PHYSIOLOGICAL RESPONSE OF BROILER CHICKENS UNDER HEAT STRESS CONDITIONS FOR SOME ORGANIC ANTIOXIDANT ADDITIVES.

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SUMMARY

One hundred and twenty 1-d old commercial broiler chicks (Cobb) were divided to four groups with six replicates, to investigate the effect of dietary supplementation with organic chromium (Cr), organic selenium (Se) and Vitamin E (Vit.E) on some physiological and immunological parameters. The first group was the control group, while the second group was fed the basal diet supplemented with organic chromium (0.8 mg/kg diet), the third group was fed the basal diet supplemented with organic selenium (0.3 mg/kg diet) and the fourth group was fed the basal diet supplemented with vitamin E (200 IU/kg diet). Temperature and humidity were recorded daily and the Temperature-Humidity Index (THI) was calculated, which demonstrated that birds were exposed to heat stress throughout the experiment period. The results showed that increasing dietary levels of organic chromium, organic selenium and Vitamin E especially Vitamin E supplementation could improve plasma total protein and globulin (p<0.05) values at 42 DOA. Supplements could significantly (p<0.05) enhance the total antibody titer against Newcastle disease Virus (NDV) either for the primary or secondary response and induce higher expression of heat shock protein 70 (HSP 70). Insulin-like Growth factor-I (IGF-I) level was improved significantly (p<0.05) due to increasing dietary organic chromium levels followed by vitamin E than organic selenium. It could be concluded that the dietary addition of organic chromium (Cr), organic selenium (Se) and Vitamin E (Vit.E) triggered improvements in the physiological and immunological measurements of broilers reared under heat stress conditions.

Key words: Broiler, Vitamin E; selenium, Chromium, IGF-I and Hsp70

INTRODUCTION

Poultry production in tropical countries is affected by many challenges especially during the hot humid summer season. Economic losses result from decreased productivity and increased mortality due to acute heat stress. Acute heat stress at marketing age especially in broiler chickens raised in open houses with reduced means of heat exchange leads to economic losses. Homeostasis is constantly challenged by intrinsic and extrinsic stressors (Lin et al., 2006). Heat stress is of major concern for poultry industry, especially in the hot regions. The important traits governing productivity (growth performance, immune suppression and high mortality rate ….. etc) are adversely affected by heat stress (Mujahid et al., 2006, 2007 and Niu et al., 2009). Biochemical and physiological events associated with hyperthermia can potentially promote reactive oxygen species formation which results in the disturbance of balance between the oxidation and antioxidants defense systems, causing lipid peroxidation (LPO), in cell membranes, free radical peroxidation and oxidative injury in biological molecules, DNA and proteins (Lin et al., 2006; Mujahid et al., 2006 and 2008 and Aslam et al., 2010). Furthermore, heat stress increases mineral and vitamin mobilization from tissues and their excretion, thus may exacerbate a marginal vitamin and mineral deficiency or an increased mineral and vitamin requirement, several methods are available to alleviate the negative effects of heat stress, mostly focused on dietary manipulation.

It could be concluded that under environmental stressful conditions, as the bird's body attempts to maintain its thermal homeostasis, increased levels of reactive oxygen species (ROS) occur. As a consequence, the body enters a stage of oxidative stress, and starts producing and releasing heat shock proteins (HSP) to try and protect itself from the deleterious cellular effects of ROS (Droge 2002). Also many HSP are expressed as an emergency response to diverse stressor agents, and that increased synthesis of these proteins seems to be involved in protection of stressed cells and organisms (Gabriel et al., 2003).
Stress thus may exacerbate a marginal chromium (Cr) deficiency or an increased Cr requirement, Cr is used in the poultry diet because of the reported benefits of Cr supplementation in broiler under heat stress because of the fact that stress condition increase chromium mobilization from the tissues that is irreversibly excreted (Sahin et al., 2002a and 2003). Cr plays a regulatory role in insulin action and consequently, in nutrient metabolism (Sands and Smith, 2002). It is also an integral part of bimolecular such as glucose tolerance factor and chromodulin, a cofactor of certain enzymes and necessary for stabilization of proteins and nucleic acids. Bahrami et al. (2012) reported that antibody titers against Newcastle virus were higher (p<0.05) in broilers received organic Cr supplements at 18 and 30 d of age. Samar et al. (2014), recorded that supplementation of Cr to broiler diets were significantly affected on serum total protein by increasing its level under heat stressed compared to the control group.

