Effects of Commutative Heat Stress on Immunoresponses in Broiler Chickens Reared in Closed System

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Abstract: Eastern region of Saudi Arabia was characterized by high temperatures especially in summer season, so this study was carried out to investigate effect of heat stress on immunoresponse in broiler chickens kept in a closed system. Thirty Cobb 500 chicken (22-day-old) were exposed to 40°C/14 hrs/day for ten days. Blood samples were collected before and after heat exposure at 3 stages during experimental, (1', 5' and 10' day) samples which were taken at the first day before heat exposure were served as control group (Basal level). The result revealed that heat stress led to significant (p < 0.01) decrease in plasma ascorbic acid, Antibodies (IgG and IgM) levels in all stage, whereas percentage lymphocyte and heterophil and H/L ratio showed significant changes during three stage of experiment. It is suggested that alteration of plasma ascorbic acid and immunoresponse may reflect heat stress in chicken.

Key word: Heat stress, immunoresponse, broiler

Introduction
Broiler production plays a major role in food security for the rapidly increasing human population. The short production cycles (35 day) of broiler are required for marketing in Saudi Arabia (Al-Ghamdi, 2005). Genetics, antibiotics, probiotics, vitamin supplements, antibodies and pelleting of feed, all decrease the time an animal requires to reach market weight, reducing feed and overall cost (Cook, 2004). Heat stress is one of the most important factors adversely affecting overall poultry production in the tropics. The domestic fowl is a homeotherm which can live comfortably only in a very relatively narrow zone of thermo-neutrality ranged from 18-22°C within which the heat from normal maintenance and productive functions of the animal in non-stressful situations offsets the heat loss to the environment without requiring an increase in rate of metabolic heat production (Ensminger et al., 1990). Any deviation especially on the upper critical temperature which reached to (40-50°C) for the greater part of the year season in Eastern region of Saudi Arabia may affect the performance of chicken (Committee of Meteorology and Environmental Protection, 2002). High environmental temperatures have deleterious effects on poultry, reducing rate of growth, feed intake, live weight gain and feed efficiency, digestibility of nutrients, egg production, egg weight, Haugh unit and yolk index (Millis et al., 1999), mortality and immunity (Naseem et al., 2005). The physiological functions of broiler are affected also by heat stress, El Husseiny and Creger (1981) who reported that a high environmental temperature (32°C) decreased concentration of some minerals. Several studies have been found that these stresses induce a cascade of neural and hormonal events beginning with hypotalamic stimulation cause the release of corticosterone, catecholamines and initiates lipid peroxidation in cell membranes (Siegel, 1995; Sahin and Kucuk, 2003). Ascorbic acid has been widely used to reduce the stress in chickens, because this vitamin could decrease corticosterone level in the blood circulation (Sheila and Cheryl, 1978). The most clearly established functional role for vitamin C involves collagen biosynthesis and corticosteroids in the adrenal glands may involve ascorbic acid related hydroxylation steps (McDowell, 1989). On stimulation of the adrenal gland by ACTH, a fall in ascorbate concentration was observed and it was suggested that vitamin C is required for steroidogenesis (Kutlu and Forbes, 1993). The function of vitamin C is also related to its reversible oxidation and reduction characteristics Recent studies revealed that broiler exposed to acute heat stress had more effects on immune response, lymphoid organs (bursa, thymus and spleen) and pathophysiology of white blood cells, increased percentage of monocytes and increased percentage of heterophil and heterophil/lymphocyte ratio (H/L ratio) (Mogenet and Youbicier-Simo, 1998; Borges et al., 1999; Altan et al., 2000 and Naseem et al., 2005). It is generally agreed that heat stress reduces immune response (Savic et al., 1993). Tuekam et al. (1994) showed that there was a positive correlation between antibody titer and ascorbic acid supplementation. Therefore, the present study was conducted in order to determine the effects of heat stress on the immune responses (IgM, IgG, H%, L% and H/L) and ascorbic acid in broiler. The results of this study will help in management of broiler in hot weather in closed system.

Materials and Methods
Management: Total 30 baby chicks of both sex (Cobb 500) were obtained from Dammam Modern Poultry Company, Saudi Arabia and housed under automatically
controlled environment with conventional ventilation. Experimental room area was 15 m². Wood shaving litter was used at 3-5 cm thickness, indoor ambient temperature started as 34°C then decreased gradually by 1°C every 2 days according to (Sainsbury, 2000). The indoor relative humidity mean is 60.7% according to (Aengwanich and Simarak, 2004) with traditional prophylactic programs.

Experimental design: Thirty birds at 22-days old were exposed to 41°C/4 hrs from 01.00-04.00 pm (4 hr heat stress episodes) in completely isolated subunit in the experimental room where the electric heater was 2000 watts placed at 50 cm height of floor. The exposure was repeated daily till 31-days old.

