condition. The highest scores of antibody titres against Proflok IBD were attained by broilers fed 50 and 100 mg ZnM/kg compared with those of 0 or 25 mg ZnM/kg. Although, no significant differences were detected in antibody titres against avian influenza H9N1, there is a numerical increase due to dietary ZnM supplementation under heat stress condition.

The results for plasma TAG, total cholesterol, HDL-cholesterol, GOT, glucose concentrations, muscle Zn concentration are shown in Table 4. Plasma TAG, total cholesterol concentrations were significantly lower in the ZnM groups (G1, G2 and G3) as compared to the control (G0) (Table 4). Plasma HDL-cholesterol, GOT and glucose were not affected by treatment \( (p > 0.05, \text{Table 4}) \). The main effect of dietary ZnM supplementation on plasma total protein, albumin and globulin.

### Table 2.

| Zinc-methionine | G0 | G1 | G2 | G3 | SEM | Value |
|----------------|----|----|----|----|-----|-------|
| BW0 (g)        | 54 | 53 | 54 | 53 | 0.825 | NS    |
| BW5 (g)        | 2045b | 2145b | 2215c | 2205b | 85 | < 0.05 |
| ADG (g/day)    | 47.60b | 49.81b | 51.45a | 51.24a | 4.6 | < 0.05 |
| FI (g/42 day)  | 3802a | 3765ab | 3673b | 3679b | 88 | < 0.05 |
| FCR            | 1.91a | 1.80a | 1.70c | 1.71c | 1.352 | < 0.01 |
| BMW (% of BW)  | 23.4a | 24.6 a | 28.1a | 27.1a | 0.85 | < 0.05 |
| Liver (% of BW)| 3.14a | 3.59a | 3.34a | 3.17a | 0.25 | NS    |
| Abdominal fat (% of BW) | 1.75a | 1.73a | 1.38b | 1.44b | 0.137 | < 0.05 |

Notes: G0: Control group (without Zn supplementation); G1: Basel diet supplemented with 25 mg/kg; G2: Basel diet supplemented with 50 mg/kg; G3: Basel diet supplemented with 100 mg/kg; BW0: Body weight at one day of age; BW5: body weight at slaughter; ADGG: average daily gain during the experiment; FI: total feed intake; FCR: food conversion ratio; BMW: breast muscle weight; a–c represents means within the same row with different superscripts differ \( (p < 0.05) \).
in broilers reared in high ambient temperature are presented in Table 4. It is clear that no significant differences between treatments were observed.

4. Discussion

Results of the present study indicated that dietary ZnM supplementation (50 or 100 mg/kg) increased BWG and BMW, improved FCR and lowered abdominal fat weight under high ambient temperature (Table 2). These results are in coincident with Rao et al. (2016) who indicated that supplementation of organic Zn increased (p < .05) body mass gain and FI compared to those fed the control in broilers reared in cyclic heat-stressed condition. Also, Sahin et al. (2005) postulated that Zn picolinate supplementation (30 or 60 mg/kg) improved growth performance and carcass quality in quails exposed to heat stress. One possible explanation for such improvements might be related to the fact that Zn is considered of critical importance in maintaining the structure of metalloproteins such as insulin, growth hormone and insulin-like growth factor-I (Macdonald 2000; Saleh, Eid, Ohtuska, et al. 2012; Khan et al. 2014; Midilli et al. 2014). Furthermore, Zn positively affects feed utilization through participating in the metabolism of carbohydrates, lipids and proteins (Macdonald 2000) which finally translated into improvements in growth performance.

As shown in Table 2, BMW was increased when ZnM was added at the rate of 50 and 100 mg/kg, and this improvement might be related to Zn requirements for normal protein synthesis and metabolism (Midilli et al. 2014). Dietary organic Zn decreased abdominal fat in the present study (Table 2). These results are in accordance with Kucuk et al. (2003) who documented that abdominal fat decreased (p < .05) upon dietary zinc and vitamin A supplementation.