Dietary Se plays an important role in all aspects of the immune system (Arthur et al., 2003). El-Sheikh et al. (2010) stated that, selenium supplementation especially at 0.2 and 0.3 mg/kg diet significantly (P≤0.05) increased serum total protein and globulin 0.2 and 0.3 selenium compared with the control group, while, no significant effects on serum albumin concentrations was observed. Chicks fed diet supplemented with sodium selenite solution at a dose of 0.23, 0.50, 0.75 and 1.5 mg/kg resulted in an increase of antibody titers against Newcastle disease (Yang et al. 2000). Mahmoud and Edens (2003 and 2005) have clearly shown that chickens provided with organic selenium in yeast are more resistant to thermal stress than chickens fed inorganic sodium selenite, and this was demonstrated by a lower expression of HSP70.

Vitamin E (Vit.E) were discovered and its role as an antioxidants was further characterized (Wolf, 2005). The Vit.E that is integrated into cellular membranes exerts its antioxidant effects by intercepting peroxyl radicals more rapidly than can polyunsaturated fatty acids (Burton and Traber, 1990). In one-day-old un-sexed Hubbard broiler chicks Metwally, (2003) found that vitamin E levels significantly (P<0.05) affected plasma albumin when supplementing the diet with either vitamin E (10, as recommended by NRC and high level as 150 IU/kg) from hatch till 6 week of age. Weberi et al. (2008) reported that haemagglutination titers against NDV increased in comparison to the controls as an effect of vitamin E supply in drinking water (10.5 mg/animal-day-1) for 14 days . Kapakin et al. (2013) when they studied Effect of Vit E on HSP 70, they found that, the highest secretion level of HSP-70 was in the control positive group (34o C), while the lowest secretion level of HSP-70 was in the control positive group (34o C), when vitamin E is administered the secretion of HSP-70 increases in control positive group (34o C) plus (DL α Tocopheryl acetate: 150 mg/kg) in addition to basal ration.

Therefore, the objective of the present study is to elucidate the effect of increasing dietary supplements levels of organic chromium, organic selenium and Vitamin E on some physiological changes in blood components, antibody titer against Newcastle Disease Virus (NDV), Insulin-like Growth factor -I (IGF-I) and heat shock protein 70 (Hsp70) of broilers under heat stress conditions. Finally, results from this study could provide a fundamental knowledge for using antioxidants to reduce oxidative stress and improve some physiological and immunological parameters in broiler production under hot climates of summer season in Egypt.

**MATERIALS AND METHODS**

**Experimental procedures:**

A total of 120 1-d old commercial broiler chicks (Cobb) were obtained from a local hatchery. Upon arrival, the chicks were divided into four groups with six replications, each of five chicks. The first group was the control group, while the second group was fed the basal diet supplemented with 0.8 mg/kg diet organic Chromium, the third group was fed basal diet supplemented with 0.3 mg/kg diet organic selenium, the fourth group was fed basal diet supplemented with 200 IU/kg diet vitamin E. At 42-d of age all groups were exposed to 41±1°C for 1h.

Maximum and minimum temperature (°F) and relative humidity in breeding houses illustrated in Table (1). The Temperature-Humidity Index (THI) was calculated using the following formula (World Meteorological Organization (W.M.O), 1996), to determine the level of stress by using the Table (2) which explained THI ranges and their corresponding stress categories (Smith et al., 1998).

\[
\text{THI} = T_d - [0.55 - (0.55 \times \text{RH/100})] \times (T_d - 58).
\]
Where: THI = Temperature Humidity Index; Td = dry bulb temperature in degrees Fahrenheit; RH = relative humidity (%).

