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# Effects of Vitamin C and Zinc on Broilers Performance of Immunocompetence under Heat Stress

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## ABSTRACT

To evaluate the effect of vitamin C and zinc on performance and immune-competence during heat stress. Four hundred and eighty, 1 day old Arbor Acres broiler males and females were used and raised in either a thermoneutral (control) or heat stressed (40°C) environment where different levels of vitamin C and zinc were supplemented. Humoral immunity was assessed by intravenous injection of Sheep Red Blood Cells (SRBC) followed by evaluation of serum for antibody titers in primary and secondary responses. Heat stressed birds that supplemented with vitamin C and Zinc showed greater ($p = 0.5$) body weight, higher daily gain, better feed conversion ratio and less mortality than the non-supplemented control group. Total, IgM and IgG antibody titers for primary and secondary responses were significantly increased in birds receiving vitamin C and zinc with variable levels according to the concentration of vitamin C and zinc. These results indicated that the immune response of broilers can be influenced under heat stress conditions by combination of different levels of vitamin C and Zinc in the diet. The recommended levels of vitamin C and Zinc depend on environmental conditions and other factors which still need further manipulation.

**Key words:** Performance, heat stress, vitamin C, humoral immunity, sheep red blood cells, antibody titers, zinc, Arbor Acres broilers

## INTRODUCTION

Broiler production in Jordan had been developed very fast in the last decades. The development covered all aspects of production in addition to the development for related industries, such as feed industry, slaughtering houses, veterinary medicines and vaccines production. One of the major problems still facing broiler production in Jordan, as well as other hot regions is the threat of huge losses due to heat stress, particularly sudden heat waves which is of great concern in all types of poultry production (Al-Fataftah and Abu-Dieyeh, 2007). These losses include the adverse effects of heat stress on feed consumption, growth rate, hatchability and survival rate. Heat loss in broilers is limited by feathering and the absence of sweat glands. When the ambient temperatures exceed the thermoneutral zone of a bird, it fails to dissipate heat efficiently.

Different physiological responses take place to mediate the effect of heat load on bird; among these is the reduction of metabolic heat production due to the reduction in total feed intake. This leads to a reduction in feed efficiency as well as low growth rate (Teeter *et al*., 1985; Montazer-Sadegh *et al*., 2008; Cinar *et al*., 2011). Geraert *et al*. (1996) reported that broilers exposed to an environmental temperature of 32°C showed a 14% decrease in feed intake by 4 weeks of age and a 24% reduction by 6 weeks of age. Other detrimental effects of high ambient temperature included the reduction of specific immune response in chickens (Thaxton *et al*., 1968;

Rao and Glick, 1970; Thaxton and Siegel, 1972; Kadam *et al*., 2010; Hashemi and Davoodi, 2012). When chicks were exposed to temperatures ranging from 32.2-43°C for short intermittent periods of constant high temperatures or cycling high temperature conditions, the resulting antibody response to SRBC was reduced significantly. The phagocytic potential of chicken macrophages decreased during *in vitro* heat stress conditions (Miller and Qureshi, 1991; De-Faria-Filho *et al*., 2007). Bauman and Currie (1980) suggested two routes for the loss of nutrients that have direct effects on immune responses, either by reduction in concentration of nutrients required to maintain health and productivity of the chicken due to the reduction in feed intake under heat stress conditions or due to the process of redirection of nutrient flow to meet the metabolic requirements of an immune or inflammatory response. For example, plasma zinc was greatly reduced and hepatic zinc was found to be more than four times the amount lost from plasma (Klasing, 1984). Several studies showed that zinc is essential in all aspects of immunity and functions through its association with the enzymes critical for the integrity of the cells involved in the immune response (Chandra and Dayton, 1982; Dardenne *et al*., 1985; Sherman, 1992; Kadam *et al*., 2010).

Vitamin C is important for the optimal function of the immune system. It has direct virucidal and bactericidal activity against a number of pathogens in vitro; also it enhances the by cells infected with Newcastle disease (Siegel, 1987). A report by Thaxton and Siegel (1972) showed that, following infection with infectious bursal disease virus, vitamin C protected the immune biological tissues in growing birds and reduced their total mortality. Moreover, vitamin C has been shown to have a role in the body as antioxidant. It was reported that supplementingas corbate *in vitro* will delay the myoglobin oxidation and by that reduces retardation (Yin *et al*., 1993).