In the present study, feeding ZnM-supplemented diet improved dry matter digestibility and crude protein utilization. These results are in harmony with other studies (Sahin and

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**Table 3.** Effect of dietary zinc-methionine supplementation on fatty acids profile in breast muscle in broilers.

| Zinc-methionine | G0   | G1   | G2   | G3   | SEM  | p-Value |
|-----------------|------|------|------|------|------|---------|
| Palmitic acid C16:0 (%) | 33.2a | 29.29ab | 25.92b | 28.04b | 3.25  | p < .05 |
| Palmitoleic acid C16:1ω9 (%) | 4.23a | 4.04ab | 3.83b | 3.23b | 0.42  | p < .05 |
| Stearic acid C18:0 (%) | 10.7a | 9.7b  | 8.7b  | 8.4b  | 0.825 | p < .05 |
| Oleic acid C18:1ω9 (%) | 38.6b | 41.6a | 43.4a | 42.8a | 4.305 | p < .05 |
| Vaccenic acid C18:1ω7 (%) | 2.4b  | 2.6b  | 3.4b  | 3.9b  | 0.18  | p < .05 |
| Linoleic acid C18:2ω6 (%) | 9.9a  | 11.9b | 13.8a | 12.7a | 0.48  | p < .01 |
| Non-identified fatty acids (%) | 9.7  | 5.87 | 0.95 | 0.93 | 0.018 | NS      |

Notes: G0: Control group (without Zn supplementation); G1: Basel diet supplemented with 25 mg/kg; G2: Basel diet supplemented with 50 mg/kg; G3: Basel diet supplemented with 100 mg/kg; a–c represents means within the same row with different superscripts differ (p < .05).
Kucuk 2003; Sahin et al. 2009) which noted that increasing supplemental Zn (0, 30 and 60 mg/kg) linearly increased digestibility of dry matter, organic matter, crude protein and ether extract. It is well known that Zn has a protective role on pancreatic tissue against oxidative damage, it might help the pancreas to function properly including secretions of digestive enzymes, thus improving digestibility of nutrients. Also, Zn is required for the activity of over 300 enzymes and participates in many enzymatic and metabolic functions in the body (Prasad and Kucuk 2002). Therefore, it could be assumed that dietary organic Zn might play a vital role in enhancing the digestion and absorption of nutrients in the gastrointestinal tract during heat stress conditions which consequently might participate in improving the growth performance in the present study.

Zinc concentration in breast muscle was increased in a dose dependent manner due to dietary ZnM supplementation (Figure 2(C)). Several previous studies (Salim, et al. 2010; Kakhki et al. 2016) reported an increase in muscle Zn concentration using dietary Zn supplementation in broilers. Kakhki et al. (2016) illustrated that muscle content of Zn in broilers has linear relationship with dietary Zn level. Interestingly, as shown in Figure 2(B), using MDA as an index of lipid oxidation, it could be observed that dietary ZnM supplementation decreased MDA values in breast meat (p < .05). These findings suggested that dietary supplementation with organic Zn increased Zn concentration in breast meat which might be involved in improving the oxidative stability and alleviated the lipid oxidation of breast meat. This positive effect might