The experimental diets and their calculated analysis are shown in Table (3). Feed and water were offered ad libitum and artificial light was provided for 23 hours daily all over the experimental period, which lasted for 6 weeks. Chicks of the four treatments were kept under similar hygienic and environmental conditions and vaccinated against common diseases.

**Measurements:**

**Plasma total protein (PTP), albumin (A), globulin (G) and albumin to globulin ratio (A/G ratio).**

Plasma total proteins (g/dl) were determined according to the method described by Henry (1974). The determination of plasma albumin (g/dl) based on a colorimetric method was conducted as described by Doumas et al. (1971). Globulin was calculated by subtraction of plasma albumin from plasma total protein, and then A/G ratio was calculated.

**Table (1): In door maximum and minimum Temperature (°F), relative humidity and Temperature Humidity index.**

| Age (Week) | Minimum Temperature(°F) | Maximum Temperature(°F) | Relative humidity (%) | THI min. | THI max. |
|------------|-------------------------|-------------------------|-----------------------|----------|----------|
| 1          | 87.8                    | 94.75                   | 68.86                 | 82.7     | 88.5     |
| 2          | 86.95                   | 89.85                   | 69.71                 | 82.1     | 84.5     |
| 3          | 83.57                   | 94.48                   | 74.43                 | 79.9     | 89.3     |
| 4          | 85.64                   | 96.12                   | 74.6                  | 81.8     | 90.8     |
| 5          | 86                      | 97.70                   | 75.4                  | 82.2     | 92.3     |
| 6          | 88.56                   | 100.31                  | 76.5                  | 84.6     | 94.8     |

**Table (2): Temperature Humidity index ranges and their corresponding stress categories.**

| Stress Category | THI range (inclusive) |
|-----------------|-----------------------|
| No stress       | <72                   |
| Mild stress     | 72-79                 |
| Heat stress     | 80-89                 |
| Severe stress   | 90-98                 |
| Death           | >99                   |

**Antibody titer against Newcastle Disease Virus (NDV).**

Vaccination program: All chicks were vaccinated with the NDV vaccine: Hitchnar B1 strain vaccine at 7 DOA and repeated with LaSota strain vaccine at 21 day of age. The primary and secondary immunization, at 20 and 30; 40 DOA were used to evaluate the primary and secondary antibody titer (Hitchner et al., 1980). Each Plasma samples were collected ten days after the first and the second vaccination to determine the immune responses for total antibodies response against NDV, using microtitre U-shape plate of 96 wells.

**Insulin-Like Growth Factors (IGF-1)**

Insulin-Like Growth Factors (IGF-1) was determined with commercial kits (Gammacoat, kits, clinical assay, Cambridge, Medical Diagnostics, and Boston, MA) as reported by Houston and Neill (1991).

**Measurement of Heat shock proteins:**

Liver tissue samples were homogenized and completely washed in ice-cold physiological saline. The Hsp70 in the liver of both heat-stressed and control broilers were measured by enzyme-linked immunosorbent assay (ELISA). Enzyme linked immunosorbent assay for liver hsp70 was performed as described by Anderson et al., (1993).

**Statistical analysis:**

Data obtained were statistically analyzed using the General Liner Model of SAS (2009). Significant differences among means were tested by Duncan’s Multiple Range Test Duncan (1955).
RESULTS AND DISCUSSION

Plasma total protein (PTP), albumin (A), globulin (G) and albumin to globulin ratio (A/G ratio).