In addition, vitamin loud to reduce the production of Reactive Oxygen Species (ROS). During heat stress, free radicals such as $O_2$ and $H_2O$ will be greated. These free radicals may damage cells membrans and by that effects body thermoregulation (Laudicina and Marnett, 1990).

It is therefore possible that the requirement for zinc and vitamin C is increased during exposure to heat stress conditions. It was believed that there are conflicting results regarding the level of zinc required to affect an immune response, Some studies indicated that supplementing the diet of broilers above 40 ppm recommended by the National Research Council enhances antibody production (Kidd *et al*., 1992), whereas others have reported no effect (Stahl *et al*., 1989a; Pimentel *et al*., 1991b). Lack of sufficient evidence showing how heat stressed broilers would respond immunologically if their diets were supplemented with varying levels of zinc has necessitated further study. Therefore, the current experiment was conducted to evaluate the effect of temperature and different levels of vitamin C and zinc on performance characteristics and immunocompetence of broilers raised under heat stress conditions.

## MATERIALS AND METHODS

**Experimental birds:** Four hundred and eighty, 1 day old Arbor Acres broiler males and females were obtained from local commercial hatchery and reared to 28 days of age in a controlled environmental poultry house using the standard brooding practices. Chicks were randomly assigned to 10 experimental units in a controlled environmental poultry house consisted of 10 identical pens located at the research station of the Faculty of Agriculture at Jerash University. Each room was provided with sufficient number of electrical heaters, which are thermostatically controlled and an electric fan for air circulation and distribution. Ambient temperature was controlled to the accuracy of ±2°C. Maximum-minimum thermometers were hanged on the wall in the opposite direction of the heaters.

**Management practices:** Birds were reared on litter with 10 birds per min. Feed and water provided ad libitum and standard commercial management practices were applied. Ambient temperature was gradually decreased from 32°C at 1 day old to reach 22°C at 21 day of age. At day 22 of age birds were weighed and randomly assigned to the different treatments. From 22-28 days of age, birds were adapted to the experimental procedure at the experimental rooms.

**Experimental rations:** A commercial broiler starter diets was used during the first 28 days of age containing 2999 Kcal ME $kg^{-1}$ and 20.9% crude protein. A finisher diets was used during the experimental period 29-56 day of age containing 3054 kcal ME $kg^{-1}$ and 18.2% crude protein. All feed ingredients were of plant sources; where no animal concentrates were used. Composition of the starter and finisher diets are shown in Table 1.

**Experimental setting:** The experiment was conducted to investigate the effect of supplementing feed with different levels of Vitamin C and Zinc on the performance and immunity of heat stressed broiler chickens. At 21 day of age, birds were weighed and moved from the brooding house to the experimental room in environmentally controlled experimental rooms. The diet used in the experiment as shown in Table 1 and the different levels of Vitamin C and Zinc were supplemented as shown in Table 2. Vitamin C was in the form of ethyl cellulose-coated Ascorbic Acid (AA) and Zinc was provided by a zinc poly amino acid complex (Hoffmann-La Roche, Inc., Nutley, NJ).

**Measured parameters:** Mortality rate was recorded on daily bases, body weight and feed consumption were recorded weekly and body gain and feed conversion were then calculated.

Table 1: Ingredients and calculated composition of the starter and finisher diets

| Item | Starter (%) | Finisher (%) |
|---|---|---|
| **Ingredients and composition** | | |
| Yellow corn | 61.60 | 69.20 |
| Soy-bean meal (44% CP) | 34.50 | 27.00 |
| Limestone | 1.00 | 1.00 |
| Dicalcium-phosphate | 2.10 | 2.00 |
| Premix* | 0.30 | 0.30 |
| DL-methionine | 0.10 | 0.10 |
| Choline | 0.10 | 0.10 |
| Salt | 0.30 | 0.30 |
| Coccidostats | 0.05 | 0.05 |
| **Nutrient composition** | | |
| ME (kcal $kg^{-1}$) | 2999.00 | 3048.00 |
| Crude protein (%) | 20.10 | 18.20 |
| ME/CP | 143.50 | 167.80 |
| Crude Fa | 2.70 | 3.03 |
| Calcium (%) | 0.96 | 0.94 |
| Phosphorus (%) | 0.76 | 0.73 |
| Lysine (%) | 1.26 | 0.99 |
| Methionin (%) | 0.48 | 0.41 |
| Methionin and cystine (%) | 0.89 | 0.39 |
| Sustain (%) | 0.40 | 0.34 |