Figure 2. Effect of feeding zinc-methionine supplementation on plasma GSH-Px (A), muscle MDA (B) and muscle Zn concentration (C). Values are means represented by vertical bar. a,b,c,d: mean values with unlike letters were significantly different (p < .05).
induces production of Zn-metallothionein, which is an effective and organic hydroperoxides (Suraï 2002; Khan et al. 2011; dination in poultry under heat stress conditions. The enzyme the activity of antioxidant enzymes and reduce lipid peroxi-
dation because it is a cofactor of the main antiox-
dsidual Zn decreased serum cholesterol concentration in heat-stressed broiler chickens. Aksu et al. (2011) also reported the decrease of total cholesterol and LDL-C, combined with the increase in HDL-cholesterol, in the plasma of chickens when the feed mixtures were supplemented with organic complexes of Zn. The decrease in plasma cholesterol due to Zn was explained by the fact that Zn is involved in lipid metabolism (Midilli et al. 2014). Abd-El-Samee et al. (2013) reported that plasma total lipids and cholesterol were decreased by feeding Zn in broiler diets. Uyanik et al. (2001) indicated that Zn supplementation decreased serum cholesterol concentration of broilers, this may be due to ZnM prevent cholesterol from absorption in gastrointestinal tract (Tizard et al. 1989; Wang et al. 2011) and can promote the growth and activity of lactic acid bacteria (Gibson and Roberfroid 1995), which reduces the cholesterol level by producing enzymes disintegrating bile salts and making them un-conjugated. Results of the present study revealed that plasma glucose concentration was not significantly affected by dietary ZnM supplementation. However, Kucuk et al. (2003) documented that supplemental Zn decreased serum glucose concentration in heat-stressed broiler chickens. In general, plasma levels of GOT and GPT were considered as liver indices for liver damage. Therefore,

### Table 4. Effect of dietary zinc-methionine supplementation on blood plasma biochemical constituents.

| Zinc-methionine | G0 | G1 | G2 | G3 | SEM | p-Value |
|-----------------|----|----|----|----|-----|---------|
| Plasma TAG (mg/dL) | 33a | 27b | 25b | 26b | 3 | < .05 |
| Plasma total protein (mg/dL) | 3.89 | 3.87 | 3.91 | 3.96 | 0.25 | NS |
| Plasma albumin (mg/dL) | 1.25 | 1.28 | 1.31 | 1.33 | 0.06 | NS |
| Plasma globulin (mg/dL) | 2.62 | 2.56 | 2.59 | 2.61 | 0.062 | NS |
| Plasma albumin/globulin ratio | 0.48 | 0.50 | 0.51 | 0.51 | 0.025 | NS |
| Plasma glucose (mg/dL) | 211 | 199 | 198 | 201 | 25 | NS |
| Plasma GOT (U/I) | 222 | 219 | 218 | 216 | 27 | NS |
| Plasma total cholesterol (mg/dL) | 146^a | 129^b | 126^b | 128^b | 7 | p < .05 |
| Plasma HDL (mg/dL) | 99 | 101 | 105 | 104 | 7 | NS |

Notes: G0: Control group (without Zn supplementation); G1: Basel diet supplemented with 25 mg/kg; G2: Basel diet supplemented with 50 mg/kg; G3: Basel diet supplemented with 100 mg/kg; a-c represents means within the same row with different superscripts differ (p < .05).

### Table 5. Effect of dietary Zinc-methionine supplementation on antibody titre against Newcastle disease (ND), Proflok infectious bursal disease (IBD) and Avian Influenza (H9N1) virus in broilers under high ambient temperature.

| Zinc-methionine | G0 | G1 | G2 | G3 | SEM | p-Value |
|-----------------|----|----|----|----|-----|---------|
| ND | 1.83^b | 2.41^ab | 2.66^ab | 2.94^a | 0.6 | p < .05 |
| Proflok IBD | 2.81 | 2.89^b | 3 | 3.36^ab | 3.97^a | 0.405 | p < .05 |
| H9N1 | 1.26 | 1.33 | 1.35 | 1.36 | 0.04 | NS |

Notes: G0: Control group (without Zn supplementation); G1: Basel diet supplemented with 25 mg/kg; G2: Basel diet supplemented with 50 mg/kg; G3: Basel diet supplemented with 100 mg/kg; a-c represents means within the same row with different superscripts differ (p < .05).
results of the present study may provide evidences for occurrence of no toxicity of feeding ZnM in broiler chickens.

5. Conclusion
Based on the data presented above and taking into account the antioxidative properties, it is possible to suggest that supplemental dietary organic Zn might be involved in enhancing growth performance, nutrient digestibility, Zn content in raw meat, meat oxidative stability, plasma antioxidative status and immune responsiveness in broilers subjected to heat stress.

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