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# Effects of dietary fumaric acid on the growth performance, immune response, relative weight and antioxidant status of immune organs in broilers exposed to chronic heat stress

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**Abstract:** This study aimed to investigate the effects of dietary fumaric acid (FA) on growth performance, immune responses, immune organ index and antioxidant status in broilers under chronic heat stress (HS). A total of 200 21-day-old Ross 308 chicks were randomly assigned in a 2 × 2 factorial arrangement with two diets (basal diet or 10 g/kg FA diet) and two temperatures (thermoneutral or HS) for 21 days. On day 42, growth performance, immune organ index, immune function and antioxidative ability were determined. HS resulted in a significant reduction in final body weight (FBW), average daily feed intake (ADFI), average daily gain (ADG), antibody titres against sheep red blood cells (SRBC) and Newcastle disease virus, IgM, IgG, relative weights of spleen, thymus and bursa of Fabricius, but a significant increase in the feed conversion ratio (FCR), activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GPx) in the bursa, contents of malondialdehyde and total carbonyl (TC) in thymus and bursa ($P < 0.05$). Dietary supplementation of FA increased FBW, ADFI, ADG, antibody titres against SRBC, IgG, relative weights of spleen and bursa, activity of GPx in thymus and bursa, whereas it decreased the FCR and TC of thymus and bursa. These results suggest that dietary 10 g/kg FA had positive effects on growth performance and immune function through improving the antioxidative capacity of immune organs.

**Keywords:** broiler; organic acid; high ambient temperature; immune function; redox status

Heat stress (HS) has become a major concern in the poultry industry along with a global temperatures rise, especially in hot regions with a long summer. Continuous selection for fast growth contributes to increased susceptibility of broiler chickens to HS. Negative impacts of HS on poultry have been well documented including decreased growth, feed efficiency, carcass characteristics, and even survival, which hinder the productivity (Lara and Rostagno 2013). HS also has negative effects on biological defence mechanisms such as development of immune organs and immune response to external factors in broilers. The reduced antibody response to sheep red blood cells (SRBC) and the ratio of helper and cytotoxic T-cells in the blood increase the risk of secondary infections and limit

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the efficacy of vaccination. On the other hand, biochemical and physiological events associated with hyperthermia potentially promote reactive oxygen species (ROS) formation which can cause oxidative injury to the immune organs like thymus, bursa of Fabricius, and spleen (Mirzaie et al. 2018). Therefore, an increase in the activities of the enzymes total superoxide dismutase (T-SOD) and glutathione peroxidase (GPx) may be beneficial to scavenge the ROS and thus improve the development and function of immune organs in broilers under HS. Previous studies have shown that the acute HS lasting hours to a few days is regulated by homeostatic regulators of the nervous and endocrine systems and the chronic HS lasting several days to weeks by homeorhetic regulators of the endocrine system (Collier et al. 2017). However, there are few studies which focus on the effects of chronic HS on the immune system in broilers. In light of the above-mentioned facts, it is necessary to take positive measures to mitigate these adverse effects on broiler chickens, especially in areas with a long period of summer.

In recent years, there has been an increase in the use of organic acids as substitutes for antibiotic growth promoters in broilers to reach higher growth performance due to their nutritional and physiological functions, protection against enteric pathogens (Yang et al. 2018). The results from the literature showed that organic acid supplementation, irrespective of the type and level of acid used, had a beneficial effect on the performance of broiler chickens. For example, dietary formic and propionic acid supplementation improved growth performance, breast meat quality, serum total antioxidant capacity, and immune responses of broilers (Durek et al. 2014; Emami et al. 2017). Fumaric acid (FA), a dicarboxylic acid, is mainly formed by the oxidation of succinate and is further converted to malic acid in the tricarboxylic acid cycle. Pirgozliev et al. (2008) reported that the dietary inclusion of FA at 1% improved the apparent metabolizable energy of the diets in broilers under natural environments. In addition, the body weight gains were significantly improved by dietary supplementation of 1% FA when compared with the control group (Banday et al. 2015). However, there is little information about the modulation effects of FA on growth performance, immune response, development and antioxidant status of immune organs in broilers under high environmental temperature. We hypothesized that FA could protect the immune function from heat stress-induced injury. Therefore, the present investigation was carried out to evaluate the effects of FA on the growth performance and modulation of immune organs in heat-stressed broiler chickens.