*: 1 kg of premix contains: 12000000 IU vitamin A, 2500000 IU vitamin D3, 10000 mg vitamin E, 2000 mg Vitamin K3, 1000 mg vitamin B1, 5000 mg vitamin B2, 10 mg vitamin U B12, 30000 mg nicotinic acid, 3000 mg Ca-pantothenat, 1000 mg folic acid, 50 mg biotin, 40000 mg Fe, 5000 mg CU, 60000 mg Mn, 100 mg I, 60000 mg Zn, 150 mg, 150 mg Co, 10000 mg B.H.T

Table 2: Layout of the experiment: ambient temperature and levels (mg $kg^{-1}$) of supplemented vitamin c and zinc to the experimental diets during the experimental period

| Ambient temperature (°C) | Vitamin C (AA) (mg $kg^{-1}$) | Zinc (mg $kg^{-1}$) |
|---|---|---|
| 40±2 | 0 | 0 |
| | | 35 |
| 40±2 | 200 | 70 |
| | | 175 |
| 40±2 | 400 | 35 |
| | | 70 |
| | | 175 |
| 40±2 | 600 | 35 |
| | | 70 |
| | | 175 |

Body weight was measured for all birds at the beginning of the experiment and was repeated weekly at the beginning of the week at the same time.

A random sample of 5 birds from each replicate was weighted as a group using a 20 kg-balance. At the end of the experiment a sample of three randomly-selected birds from each treatment were slaughtered and the bursa of Fabricius and the spleen were removed and weighed.

**Immunocompetence analysis:** Sheep Red Blood Cells (SRBC) were used as test antigens to quantitatively analyze specific antibody response as a measure of humoral immunocompetence. At 28 of age, 5 brids were randomly selected from each treatment, marked and received an antigen challenge with SRBC. The antigen consists of 7.0% suspension of SRBC in 0.85% NaCl. Each bird was intravenously given 1 mL. Seven days later (on day 35) one milliliter of whole blood was collected via the wing veins of birds to evaluate the primary hemagglutination response. A second SRBC challenge with the same antigenic dose was given 16 days after the first SRBC challenge (on day 44 of age).

Blood samples were collected 1 week later (on day 51) to determine secondary hemagglutination response. Blood samples were allowed to stand at room temperature for approximately 2 h. The samples were then centrifuged for 15 min at 3500 round per min. The resulting serum samples were decanted and stored at -20°C until serological analyses were performed. Total antibody, IgM and IgG anti-SRBC antibody titers were determined using microhemagglutination assay in 96 well plates as previously described (Bartlett and Smith, 2003). Briefly, 2-mercaptoethanol-resistant antibody (presumably IgG) were determined by incubating 0.05 mL serum with an equal volume of Phosphate Buffered Saline (PBS) pH 7.5 and 0.2 molarity, 2-mercaptoethanol at 37°C for 30 min prior to the hemagglutination test. The 2-mercaptoethanol-sensitive antibody (presumably IgM) levels were determined by subtracting the 2-mercaptoethanol-resistant antibody titers from the total titers. The antibody titers were expressed as the $\log^2$ of the highest dilution of serum that agglutinated 0.05 mL of 2% suspension of SRBC. All antigen administrations and bleeding was performed intravenously from the wing veins.

**Statistical analysis:** A two factor factorial design was used to show the effect of vitamine C and zinc suppliment with broilers during heat stress. All means of the experimental treatments were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS (2001). Anti-SRBC antibody titers, as well as titers following the mercaptoethanol treatments, were

transformed to log² prior to statistical analysis. Significant F-statistic was indicated by analysis of variance. Means were separated by the method of Least Significant Differences (LSD). All results are presented as Mean±Standard Error (SE) of the means.

## RESULTS

**Broiler performance:** Means±SE of different performance parameters measured during the experimental period are presented in Table 3. Generally, body weight of broilers fed diet supplemented with combinations of different levels of AA and zinc and reared at 40°C are significantly ($p<0.05$) higher than those fed non-supplemented diet, with the maximum weight being recorded at 200 mg AA diet and 70 mg zinc per kg of diet. Similar trends were observed in body weight gain. The differences in body weight gain are mainly due to differences in feed consumption. The feed consumption of broilers fed supplemented diet with combination of different levels of AA and zinc are higher than those fed the non-supplemented diet but not at all levels. During the second week of the experiment, the trend in feed consumption was the same as that shown during the last three weeks of experiment but insignificant differences appeared in broiler fed supplemented and non-supplemented diet. At the end of the four weeks of experiment (8 weeks of age) the average amounts of feed consumption at acute high temperature 40°C increased significantly ($p<0.05$) in the broilers fed supplemented diet with combination of AA and zinc than those fed the non-supplemented diet, with the maximum feed consumption being recorded at (600 mg AA $kg^{-1}$ diet and 35 mg of zinc $kg^{-1}$ diet).