Immunological parameters: At 1st, 5th and 10th day of exposure (22, 26 and 31 days old), individual blood samples collected from the same 10 birds (wing vein) in plastic tubes contained anticoagulant (EDTA) then it centrifuged at 3000 r.p.m./15 min. to separate plasma which kept in -18°C until the following assays. Immunoglobulin estimation (IgG and IgM) was done immunochromically using triantigen plates (Mancini et al., 1965) kits reference (CU 50045 SD, Serotec, Oxford, UK). Total ascorbic acid concentrations were estimated spectrophotometrically by method of Maical, 1960. Immunoglobulins were measured by nephelometry using Beckman Array Analyzer (Beckman, Instruments Inc, California, USA). All assays included manufactures calibration standards for comparison with chicken samples. Blood smears were made and stained with Gemsa for differential leukocytes count recording H%, L% and H/L ratio (Gross and Siegel, 1983). Statistical analysis for collected data were done using personal computer and SPSS (descriptive and correlation using one-way ANOVA, then post Hoc test were done to obtain LSD. (Hollander and Douglas, 1973).

Results and Discussion
Table 1 shows that plasma ascorbic acid in broiler chickens decreased from 53.94 ± 156 mg/dl before first heat exposure (basal level: stage 1) to 27.10 ± 0.346 mg/dl after last exposure to heat stress (stage 3), it is clearly observed that ascorbic acid significantly (p = 0.01) decreased after exposure to heat stress when compared to its levels before chickens subjected to heat and with based level (zero time). It was noticed that the lowest value of ascorbic acid was found after last repeated exposure to heat stress. The results were similar to (Klausing, 1996; Sahin and Kucuk, 2001; Sahin et al., 2002) where they found that the negative effect of heat stress was the decreased concentration of vitamins C, E and A, iron, zinc and chromium in the serum and liver. Stress increased mineral and vitamin mobilization from tissue and their excretion (McDowell, 1988; Siegel, 1995) and thus may exacerbate a marginal vitamin and mineral deficiency or lead to increased mineral and vitamin requirements. High ambient temperatures were documented to suppress broiler carcass weight and vitamin C supplementation was observed to alleviate such effect (Hurwitz et al., 1980; Donkoh, 1989; McKee et al., 1997; Seehawer, 2001). Vitamin C and E are used in the poultry diet because of their anti-stress effects and because their level is reduced during the heat stress (Richards, 1997). It is involved in a number of biochemical processes. Ascorbic acid is necessary for various biosyntheses (L-carnitine, 1, 25 dihydroxyvitamin D, adrenaline etc.) as well as for the regulation of diverse reactions (secretion of corticosterone, regulation of body temperature, activation of the immune system). (Sahin and Kucuk, 2003). Regarding the antibodies (Table 1) there were non-significant differences in IgG concentrations at stage 2 and 3 before chicken exposed to heat stress when compared with based level (zero time) although the chicken were exposed to heat daily, IgM concentrations before exposure to heat in stage 2 showed significant decrease (p = 0.01) when compared with based level but in stage 3 it showed significant increase in comparison with based level. After exposure to heat stress IgM and IgG concentrations were significantly (p = 0.01) decreased in all stages especially at the 3rd stage in which extremely lowered concentration were recorded when compared with basal level. Lowered concentration of antibodies (IgM, IgG) compared with basal level were attributed to the negative effects of heat stress as reported by (Zulkifi et al., 2000). Heat stress was also reported to cause a reduction in antibody production in young chickens (Zulkifi et al., 2000). However Donker et al. (1990) found that heat exposure did not reduce antibody production to SRBC and Heller et al. (1979) even found significantly increased antibody titer to SRBC following heat exposure. Mashay et al. (2004) explained the difference in these findings could be associated with age and type of bird used or due to the experimental methodology that was applied. Heat-induced immunosuppression may depend on breed of bird (Regnier et al., 1980) and on the length and intensity of the heat exposure (Kelley, 1983). The reduction in antibodies synthesis could be indirectly due to an increase in inflammatory cytokines under heat stress (Ogle et al., 1997), which stimulates the hypothalamic production of corticotropin releasing factor which is known to increase adrenocorticotropic hormone from the pituitary, which then stimulates corticosterone production. The latter inhibits antibody production (Gross, 1992). Furthermore, heat stress is known to decrease T-helper 2 cytokines (Wang et al., 2001), which are important for antibody production (Lebman and Coffman, 1988). In this study it was clearly noticed that heat exposure to 40°C episodes had fast, direct and intensive effect (after four hours from heat
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Table 1: Effect of heat stress on immune parameters in plasma and blood of broilers (cobb500)