## MATERIAL AND METHODS

### Experimental design, broiler chickens, and diets

The animal protocol used in the present study was approved by the Animal Care and Use Committee. A total number of 200 day-old Ross 308 broiler chicks (average weight of 45 g, males to females at an equal ratio) were obtained from a commercial hatchery. The birds were fed the basal diet to match the requirements of the Ross 308 recommendations during the starter (1–21 days) and grower (22–42 days) phases (Table 1). Broilers were raised under standard envi-

Table 1. Composition and nutrient levels of basal diets (air-dry basis; %)

| Item | 0–21 d | 22–42 d |
|---|---|---|
| **Ingredients** | | |
| Corn | 62.0 | 64.0 |
| Soybean meal | 29.1 | 27.8 |
| Soybean oil | 3.0 | 3.0 |
| Fish meal | 3.0 | 2.5 |
| Calcium hydrogen phosphate | 1.6 | 1.4 |
| Sodium chloride | 0.30 | 0.30 |
| Premix[1] | 1.0 | 1.0 |
| **Nutrient level** | | |
| ME/(MJ/kg)[2] | 12.96 | 13.03 |
| Crude protein | 20.50 | 20.02 |
| Lysine | 1.05 | 1.02 |
| Methionine + Cystine | 0.80 | 0.78 |
| Calcium | 1.01 | 1.00 |
| Total phosphorus | 0.71 | 0.67 |

[1]The premix provided the following nutrients per kilogram of diets: Mn (as manganese sulphate) 66 mg, Zn 44 mg, Cu (as copper sulphate) 9 mg, Fe (as ferrous sulphate) 50 mg, I (as potassium iodide) 0.4 mg, vitamin A 7 000 IU, vitamin $D_3$ 875 IU, vitamin E 20 IU, vitamin $K_3$ 1 mg, vitamin $B_1$ 2 mg, vitamin $B_2$ 4.5 mg, D-pantothenic acid 12 mg, nicotinic acid 50 mg, vitamin $B_6$ 2.5 mg, vitamin $B_{12}$ 0.6 mg.
[2]ME was a calculated value

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ronmental conditions for the first 21 days before the beginning of the study. From days 22 to 42 of age, the birds were randomly allocated to four treatment groups with 5 replicates of ten broilers per replicate. Every replicate was reared in one cage (90 cm length × 90 cm width × 38 cm height) with relative humidity of 60 ± 5% and a light-dark (23 : 1) photoperiod program with the luminous intensity of 10 lx. Proper ventilation was ensured by means of the exhaust fans and wind speed in the range of 1.5 m/s to 2.5 m/s. Fresh feed and water were provided daily *ad libitum*. Given that the dietary inclusion of FA at 1% improved body weight gains and apparent metabolizable energy of the diets, herein we prepared FA-supplemented basal diet at the dose of 10 g/kg (Pirgozliev et al. 2008; Banday et al. 2015). The experiment consisted of four treatment groups in a 2 × 2 factorial arrangements with two temperatures (thermoneutral or HS) and two diets (basal diet or 10 g/kg FA diet). Each treatment included five replicates with 10 broiler chickens per replicate. The thermoneutral and heat treatments were assigned into separate rooms. Both thermoneutral groups, including one group fed basal diet and one group fed diet supplemented with FA were raised at 24 ± 1 °C for 21 days. The heat-stressed treatment groups, including one group fed basal diet and one group fed diet supplemented with FA were kept for 21 successive days under 32 ± 1 °C. They were also vaccinated against the Newcastle disease (on days 7 and 21) using the Lasota vaccine. The temperature was monitored several times daily during the HS period at different locations of the pens. At the end of the feeding trial, ten birds per treatment (2 per replicate at random) were selected and used for the determination of serum and lymphoid organ parameters. To calculate the weight gain of the broilers from the thermal comfort and HS groups, the broilers were weighed at 42 days of age. The cumulative feed consumption per replicate was also recorded. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated.