**Immunocompetence and blood constituents:** The relative weights of spleen and bursa of Fabricius of birds are shown in Table 4. Weights are expressed as milligrams of organ per 100 g of body weight. The spleen weight of broilers fed supplemented diet with combination of different levels of AA and Zinc was higher ($p>0.05$) than those fed non-supplemented diet, with the peak being recorded at the 200 mg AA $kg^{-1}$ diet and 175 mg zinc $kg^{-1}$ of diet. However, there were insignificant differences in spleen weight between the broilers fed supplemented diet with AA and zinc and those fed the non-supplemented diet, these results indicated that supplemented diet with ascorbic acid at level 200 mg $kg^{-1}$ diet was recommended to increase the relative weight of spleen

Table 3: Means±SE of different performance parameters measured during the experimental period

| Temperature (°C) | Vitamin C (AA) (mg $kg^{-1}$) | Zinc (mg $kg^{-1}$) | Body weight (g) | Body gain (g/bird) | Feed consumption (g/bird) | Feed conversion ratio | Mortality rate (%) |
|---|---|---|---|---|---|---|---|
| 40±2 | 0 | 0 | 1601+19$^{e}$ | 210±24$^{b}$ | 637.5±37$^{bc}$ | 3.1±0.32$^{a}$ | 65.5±1.5$^{a}$ |
| | | 35 | 1911+23$^{ab}$ | 259±26$^{ab}$ | 697.6±36$^{ab}$ | 2.7±0.54$^{ab}$ | 24.5±2.3$^{cd}$ |
| 40±2 | 200 | 70 | 1992+44$^{a}$ | 275±23$^{a}$ | 653.4±35$^{bc}$ | 2.4±0.45$^{c}$ | 41.6±2.1$^{ab}$ |
| | | 175 | 1886+34$^{ab}$ | 257±22$^{ab}$ | 686.1±36$^{ab}$ | 2.7±0.26$^{ab}$ | 33.4±4.3$^{bc}$ |
| | | 35 | 1894+86$^{ab}$ | 251±21$^{ab}$ | 598.9±34$^{c}$ | 2.4±0.78$^{c}$ | 33.2±5.2$^{bc}$ |
| 40±2 | 400 | 70 | 1879+129$^{ab}$ | 257±26$^{ab}$ | 677.6±30$^{ab}$ | 2.7±0.24$^{ab}$ | 36.8±2.3$^{b}$ |
| | | 175 | 1737+2$^{b}$ | 259±21$^{ab}$ | 646.0±31$^{bc}$ | 2.6±0.23$^{b}$ | 27.3±2.3$^{bc}$ |
| | | 35 | 1925+127$^{ab}$ | 276±25$^{a}$ | 737.0±30$^{a}$ | 2.7±0.14$^{ab}$ | 18.2±2.4$^{d}$ |
| 40±2 | 600 | 70 | 1587+67$^{a}$b | 256±26$^{ab}$ | 669.9±38$^{abc}$ | 2.7±0.25$^{ab}$ | 18.6±4.1$^{d}$ |
| | | 175 | 1787+9$^{abc}$ | 257±27$^{ab}$ | 662.4±36$^{bc}$ | 2.6±0.25$^{b}$ | 23.4±3.5$^{d}$ |

Means with different superscripts in the same column are significantly different at $p<0.05$

Table 4: Means±SE of the relative weights of the spleen and bursa of Fabricius (milligram of organ/100 g of live body weight) as indicator of immune system response as affected by combination of different levels of Vit C and zinc of broiler reared at 40°C during the experiment