Results of plasma total protein fractions as influenced by dietary supplements of organic chromium, organic selenium and Vitamin E are presented in Table (4). At 21 and 42 DOA, the present data show significantly (p<0.05) higher values of plasma total protein and globulin for treated chicks compared with control ones, with insignificant difference between the Cr fed chicks and control. Albumin levels were lower for the control and Cr group compared with Se and Vit.E groups; however the differences were not significant. Albumin to globulin ratio (A/G ratio) was significantly lower in the treatment groups compared with the control group which may suggest an improvement in the immune responses of chicks from the treatments groups. These results are consistent with previous studies by Sahin et al. (2002b) and Rashidi et al. (2010); found that vitamin E supplementation increased plasma total protein, albumin and globulin concentration. Also Cetin et al. (2002) found that when broilers were fed diets supplemented with 0.3 ppm organic Se total protein was significantly increased compared with the control. It appears that, Vit.E as an antioxidant and selenium reduces free- radical induced pathological changes during both normal metabolic states and inflammation. (Packer and Suzuki, 1993). As a primary antioxidant of cell membranes, Vit.E is particularly important for the prevention of fatty acid peroxidation (Benedich, 1990). Fatty acids can act as immunoregulatory molecules that mediate cellular communication, this prevents have made a good indicator for increasing plasma total protein, albumin and globulin concentration (Leshchinsky and Klasing 2001). The role of Cr supplementation to diet was also observed and it confirms the findings by Uyanik et al., (2002) and El-Hommosany, (2008) who obtained comparable results. They found that Cr increased serum protein, albumin and globulin and attributed the positive effects of Cr on plasma protein and its fractions to the anabolic action of insulin mediated through increasing the amino acids synthesis by liver and enhancement the incorporation of several amino acids into protein.

### Table (3). Composition and calculated analysis of the experimental diets.

| Ingredient                      | Starter % | Grower % |
|---------------------------------|-----------|----------|
|                                 | 1-21 D    | 22-42 D  |
| Yellow corn                     | 55.35     | 60.45    |
| Soybean meal (44%)              | 27.5      | 25       |
| Corn gluten meal (62%)          | 8.9       | 6        |
| Mono-Ca phosphate               | 1.65      | 1.6      |
| Limestone                       | 1.7       | 1.65     |
| Vegetable oil                   | 3.7       | 4.15     |
| Salt                            | 0.45      | 0.4      |
| Methionine                      | 0.15      | 0.15     |
| Lysine                          | 0.3       | 0.3      |
| Vitamin and mineral mixture     | 0.3       | 0.3      |
| Total                           | **100**   | **100**  |

**Calculated analysis**

|                        | CR kcal/kg | ME kcal/kg |
|------------------------|------------|------------|
| Crude protein %        | 22.77      | 20.31      |
| Lysine %               | 1.27       | 1.19       |
| Methionine %           | 0.55       | 0.51       |
| Methionine+ Cystine %  | 0.93       | 0.85       |
| Calcium %              | 1.00       | 0.97       |
| Av.Phosphorus %        | 0.47       | 0.47       |

*: Vitamin and mineral mixture per kg of diet:

- VA, 12000 I.U. ,
- D3, 2500 I.U. ,
- E, 10mg. ,
- B1, 2mg. ,
- B2, 5mg. ,
- B6, 4mg. ,
- B12, 10µg.,
- Niacin, 25mg.,
- Pantothenic acid, 10mg.,
- Biotin, 50µg.,
- Folic acid, 1000µg.,
- Colline chloride, 255mg.
- Selenium, 300µg.,
- Copper, 10mg.,
- Iodine, 1.0mg.,
- K, 2.0mg.,
- Iron, 33mg.,
- Manganese, 60mg.
- Zinc, 60mg.
Table (4). Effect of dietary supplementation with organic chromium, organic selenium and Vitamin E on plasma total protein (PTP), albumin (A) globulin (G) and albumin to globulin ratio (A/G ratio).

