Alleviating Transport Stress of Broiler Using Vitamin C and Acetyl Salicylic Acid

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ABSTRACT

Alleviating the effects of transportation stress was the purpose of this study by using 270 thirty-five-day-old Ross 308 broiler chickens to check out the effects of vitamin C and acetyl salicylic acid (ASA) at different levels via drinking water. Birds in the experiment were randomly allocated to 27 identical pens (140×120×90 cm) with 10 birds each (5 males and 5 females -almost equal in weight-). At the last 24 hours of experiment pens were assigned to 9 water treatments: at levels of 0 (Control), 50mg/L vitamin (T1), 100mg/L vitamin (T2), 1 gm/L salicylic acid (T3), 1.5 gm/L salicylic acid (T4), 50mg/L vitamin C with 1 gm/L salicylic acid (T5), 50mg/L vitamin C with 1.5 gm/L salicylic acid (T6), 100mg/L vitamin C with 1 gm/L salicylic acid (T7) and 100mg/L vitamin C with 1.5 gm/L salicylic acid (T8). Studied traits were: glucose, total protein, uric acid, albumin, calcium, globulin, ALT, AST, Creatine Kinase and hormones (T3 and T4) studied in this experiment. Results indicate that incorporating Vit. C and ASA into broilers water compared to control group decrease the value of all stress indicator in a way that each of (glucose, albumin, globulin, uric acid, calcium, ALT, AST, Creatine Kinase and T3) significantly decreased. However, T4 was significantly increased while total protein was not changed. Results concluded that supplementation of the broiler drinking water with Vitamin C and ASA before transportation can be used as effective way for alleviating transporting stress and supporting the broilers to have more stable physiology during transportation.

Keywords: Broiler Chicken, Transport stress, Vitamin C, Acetyl salicylic acid

INTRODUCTION

The growth of human population, growth of cities and improved incomes are the reasons for the developing countries’ demand for food of animal origin (Abdullah et al., 2011). Based on this need, a rise in the production of the poultry sector has been faster growing than any other livestock sectors. (Bessei, 2018). Therefore, increasing demand for poultry meat has obliged the founding of new farms and slaughter houses out of their original production area, resulting in a significant increase in poultry transport (Metheringham and Hubrecht, 1996). Conditions of transportation are always considered as source of physiological stress since the very act of handling, crating, loading and transporting poultry has a detrimental effect on the health, physiology and the productivity of the birds (Scope et al., 2002).

The technological progression in commercial vehicles used to transport broilers has not reached yet to Iraq and especially Kurdistan region in terms of module materials, size, and weatherization measures. Therefore, with increased transportation, there is also increased demands for ways to alleviate the stress, particularly with hot climatic conditions. Due to the high mechanical cooling costs of the vehicle, efforts are mainly focused on nutritional correction (Abidin and Khatoon, 2013).

Vitamin C and acetylsalicylic acid (ASA) are known to have immunomodulatory effects. They have been used as a remedy for many diseases, especially stress in human (Pardue and Thaxton 1986; Balz, 2003), and poultry (Minka and Ayo, 2008; McKee and Harrison, 1995; Ayo and Sinkalu 2003) and other livestock (Ayo et al. 2006). However, the combined effect of ascorbic acid and ASA for reducing the stress caused by broiler transport is better to study. Therefore, this study was to designed to know the alleviating effect of vitamin C and ASA supplementation via drinking water on broilers during transport stress.

MATERIALS AND METHODS

270 Ross 308 (135 males and 135 females) broilers were evaluated from 35 to 42 days of age. Birds were housed at clean well–ventilate room previously disinfected and the management of the nine treatment groups with three replicates were identically carried out. No reused wood shavings were used as litter with the thickness of 5cm.