| Temperature (°C) | Vitamin C (AA) (mg $kg^{-1}$) | Zinc (mg $kg^{-1}$) | Spleen mg 100 $g^{-1}$ of live body weight | Bursa of fabricius mg 100 $g^{-1}$ of live body weight |
|---|---|---|---|---|
| 40±2 | 0 | 0 | 82.1±3.85$^{c}$ | 54.1±5.8$^{c}$ |
| | | 35 | 106.7±11.5$^{a}$ | 47.2±7.2$^{c}$ |
| 40±2 | 200 | 70 | 98.4±15.3$^{b}$ | 49.3±12.3$^{c}$ |
| | | 175 | 109.3±9.8$^{a}$ | 74.1±10.3$^{b}$ |
| 40±2 | 400 | 35 | 101.2±8.8$^{a}$ | 83.4±11.4$^{a}$ |
| | | 70 | 98.6±7.6$^{b}$ | 56.3±12.1$^{c}$ |
| | | 175 | 102.3±5.9$^{a}$ | 107.5±11.4$^{a}$ |
| 40±2 | 600 | 35 | 92.6±11.2$^{b}$ | 59.4±11.8$^{b}$ |
| | | 70 | 94.9±10.6$^{b}$ | 68.9±11.7$^{b}$ |
| | | 175 | 98.7±9.7$^{b}$ | 61.2±11.6$^{b}$ |

Means with different superscripts in the same column are significantly different at $p<0.05$

Table 5: Means±SE of antibody titers ($log^{2}$) as indicator of immune system response as affected by combination of different levels of ascorbic acid and zinc of broiler reared at 40°C during the periods of experiment

| Ambient temperature (°C) | Vitamin C (AA) (mg $kg^{-1}$) | Zinc (mg $kg^{-1}$) | Primary total | Secodary IgG | IgM | Total | IgG | IgM |
|---|---|---|---|---|---|---|---|---|
| 40±2 | 0 | 0 | 2.05±0.09$^{a}$ | 0.85±0.04$^{a}$ | 1.20±0.03$^{a}$ | 2.33±0.12$^{a}$ | 1.11±0.06$^{a}$ | 1.22±0.08$^{a}$ |
| | | 35 | 3.60±0.09$^{b}$ | 0.95±0.03$^{b}$ | 2.65±0.03$^{b}$ | 3.08±0.11$^{b}$ | 1.55±0.05$^{b}$ | 1.53±0.08$^{b}$ |
| 40±2 | 200 | 70 | 4.15±0.08$^{c}$ | 1.55±0.04$^{c}$ | 2.60±0.05$^{b}$ | 3.06±0.11$^{b}$ | 1.41±0.05$^{b}$ | 1.65±0.09$^{b}$ |
| | | 175 | 4.65±0.09$^{c}$ | 1.95±0.05$^{d}$ | 2.70±0.04$^{b}$ | 3.09±0.09$^{b}$ | 1.35±0.04$^{b}$ | 1.74±0.04$^{b}$ |
| | | 35 | 3.80±0.09$^{b}$ | 1.10±0.05$^{c}$ | 2.70±0.04$^{b}$ | 3.18±0.08$^{b}$ | 1.65±0.03$^{b}$ | 1.53±0.05$^{b}$ |
| 40±2 | 400 | 70 | 4.35±0.10$^{c}$ | 1.65±0.06$^{d}$ | 2.70±0.04$^{b}$ | 3.18±0.09$^{b}$ | 1.47±0.07$^{b}$ | 1.71±0.05$^{b}$ |
| | | 175 | 4.75±0.10$^{c}$ | 1.95±0.04$^{d}$ | 2.80±0.09$^{b}$ | 3.47±0.10$^{b}$ | 1.55±0.07$^{b}$ | 1.92±0.06$^{b}$ |
| | | 35 | 3.95±0.09$^{bc}$ | 1.30±0.03$^{c}$ | 2.65±0.08$^{b}$ | 2.92±0.06$^{b}$ | 1.44±0.08$^{b}$ | 1.48±0.04$^{b}$ |
| 40±2 | 600 | 70 | 4.68±0.04$^{c}$ | 1.98±0.08$^{d}$ | 2.70±0.09$^{b}$ | 3.00±0.08$^{b}$ | 1.45±0.07$^{b}$ | 1.55±0.09$^{b}$ |
| | | 175 | 4.55±0.05$^{c}$ | 1.90±0.07$^{d}$ | 2.65±0.04$^{b}$ | 3.22±0.09$^{b}$ | 1.61±0.07$^{b}$ | 1.61±0.07$^{b}$ |

Means with different superscripts in the same column are significantly different at $p<0.05$

of broilers reared at 40°C. Broilers fed diet supplemented with AA and zinc had significantly ($p<0.05$) higher bursa weight than those fed the non-supplemented diet, with the peak being recorded at the 400 mg AA $kg^{-1}$ diet and 175 mg zinc $kg^{-1}$ of diet.

Means±SE of total antibody, IgG and lgM titers as indicators of immune system response to SRBC during primary and secondary responses of broilers kept at 40°C and fed diet supplemented with combination of different levels of AA and Zinc are shown in Table 5.