### Antibody titres against SRBC

At 35 days, in each experimental unit, two broiler chickens were randomly selected and 0.1 ml of 25% SRBC was injected into the breast muscle of the birds. To determine the antibody titre against SRBC, 2 ml of blood was taken from the wing vein of the chickens on day 42. Briefly, the serum was exposed to a temperature of 56 °C for 30 minutes. Then 50 µl of serum and 50 µl of PBS buffer were added to the first well of 96-well ELISA (U-shaped) and incubated at 37 °C for 30 minutes. After 30 min, the pellets were removed from the incubator and 50 µl of PBS was added to the other wells and thus serial dilutions were prepared for each sample. After preparing the solution, 50 µl of 2% SRBC was added to each well and then incubated at 37 °C for 30 minutes. Antibody values were expressed as $\log_2$ of the reciprocal of the highest dilution where visible agglutination was observed.

### Serum Newcastle disease virus (NDV) antibody and immunoglobin determination

The NDV antibody, serum IgA, IgG and IgM concentrations were determined using a commercial ELISA kit (Shanghai Enzyme Linked Immunosorbent Assay; Institute of Biotechnology, Shanghai, P. R. China), according to the manufacturer's instructions. The NDV antibody and immunoglobulin levels were determined using a standard curve and were expressed in nanograms per millilitre of serum.

### White blood cell count (WBC), heterophils, lymphocytes and heterophil to lymphocyte ratio (H/L)

On day 42, two broiler chickens from each replicate unit (other than those that received SRBC) were randomly selected for white blood cell count. Blood was drawn from a vein and fixed via methanol 99.5% on the slide. The blood smears were then covered with water-diluted Wright-Giemsa staining. After 5 min, the slides were washed and observed under an optical microscope at a high magnification (100 × objective lens and 10 × eyepieces), the type of white blood cells was recorded and counting was performed up to 100 white blood cells. Then, the heterophil to lymphocyte ratio was calculated for each sample.

### Relative weight of lymphoid organs

At the end of the experimental period, for each experimental unit, two broiler chickens close to the

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average weight were selected and then they were euthanized by sodium pentobarbital. The lymphoid tissues such as thymus, spleen and bursa of Fabricius were separated from the body and weighed on a digital scale with an accuracy of one thousandth of a gram. The relative weight of lymphoid organs was expressed by the ratio of organ weight (g) to body weight (kg).

### Oxidative status

All the spleen, thymus and bursa samples were homogenized (1 : 9, w/v) with ice-cold 0.9% saline solution, and then centrifuged at 3 000 × *g* for 10 min at 4 °C to collect the supernatant. The total antioxidant capacity (T-AOC), activities of T-SOD and GPx in the supernatant were determined by the chemical colorimetry, xanthine oxidase method and hydrogen peroxide degradation method, respectively. The contents of total carbonyl (TC) and malondialdehyde (MDA) were quantified by the 2, 4-dinitrophenylhydrazine method and the thiobarbituric acid method, respectively. All the reagent kits were purchased from Nanjing Jiangcheng Bioengineering Institute (Nanjing, P. R. China). The absorbance of T-AOC, SOD, GPx, TC and MDA was measured at 520, 550, 412, 370 and 532 nm, respectively. Total protein concentration was determined according to the Bradford method using bovine serum albumin as the standard protein. All data were normalized against total protein for inter-sample comparison.

### Statistical analysis

Results are expressed as mean values with pooled SEM. Data were analyzed using two-way analysis of variance (ANOVA) with the General Linear Models (GLM) procedure of PASW (PASW Statistics; v18.0) that included the effects of temperature, diet and their interactions. The differences between treatments were evaluated by the least significant difference Bonferroni's multiple comparisons test. For performance traits, each pen was considered as the experimental unit. Meanwhile, each bird acts as the experimental unit for the other traits. Differences were statistically significant when $P < 0.05$.

## RESULTS

In the present study, no interaction effect between temperature and diet was observed in any evaluated parameter.

### Growth performance

The effect of environmental temperature and dietary FA on growth performance is summarized in Table 2. Broilers exposed to HS exhibited a significant reduction in final body weight (FBW), ADFI, ADG, but a significant increase in FCR when compared with broilers under thermoneutral conditions ($P < 0.01$). However, dietary FA increased the FBW, ADFI, ADG, but decreased the FCR ($P < 0.01$) in comparison with the basal diet group.