<table>
<thead>
<tr>
<th>Age</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma parameters</td>
<td>before</td>
<td>after</td>
<td>before</td>
</tr>
<tr>
<td>ascorbic acid</td>
<td>53.64</td>
<td>30.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>mg/dl</td>
<td>0.198</td>
<td>0.336</td>
<td>0.208</td>
</tr>
<tr>
<td>IgG</td>
<td>1237.70</td>
<td>1010.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1320.00</td>
</tr>
<tr>
<td>(g/L)</td>
<td>122.471</td>
<td>2.319</td>
<td>5.164</td>
</tr>
<tr>
<td>IgM</td>
<td>120.00</td>
<td>105.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(g/L)</td>
<td>0.518</td>
<td>0.277</td>
<td>0.775</td>
</tr>
<tr>
<td>Blood parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterophil</td>
<td>51.20</td>
<td>38.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(H %)</td>
<td>5.190</td>
<td>3.600</td>
<td>4.180</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>43.60</td>
<td>56.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(L %)</td>
<td>5.180</td>
<td>3.760</td>
<td>4.820</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>1.68</td>
<td>0.704</td>
<td>0.6330</td>
</tr>
<tr>
<td></td>
<td>.410</td>
<td>.119</td>
<td>.154</td>
</tr>
</tbody>
</table>

Data indicate mean ± SE, *there was significant difference with baseline level in the same row at (p = 0.01), *there was significant difference in every stage before and after in the same row at (p = 0.01), c there was significant difference in every stage between before and after in the same row at (p = 0.05), Stage 1: Basal level (Zero time) before heat stress exposure. At 1<sup>st</sup> day after heat stress exposure. Stage 2: At 5<sup>th</sup> day before daily heat stress exposure. At 5<sup>th</sup> day after daily heat stress exposure. Stage 3: At 10<sup>th</sup> day before daily heat stress exposure. At 10<sup>th</sup> day after daily heat stress exposure.

exposure) on the antibodies concentrations (IgM and IgG). Theses results were sustained by Bobeck and Cook, 2005 who reported the antibody was nearly denatured by five minutes (79% lost) their results showed a downward slope in the loss of antibodies as the time in heat increases. On regarding to H% and H/L ratio the current results revealed same behaviour for the two parameters, in comparison with basal level. It appeared that there were gradually decrease in values until stage 3 (10 days of heat stress) which showed dramatic and significant (p = 0.01) increase when compared with values at stage 1 and 2 significantly H% and non-significantly and H/L ratio were observed. Zulkiﬁ and Siegel (1995); Borges (1997) Gross and Seigel (1983) reported that H/L ratio has been indicated to be a good quantitative measures of stress. The decrease of H% could be explained by inflammation as reported by Ritchie et al. (1994). Whereas, L% showed highly significant decrease in last stage. This is in agreement with Wang et al. (2001). The previously mentioned results were similar to results of Borges et al. (2003); Borges et al. (2004) for H% and L% and with McFarlane and Curtis (1989) for H/L ratio, but Mashaly et al. (2004) found that birds exposed to acute heat stress (1 week) showed no differences in WBC and H/L ratio compared with birds exposed to acute cyclic or controlled temperature. They added that birds exposed to chronic heat stress (4 weeks) had a lower WBC count and higher H/L ratio compared with birds exposed to chronic cycle or controlled temperature and Koellebeck et al. (1988) reported the differences in results could be due to differences in heat stress treatments or the type of birds used. This might be due to the reason that chicks up to 3 weeks were more tolerant to heat stress (Jordan, 1990). It was noticed that L% was significantly increased (p = 0.05 and p = 0.01) in stage 1 and 2 Respectively and decreased (p = 0.01) in stage 3. This could be explained by Makkawy (2000) who reported that increased level of glucocorticoids in blood lead to the increase number of cells producing immunoglobulins. The decrease in immuno-response during treatment with large amount of glucocorticoids for long period of time has lead to the loss of immune cell which are able to synthesis lymphokine and monokine necessary to produce antibodies. It could be said that immunoglobulin producing specialized cells where sited among more types of cells that which degradation and death occur under effect of glucocorticoids rise (for long period) then antibodies production was affected to extant limit. Similarly, Gross (1992) and Naseem et al. (2005) reported that ascorbic acid could improve immune response in birds under stress and disease condition. The leukocytocye count aids in the assessment of the leukocytosis, because a heterophilia is usually present in leukocytosis caused by inflammation (Ritchie et al., 1994). Furthermore, Jain, (1993) reported that corticosteroid induced lymphopenia attributed to lymphocysis in blood and lymphoid tissue, increased shift of lymphocytes from blood to other body compartments. Mashaly et al., 2004 found that the heat stress group not only had an increased in the H/L rates, indicating the birds were under increased stress, but also a decrease in antibody titer. It could be concluded that heat stress has fast, direct and severe effects on ascorbic acid, IgG and IgM of plasma concentration on the same day of heat exposure. This effect was repeated on stage 1, 2 and 3. The effects of heat stress on H%, L% and H/L ratio were obtained later at stage 3, which indicate that heat stress effect on these parameters are accumulative. Heat stress in this study has a two-way action, direct on antibodies and indirect on it by decreasing L% count. So, it suggested that antibodies
concentration (IgG, IgM) and ascorbic acid in plasma are good indicators for fast direct and severe action of heat stress (41°C) more than H/L ratio. The results of this study may help in management of broiler in hot weather in closed system.

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