## DISCUSSION

The results indicate that supplementing diet with combination of 600 mg AA $kg^{-1}$ and 35 mg of zinc $kg^{-1}$ of diet is very beneficial to increase the feed consumption of broilers reared at acute high temperature (40°C). These results were in general agreements with the previous findings of Pimentel *et al.* (1991b), Stahl *et al.* (1989b) and Teeter *et al.* (1985).

However, this is not always the case. Previous researches reported that the level of zinc in the diet did not significantly influence broiler growth performance, feed intake, feed conversions ratio and growth rate (Pimentel *et al.*, 1991b; Bartlett and Smith, 2003; Hamidi and Pourreza, 2009; Hamidi *et al.*, 2010).

Due to the considerable variation found in the above reports, these researchers believe there are apparent differences in susceptibility to heat stress based on genetic lines.

At the end of the four weeks of experiment (8 weeks of age) the average feed conversion ratio increased significantly ($p<0.05$) for broilers fed the non-supplemented diet. In general, the broilers fed non-supplemented diet and reared at hot environmental temperature (40°C) achieved the poorest feed efficiency compared to broilers fed supplemented diet with combination of different levels of AA and zinc. Broilers fed supplemented diet with combination of AA and zinc at 400 mg AA $kg^{-1}$ diet and 35 mg of zinc or 200 mg AA $kg^{-1}$ and 70 mg zinc had significantly ($p<0.05$) the best feed conversion ratios. These results are similar to the results of Bartlett and Smith (2003), Teeter *et al.* (1985) and Deif *et al.* (2007).

The mortality rate of broilers fed supplemented diet with combination of different levels of AA and zinc are lower than those fed the non-supplemented diet. At the end of the four weeks of experiment (8 weeks of age) the total mortality rate increased significantly ($p<0.05$) in broilers fed the non-supplemented diet. Supplementing diet with 600 mg AA $kg^{-1}$ and 35 mg zinc $kg^{-1}$ of diet. Similar results were obtained by Bartlett and Smith (2003), Pimentel *et al.* (1991a), Puthpongsiriporn *et al.* (2001) and Teeter *et al.* (1985).

The data show the means of $log^2$ the reciprocal of the last dilution exhibiting agglutination. Before the antigenic challenge, sera from birds were analyzed for prechallenge antibody titers that could influence the results and were found to be negative. During the first response, broilers reared at hot ambient temperatures (40°C) and fed non-supplemented diet had significant lower total antibody, IgG and IgM titers compared with those fed diet supplemented with AA and zinc, there were also significant differences in total antibody, IgG and IgM titers among the broilers fed supplemented diet with combination of different levels of AA and zinc and those fed the non-supplemented diet during secondary response. This could be explained by the increases in feed intake for the supplemented groups and improved in feed conversion ratio that leads to an increase in the nutrients available to exhibit an effective immune response. The total titers appear to be more correlated to zinc level than to AA level; birds receiving high levels of zinc had significantly higher ($p>0.05$) titers of total antibodies than those receiving other diets for the primary response. These results are in agreement with previous findings (Beach *et al.*, 1980; Burns, 1983; Pimentel *et al.*, 1991a) that diets supplemented with zinc tend to improve the ability of the birds to produce antibodies. In the current study, broiler birds kept under acute high ambient temperature were able to exhibit significant reduction in all three parameter during primary and secondary responses. Similar reports were provided by Thaxton and Siegel (1970), Donker *et al.* (1990), Bartlett and Smith (2003) and Akharaiyi and Gabriel (2007).

When birds were exposed to high ambient temperature. Broilers reared at 40°C and fed supplemented diet with combination of different levels of AA and zinc at (200 mg AA $kg^{-1}$ diet+35 mg zinc $kg^{-1}$ of diet, 400 mg AA $kg^{-1}$ diet+35 mg zinc $kg^{-1}$ and 600 mg AA $kg^{-1}$ diet+175 mg zinc $kg^{-1}$ of diet) levels, had showed insignificant higher total antibody, IgG and IgM titers compared with those fed non-supplemented diet during secondary response.

The results of the current study support supplementation of vitamin C and Zinc in broiler diets to reduce the negative effects of heat stress. Supplementation of poultry diets with different levels of vitamin C and zinc, may protect cells from damage stimulated by heat stress, the levels are highly dependant on genetic line, age and stressor level. However, we can only postulate that the level of vitamin C and zinc could be manipulated further before a definite recommendation is made.

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