### Antibody responses

Effects of environmental temperature and dietary FA on serum antibody titres against SRBC, levels of NDV antibody and immunoglobins are pre-

Table 2. Effects of environmental temperature and dietary FA on body weight, feed intake, and FCR in broilers

| | Thermoneutral | | HS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | basal diet | FA | basal diet | FA | | temperature | diet | temperature*diet |
| IBW (g) | 650.61 | 644.11 | 646.1 | 651.47 | 1.53 | – | – | – |
| FBW (g) | 2 202.72 | 2 494.16 | 1 746.71 | 2 000.51 | 136.96 | < 0.001 | 0.007 | 0.905 |
| ADFI (g) | 155.22 | 171.25 | 118.62 | 136.66 | 9.87 | < 0.001 | < 0.001 | 0.648 |
| ADG (g) | 73.91 | 88.05 | 52.41 | 64.24 | 6.54 | < 0.001 | < 0.001 | 0.189 |
| FCR | 2.10 | 1.95 | 2.26 | 2.13 | 0.06 | < 0.001 | < 0.001 | 0.076 |

ADFI = average daily feed intake; ADG = average daily gain; FA = fumaric acid; FBW = final body weight; FCR = feed conversion ratio; HS = heat stress; IBW = initial body weight

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sented in Table 3. Broilers exposed to HS showed a significant reduction in the SRBC antibody titre and concentration of NDV antibody, IgM and IgG when compared with broilers under thermoneutral conditions ($P < 0.05$ or $P < 0.01$). Broilers fed FA-supplemented diet exhibited an increase of the SRBC antibody titre and IgG concentration when compared with the birds fed basal diet ($P < 0.05$ or $P < 0.01$).

## WBC, heterophils, lymphocytes and H/L ratio

The WBC, heterophil, lymphocyte counts and H/L ratios obtained in the present study are shown in Table 4. There was no significant difference in WBC, heterophils, lymphocytes and H/L ratio, regardless of the temperature and diet.

## Relative organ weights

Relative immune organ weights are shown in Table 5. All relative organ weights were reduced significantly when the birds were exposed to HS ($P < 0.05$). Supplementing the diet with FA significantly improved the relative weights of spleen and bursa of Fabricius when compared with the basal diet group. Moreover, broilers fed FA-supplemented diet had a trend of the increased relative weight of thymus as compared with the birds fed basal diet, although the difference was not significant.

## Antioxidant status

The antioxidant status of immune organs is presented in Table 6. T-AOC and activities of T-SOD

Table 3. Effects of environmental temperature and dietary FA on serum antibody titres against SRBC, levels of NDV antibody and immunoglobins

| | Thermoneutral | | HS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | basal diet | FA | basal diet | FA | | temperature | diet | temperature*diet |
| SRBC antibody ($Log_2$) | 3.31 | 3.63 | 2.25 | 3.18 | 0.26 | 0.001 | 0.004 | 0.126 |
| NDV antibody (ng/ml) | 299.88 | 302.71 | 237.07 | 260.98 | 13.75 | 0.035 | 0.069 | 0.542 |
| IgA (ng/ml) | 104.81 | 111.59 | 88.08 | 90.64 | 4.88 | 0.123 | 0.269 | 0.684 |
| IgM (ng/ml) | 342.61 | 369.68 | 263.87 | 289.17 | 20.96 | 0.031 | 0.523 | 0.852 |
| IgG (ng/ml) | 1 115.16 | 1 242.37 | 928.47 | 984.57 | 60.76 | 0.032 | 0.042 | 0.276 |

FA = fumaric acid; HS = heat stress; NDV = Newcastle disease virus; SRBC = sheep red blood cells

Table 4. Effects of environmental temperature and dietary FA on counts of WBC, heterophils and lymphocytes, and H/L ratio

| | Thermoneutral | | HS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | basal diet | FA | basal diet | FA | | temperature | diet | temperature*diet |
| WBC ($10^8$/l) | 222.11 | 229.15 | 220.44 | 221.19 | 1.74 | 0.389 | 0.485 | 0.572 |
| Heterophils ($10^8$/l) | 50.86 | 53.21 | 48.39 | 49.33 | 0.91 | 0.298 | 0.586 | 0.817 |
| Lymphocytes ($10^8$/l) | 151.71 | 155.31 | 152.33 | 152.92 | 0.68 | 0.702 | 0.367 | 0.517 |
| H/L (%) | 33.36 | 34.25 | 31.66 | 32.07 | 0.52 | 0.243 | 0.692 | 0.884 |