| Treatment          | Control       | Chromium      | Selenium     | Vit. E | Significance |
|--------------------|---------------|---------------|--------------|--------|--------------|
| Age (days)         |               |               |              |        |              |
| (at 21 D.O.A)      | PTP (g/dl)    | 4.01 a        | 4.67 ab      | 5.07 a | 5.19 a       |
|                    | ± 0.303       | ±0.705        | ±0.225       | ±0.494 | *            |
|                    | Albumin (g/dl)| 1.83          | 1.60         | 1.87   | 1.99         |
|                    |              | ±0.304        | ±0.146       | ±0.265 | ±0.220       | NS           |
|                    | Globulin (g/dl)| 2.17 a       | 3.07 b      | 3.20 b | 3.20 b       | *            |
|                    |              | ±0.272        | ±0.626       | ±0.232 | ±0.274       | *            |
|                    | A/G ratio    | 0.85 a        | 0.53 b      | 0.59 b | 0.62 b       | *            |
|                    |              | ±0.200        | ±0.093       | ±0.110 | ±0.017       | *            |
| (at 42 D.O.A)      | PTP (g/dl)    | 4.58b         | 6.36a        | 6.88a  | 6.54a        | *            |
|                    | ±0.494       | ±0.793        | ±0.390       | ±0.538 | *            |
|                    | Albumin (g/dl)| 2.80          | 3.03         | 3.10   | 2.77         |
|                    |              | ±0.203        | ±0.298       | ±0.450 | ±0.257       | NS           |
|                    | Globulin (g/dl)| 1.78 b       | 3.33a        | 3.77a  | 3.76c        | *            |
|                    |              | ±0.353        | ±0.495       | ±0.139 | ±0.572       | *            |
|                    | A/G ratio    | 1.613a        | 0.933 b      | 0.826c | 0.753b       | *            |
|                    |              | ±0.286        | ±0.180       | ±0.141 | ±0.163       | *            |

Means± SD within a row with different superscripts are significantly different (P≤0.05).
NS = non-significant, *= P ≤ 0.05.

Table (5). Effect of dietary supplementation with organic chromium, organic selenium and Vitamin E on Titration against Newcastle Disease Virus (NDV) of broiler chicks at 42 day of age.

| Trait               | Age (days) | Control | Chromium | Selenium | Vit. E | Significance |
|---------------------|------------|---------|----------|----------|--------|--------------|
| Primary             | 20 d       | 4.33 a  | 5.66 b   | 7.00 a   | 7.33 a | *            |
|                     | ±0.57      | ±0.57   | ±1.0     | ±0.57    | ±0.57  | *            |
| Secondary           | 30 d       | 5.1 c   | 6.7 b    | 8.1 a    | 8.2 a  | *            |
|                     | ±0.57      | ±0.57   | ±1.0     | ±0.57    | ±0.57  | *            |
|                     | 40 d       | 3.2 c   | 4.61 b   | 6.01 a   | 6.23 a | *            |
|                     | ±0.57      | ±0.57   | ±1.0     | ±0.57    | ±0.57  | *            |

Means± SD within a row with different superscripts are significantly different (P≤0.05).
NS = non-significant, *= P ≤ 0.05.

Insulin-like Growth factor -I (IGF-I)

Table (6) presents the results of IGF-I level as affected by dietary supplements of organic chromium, organic selenium and Vitamin E. It appears that the higher level was obtained for chicks that fed organic...
chromium supplemented group followed by vitamin E and then organic selenium supplemented groups, however the lowest level was obtained from the control group. It is well known that increasing dietary Cr level has increased plasma insulin hormone concentration (Sahin et al., 2001 and 2002a; and El-Hommosany, 2008), and this effect may be enhanced by dietary Vit.E and Se addition. Moreno, et al. (2001) indicate that Se is particularly important for IGF’s bioactivity. Moreover, IGF-I level was significantly increased in chicks produced from young than old hens. This may be related to the relationships between the anabolic hormones, T3, T4, GH...etc. during the growing periods and the possible effect of vitamin E and organic selenium as antioxidant. Christensen et al. (2001) found that the control line of turkey had elevated IGF-I concentrations in response to higher temperatures.