Birds have been fed manually and feed and water were available at all times during the experiment period which lasted one week. This period was used as adaptation period. All broilers in this experiment were provided the same commercial diet “grower diet” as shown in tables 1. At the last day Vitamin C and acetylsalicylic acid were added into drinking water at levels of 0 (Control), 50mg/L vitamin (T1), 100mg/L vitamin (T2), 1 gm/L salicylic acid (T3), 1.5 gm/L salicylic acid (T4), 50mg/L vitamin C with 1 gm/L salicylic acid (T5), 50mg/L vitamin C with 1.5 gm/L salicylic acid (T6), 100mg/L vitamin C with 1 gm/L salicylic acid (T7) and 100mg/L vitamin C with 1.5 gm/L salicylic acid (T8). Broiler chickens were transported from Bakrajo Poultry Breeding Field, to a slaughter house in Piramagroon town, by truck with
average speed (60 km/h). Transportation was carried out at the end of the day. 42. Transportation included broilers from all chickens with numbered legs. The broilers were carried with two hands to hold the wings against the body. Each crate (0.940 x 0.50 x 0.27 m3) was contained all birds from of one replicate which makes the number of all crates was (27) containing broilers from different groups and they all were randomly distributed in the truck.

Table 1. Ingredient of the grower diet used in the experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow Corn</td>
<td>61</td>
<td>61</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Protein conc.*</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Wheat</td>
<td>4.97</td>
<td>4.96</td>
<td>4.94</td>
<td></td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>DCP** phosphate</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>0</td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* Protein concentrate used in the diets was produced in Holland (WAFF) which contains: 40 % crude protein, 2100 Kcal ME / Kg, 5 % crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, %3.85 lysine, 3.70 % methionin.

** DCP: Di Calcium Phosphate.

Each crate contained 8 chickens (body weight: approximately 2.840 Kg). So, each crate approximately had the density of 22.720 kg/crate. Before the trip, birds were individually weighed and from brachial vein (1 mL) of blood was collected from each broiler. Transportation began at 8:30pm) outside temperature was recorded at (34.3 ± 3 °C) and inside temperature was (33.6 ± 1.5 °C with 40.0 RH). The crates were randomly set inside the truck to ensure that all broilers get the same microenvironment of the truck. The route was approximately 35 km. After the bird reached to the slaughter house in Piramagroon.

Again, the weight of all birds were individually recorded and blood samples were collected.

Blood samples were centrifuged at (1500 rpm for 10 minutes) to separate serum. The serum was used to measure glucose, globulin, uric acid, calcium, albumin and total protein and activities of (ALT), (AST) and Creatine Kinase (CK), also hormones like T3 and T4. By using the procedures from the instructions of each kit of these assays all the parameters were assayed. These kits were prepared by (Fabricante: Biolabo Sa, 02160, Maizy, France).

Data collected from this experiment were analyzed by the single factor analysis using software XLStat, version 2019.2.2 59614. The significant differences between means of traits included in this study were determined using Duncan's multiple range test (DMRT) under the probability (p<0.05).

RESULTS AND DISCUSSION

Results

Changes in concentration of Total protein, Albumen, Globulin and Glucose

Effect of Vit. C and ASA on plasma total protein, albumen, globulin and glucose is summarized in (Table 2).

Table 2. Effect of Vit. C and ASA on total protein, albumin, globulin and glucose before and after the journey stress

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Before the Journey</th>
<th>Before the Journey</th>
<th>After the Journey</th>
<th>After the Journey</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>28.3 ± 0.24a</td>
<td>1.60 ± 0.22a</td>
<td>1.54 ± 0.24a</td>
<td>1.23 ± 0.15a</td>
</tr>
<tr>
<td>T1</td>
<td>28.2 ± 0.22a</td>
<td>1.59 ± 0.19a</td>
<td>1.54 ± 0.17ab</td>
<td>1.23 ± 0.13ab</td>
</tr>
<tr>
<td>T2</td>
<td>28.3 ± 0.2a</td>
<td>1.57 ± 0.24a</td>
<td>1.54 ± 0.18ab</td>
<td>1.25 ± 0.15a</td>
</tr>
<tr>
<td>T3</td>
<td>28.2 ± 0.19a</td>
<td>1.56 ± 0.25a</td>
<td>1.53 ± 0.22ab</td>
<td>1.25 ± 0.16ab</td>
</tr>
<tr>
<td>T4</td>
<td>28.4 ± 0.21a</td>
<td>1.56 ± 0.15a</td>
<td>1.52 ± 0.23abc</td>
<td>1.24 ± 0.17ab</td>
</tr>
<tr>
<td>T5</td>
<td>28.4 ± 0.24a</td>
<td>1.59 ± 0.18a</td>
<td>1.52 ± 0.14abc</td>
<td>1.24 ± 0.11ab</td>
</tr>
<tr>
<td>T6</td>
<td>28.3 ± 0.23a</td>
<td>1.57 ± 0.23a</td>
<td>1.51 ± 0.18bc</td>
<td>1.25 ± 0.14ab</td>
</tr>
<tr>
<td>T7</td>
<td>28.1 ± 0.21a</td>
<td>1.58 ± 0.21a</td>
<td>1.51 ± 0.2bc</td>
<td>1.23 ± 0.14ab</td>
</tr>
<tr>
<td>T8</td>
<td>28.4 ± 0.18a</td>
<td>1.70 ± 0.23a</td>
<td>1.50 ± 0.18c</td>
<td>1.24 ± 0.15ab</td>
</tr>
</tbody>
</table>