FA = fumaric acid; H/L = heterophils/lymphocytes × 100%; HS = heat stress; WBC = white blood cells

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Table 5. Effect of environmental temperature and dietary FA on relative weights of immune organs (g/kg)

| | Thermoneutral | | HS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | basal diet | FA | basal diet | FA | | temperature | diet | temperature*diet |
| Spleen | 1.68 | 2.49 | 0.96 | 1.16 | 0.30 | 0.000 | 0.037 | 0.198 |
| Thymus | 2.90 | 3.06 | 2.20 | 2.73 | 0.17 | 0.015 | 0.093 | 0.063 |
| Bursa | 1.03 | 1.22 | 0.88 | 1.11 | 0.06 | 0.009 | 0.042 | 0.753 |

FA = fumaric acid; HS = heat stress

Table 6. Effect of environmental temperature and dietary FA on the antioxidant status of immune organs in broilers

| | Thermoneutral | | HS | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | basal diet | FA | basal diet | FA | | temperature | diet | temperature*diet |
| **Thymus** | | | | | | | | |
| T-AOC (IU/mg protein) | 3.56 | 3.80 | 4.09 | 4.92 | 0.51 | 0.042 | 0.041 | 0.096 |
| T-SOD (IU/mg protein) | 10.81 | 12.90 | 11.90 | 16.46 | 1.06 | 0.561 | 0.021 | 0.210 |
| GPx (IU/mg protein) | 14.80 | 18.25 | 12.62 | 23.89 | 2.13 | 0.852 | 0.014 | 0.562 |
| MDA (nmol/mg protein) | 1.22 | 1.19 | 1.99 | 2.13 | 0.23 | 0.020 | 0.850 | 0.748 |
| TC (mg/mg protein) | 0.11 | 0.10 | 0.20 | 0.15 | 0.02 | 0.037 | 0.042 | 0.061 |
| **Spleen** | | | | | | | | |
| T-AOC (IU/mg protein) | 2.11 | 2.21 | 2.08 | 2.08 | 0.03 | 0.561 | 0.652 | 0.693 |
| T-SOD (IU/mg protein) | 8.12 | 7.92 | 9.52 | 7.74 | 0.35 | 0.695 | 0.752 | 0.785 |
| GPx (IU/mg protein) | 9.10 | 9.03 | 8.83 | 7.03 | 0.43 | 0.712 | 0.420 | 0.635 |
| MDA (nmol/mg protein) | 0.88 | 0.71 | 0.85 | 0.79 | 0.03 | 0.854 | 0.085 | 0.752 |
| TC (mg/mg protein) | 0.14 | 0.14 | 0.13 | 0.09 | 0.01 | 0.745 | 0.066 | 0.185 |
| **Bursa** | | | | | | | | |
| T-AOC (IU/mg protein) | 3.30 | 3.85 | 4.56 | 4.08 | 0.23 | 0.047 | 0.578 | 0.649 |
| T-SOD (IU/mg protein) | 7.42 | 7.57 | 10.94 | 12.50 | 1.09 | 0.040 | 0.094 | 0.245 |
| GPx (IU/mg protein) | 11.04 | 15.85 | 18.75 | 21.71 | 1.97 | 0.008 | 0.044 | 0.612 |
| MDA (nmol/mg protein) | 0.18 | 0.16 | 0.32 | 0.33 | 0.04 | 0.024 | 0.342 | 0.421 |
| TC (mg/mg protein) | 0.14 | 0.10 | 0.19 | 0.12 | 0.02 | 0.035 | 0.015 | 0.096 |

FA = fumaric acid; GPx = glutathione peroxidase; HS = heat stress; MDA = malondialdehyde; T-AOC = total antioxidant capacity; TC = total carbonyl; T-SOD = total superoxide dismutase

and GPx in the bursa of Fabricius exhibited a significant increase ($P < 0.05$ or $P < 0.01$) when compared with birds reared under thermoneutral conditions. Meanwhile, HS increased the T-AOC in thymus and the levels of MDA and TC in both thymus and bursa. Supplementing the diet with FA increased the T-AOC and activities of T-SOD and GPx in thymus when compared with the basal diet group. Moreover, broilers fed the FA-supplemented diet had decreased contents of TC in thymus and bursa when compared with broilers fed the basal diet. Additionally, there were weak interactions between temperature and diet in terms of T-AOC of the thymus and TC of the bursa.