Table (6). Effect of dietary supplementation with organic chromium, organic selenium and Vitamin E on Insulin-like Growth factor -I (IGF-I) at 42 DOA.

| Trait          | Treatment | Significance |
|----------------|-----------|--------------|
|                | Control   | Chromium     | Selenium    | Vit. E      |             |
| IGF-I          | 39.16b    | 46.46a       | 40.46b      | 41.36b      | *           |

***Means± SD within a row with different superscripts are significantly different(P≤0.05).
NS = non-significant, *= P≤ 0.05.

Heat shock protein 70 (Hsp70).

Results of the Hsp70 quantity in the liver tissues of heat exposed broilers at 21 and 42 DOA are shown in Table (7). The results at 21 DOA showed that dietary supplements have non-significant effect on Hsp70 at this age. However, there is an increase due mainly to dietary supplements. This was not observed at 42 DOA where all birds were exposed to 41°C±1 for one hour, which may increase Hsp70 production. This resulted in significant increases in Hsp70 for chicks of the treatment groups compared by control. The results of the present study showed similar trends for the effects of vitamin E on Hsp70, it was also suggested that vitamin E act synergistically (McDowell, 1989). In addition, the overall antioxidant potential was reported to possibly be more efficient and crucial than single antioxidant nutrients (Gallo-Torres, 1980). Selenium had the ability to reduce the impact of a heating episode. Mahmoud and Edens., (2003) have shown that heat distress induces oxidative stress, and oxidative stress is characterized by production of excessive reactive oxygen species (ROS), which are known to induce Hsp70. Furthermore, there are data that suggest a strong relationship among glutathione depletion, thiol-oxidation and Hsp70 synthesis in stressed cells that would affect the structure of proteins by allowing the unfolding of a fraction of these proteins leading to heat shock factor activation (Zou et al. 1998).

Table (7). Effect of dietary supplementation with organic chromium, organic selenium and Vitamin E on Heat shock protein 70 (Hsp70) of broiler chicks at 42 day of age.

| Trait                | Treatment          | Significance |
|----------------------|--------------------|--------------|
| HSP70 ( ng/mg TP)    | Control            | Chromium     | Selenium    | Vit. E      |             |
| (at 21 DOA)          | 3.93 ±0.64         | ±0.35        | ±0.51       | ±0.76       | NS          |
| HSP70 ( ng/mg TP)    | 4.66b ±0.62        | 5.91b ±0.62  | 6.01a       | 6.04a       | *           |
| (at 42 DOA)          | ±0.35              | ±0.30        | ±0.55       |             |             |

*a, b, c Means ± SD within a row with different superscripts are significantly different(P≤0.05).
NS = non-significant, *= P≤ 0.05.

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الاستجابَ الفسيْلْجيِ لدجاج التسْمِن تحت ظرَفَ الاجِاد الحراري لبعض اضافات هضاد الاكسدٍ العضْيَ

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استهدفت الدراسة محاولة تخفيف التأثيرات الضارة لارتفاع درجات الحرارة على الأداء الفسيولوجي والمناعي لدجاج التسمين خلال فترة الاكتاج و ذلك عن طريق اتباع اضافات الكروم العضوي، والسلوئوم العضوي وفيتامين ه د لعلاج دجاج التسمين و تم تربيع ككمايات المسمى في بطرات مناسبة مع تقديم كافة اساليب العناية المتكاملا له. 

و كانت أهم النتائج المتخصصة ان الاضادات العضوية (فيتامين ه د، السلوئوم العضوي والكروم العضوي) حصنت بصورة معنوية مفيدة بالكترول بمعدل 1 كيلو ردوان اضداد بروتينات الدم الكلية وكذلك مستوى الجلوبوليات المناعية، مستوى الأجسام المناعية لمرض البوكسال، وكذلك بروتينات الصممة الحرارية 70 كيلو ردوان. 

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