## DISCUSSION

During HS, the chickens divert energy from efficient production to reducing their body temperature through different metabolic mechanisms. In the present study, HS decreased FBW, ADFI and ADG of broilers, which could be related to decreased feed intake and feed efficiency, as reported by Sohail et al. (2012). These results were also similar to the results of Shakeri et al. (2018), who reported the reduced production efficiency in broilers exposed to cyclic heat. Based on the mechanism of HS-induced injury, the changes in metabolic

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utilization of nutrients account for these negative results, regardless of the type of HS. FA supplementation partly alleviated the negative effects of HS on growth performance, as reflected by increasing BWG and a decrease in FCR. These results are similar to the previous study showing that FA improves the growth performance in broilers reared under thermoneutral conditions (Adil et al. 2010). Such a positive impact of dietary FA on growth performance may be related to the intestinal protection effects of FA which improves the pH values in feed, development of digestive tract, digestive enzyme activities, and gut microflora (Adil et al. 2010; Liu et al. 2017). In addition, FA is an intermediate product of the citric acid cycle which is a source of intracellular energy in the form of adenosine triphosphate. Therefore, FA was added to the diet and thus the metabolic energy of this particular diet was slightly increased, which may partly explain such beneficial effects.

Chronic HS also decreased the SRBC antibody titre and the levels of NDV antibody, IgM and IgG when compared with birds reared under thermoneutral conditions. This response was similar to observations by Niu et al. (2009) and Park et al. (2013), when birds were exposed to acute HS. This phenomenon may be explained by Kamel et al. (2017), who reported thaT-cells exposed to elevated temperature showed an inhibition of protein synthesis through alterations in the phosphorylation state of many components of the translational process. Meanwhile, high plasma corticosterone concentrations induced by HS enable a decrease in the formation of antibodies against SRBC and some kinds of immunoglobins. In addition, the decrease in feed intake caused by HS led to a reduction in the nutrients available to mount an effective immune response. However, our results are in contrast with those of Honda et al. (2015), who reported that the increased levels of IgM and IgG were observed in broilers exposed to short-term HS. These inconsistent results may be explained by the duration and severity of HS. In addition, there is a significant increase in the SRBC antibody titre and IgG concentration when compared with the birds fed basal diet. These results indicated that different types of immunoglobins have no uniform responses to heat and FA-supplemented diet. These results are similar to the findings of Emami et al. (2017), who showed that dietary formic acid can increase most of the primary and secondary humoral, as well as cellular, immune responses in broilers challenged with *E. coli* K88. The beneficial effects of FA on the improvement of the immune response may be due to the effects of FA on the increased availability of minerals and amino acids, which are nutrients needed for an effective and vigorous immune response. In addition, organic acids reduce Gram-negative bacteria and increase bacteria in the gastrointestinal tract of broilers, and lactobacillus bacteria have been reported to have beneficial effects on the host immune system (Khodambashi Emami et al. 2013).

The WBC, heterophils and lymphocytes are indicators of the immune status in animals. The results of the present study demonstrated that broiler chickens in treatment groups had no significant difference in WBC, heterophils, and lymphocytes. These findings may suggest that the birds activated thermoregulatory mechanisms to cope with the chronic HS conditions according to the haematological parameters. However, Habibu et al. (2018) reported that HS decreased lymphocytes implicating the immunosuppressive effect of HS during the hot season. Hence, depending on animal species, duration and severity of heat exposure, and adaptive thermal traits in farm animals, the findings may vary in their leucocytic response to HS. The increased H/L ratio has been used as a more reliable indicator of stress in birds than plasma corticosteroids because it is also less variable than total cell numbers. However, we did not observe a significant difference in the H/L ratio due to higher ambient temperature or dietary FA supplement. The results are in agreement with those of Lentfer et al. (2015), who observed no significant changes in the H/L ratio due to stress in laying hens. Contrary to our results, Aengwanich (2007) found that the H/L ratio decreased after 21 days of HS treatment in Thai indigenous chickens. Types, duration and intensity of HS contribute to explain this discrepancy.

Relative weights of immune organs reflect the development and function of the immune system including humoral and cellular immunity. In the present study, all relative organ weights were reduced significantly when birds were exposed to chronic HS. These results demonstrated that HS suppressed the immune system, leading to failures in the chickens' response to vaccination and immune organ involution. Shini et al. (2010) reported that the cortical region of the bursa of Fabricius

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increased while the medullary region decreased in broilers under stressful conditions. Moreover, the spleen and thymus are also atrophied in broilers under extreme HS (Park et al. 2013; Liang et al. 2016). The elevated levels of circulating corticosterone in heat-stressed broilers were reported to inhibit the growth and involution of immune organs (Aschbacher et al. 2013). In addition, this could have been a result of the reduction in feed intake, thereby providing fewer nutrients for the proper development of these organs. Supplementing the diet with FA has shown a tendency to improve the relative weights of these organs under HS conditions. The increased relative organ weights may be due to the effects of FA on the increased availability of nutrients which are needed for immune organ growth and development.

HS disturbs the balance between the production of ROS and enzymatic scavenging systems. Excessive levels of ROS result in the disturbance of the balance between oxidation and antioxidant defence systems, causing lipid peroxidation, oxidative damage to proteins and DNA. HS increases levels of ROS in plasma and skeletal muscle and they will cause damage including protein carbonylation and lipid peroxidation. In the present study, SOD and GPx activities were used to estimate the responses of enzymatic scavenging systems in immune organs. HS increased the T-AOC and activities of T-SOD and GPx in the bursa of Fabricius. These results are similar to those of Mahmoud and Edens (2003), who found that GPx activity increased in red blood cells after the exposure of broilers to HS. The increased T-AOC and activities of antioxidative enzymes imply that the balance between the production and scavenging of hydroxyl radicals is disrupted, resulting in their compensatory increase and an adaptive mechanism underlying the increase in oxidative stress. The increased activities in animal's tissues were used to eliminate the higher levels of ROS under HS conditions. Lipid bilayers of the cell membrane and functional proteins are the common biological targets of ROS. There is a significant increase in MDA and TC in the bursa and thymus at the same time, regardless of the diet. The results imply that the activation of antioxidative enzymes cannot prevent the oxidative injury induced by heat exposure. It should be noted that oxidative stress is associated not only with the changes in the scavenging capacity of antioxidant systems but also with the elevated production of free radicals. The net result of an imbalance between the production and ROS scavenging determines the levels of MDA and TC in different immune organs. Also, the spleen in broilers exposed to HS did not show any significant changes in selected antioxidative parameters. These results suggested that heat-stressed broilers had different oxidative stress profile because of different physiological and biochemical characteristics of different immune organs. In fact, the responses of antioxidant enzymes during heat exposure were different in the context of different frequency, duration and severity of HS, broiler breeds, tissues, and so on. Dietary supplement of 10 g/kg FA increased GPx activity, but decreased TC content in the thymus and bursa of Fabricius, suggesting that FA improved the oxidative status of particular immune organs. These results are similar to those of Abudabos and Al-Mufarrej (2014), who reported that formic acid improved T-AOC and reduced the hydrogen peroxide concentration in the serum of broilers challenged orally with pathogenic bacteria. This phenomenon may be explained by Moharregh-Khiabani et al. (2009), who reported that FA interferes with the cellular redox system by modulating intracellular thiols and thereby increasing the level of reduced glutathione. Hence, FA that interferes with free radicals may restore the balance of oxidants/antioxidants and lead to the improvement of health, growth and immune function.

## CONCLUSION

In conclusion, our results confirm the efficacy of FA in increasing the growth performance, immune response to SRBC and NDV and serum IgG level. These effects are accompanied by an increase in the weight of immune organs such as thymus and bursa of Fabricius. Moreover, supplemental FA exerts beneficial effects on the antioxidant status of thymus and bursa in broilers reared under HS conditions. Dietary supplementation with FA may be a potential strategy to improve the productivity and immunity of broilers, especially under high ambient temperature.

### Conflict of interest

The authors declare no conflict of interest.

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