C: control group; T1 T2 adding Vitamin C to the drinking water of broiler chicken at levels of 50 mg/L and 100 mg/L respectively. T3 and T4 adding ASA (1 gm/L and 1.5 gm/L) respectively. T5 and T6 adding (50 mg Vit. C and 1 gm of ASA/L) and (50 mg Vit. C and 1.5 gm of ASA/L) respectively. T7 and T8 adding (100 mg Vit. C and 1 gm of ASA/L) and (100 mg Vit. C and 1.5 gm of ASA/L) respectively.

Values within columns followed by different letters differ significantly (p<0.05).

Results revealed that before the stress journey supplementing broiler via drinking water didn’t significantly (P> 0.05) changed any biochemical traits. Furthermore, after the stress of transportation all traits except (total protein) significantly (P<0.05) changed in a way that plasma albumen decreased in almost all supplemented groups which the highest mean value for this trait was recorded in control group (1.549 g/dl) meanwhile in contrast to albumin, globulin was increased (P< 0.05) and the mean value for this trait was recorded in T7 (1.310 g/dl) and T7 comes after it, then the increase of these traits was decreases up to lowest level of supplementation. The highest level of glucose was recorded in control group and the lowest in T8, and mean value of this trait was (259.13and 225.28) g/dl for (control and T8) respectively.

Changes in Calcium, Uric acid, cholesterol

Results from Table 3 revealed that adding Vit. C and ASA to the water of broiler chickens significantly decreased.
(P < 0.05) uric acid and calcium during the transportation; However, the effects of treatments weren’t the same for cholesterol because this trait wasn’t different (P > 0.05) after the journey. The highest mean values of uric acid (2.984 g/dl) and calcium (10.829 g/dl) were in control group while the lowest value for both were recorded in T8.

Changes in Enzyme activity

Means of studied enzymes are displayed in (Table 4). After transportation, there is a significant decrease in the activity of AST, ALT, and CK in the treated broilers.

### Table 3. Effect of Vit. C and ASA on calcium, uric acid and cholesterol before and after the journey stress (Means ± S.E.)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Calcium (g/dl) Before the Journey</th>
<th>Calcium (g/dl) After the Journey</th>
<th>Uric Acid (g/dl) Before the Journey</th>
<th>Uric Acid (g/dl) After the Journey</th>
<th>Cholesterol (g/dl) Before the Journey</th>
<th>Cholesterol (g/dl) After the Journey</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10.818 ± 1.23 a</td>
<td>10.829 ± 1.31 a</td>
<td>2.906 ± 0.31 a</td>
<td>2.984 ± 0.21 a</td>
<td>97.938 ± 9.76 a</td>
<td>98.185 ± 10.55 a</td>
</tr>
<tr>
<td>T1</td>
<td>10.854 ± 1.2 a</td>
<td>10.777 ± 1.23 ab</td>
<td>2.906 ± 0.53 a</td>
<td>2.980 ± 0.20 a</td>
<td>98.916 ± 9.60 a</td>
<td>98.109 ± 9.21 a</td>
</tr>
<tr>
<td>T2</td>
<td>10.879 ± 1.24 a</td>
<td>10.765 ± 1.20 ab</td>
<td>2.918 ± 0.43 a</td>
<td>2.939 ± 0.24 ab</td>
<td>97.679 ± 9.41 a</td>
<td>98.004 ± 9.71 a</td>
</tr>
<tr>
<td>T3</td>
<td>10.856 ± 1.2 a</td>
<td>10.738 ± 1.23 ab</td>
<td>2.909 ± 0.60 a</td>
<td>2.938 ± 0.31 b</td>
<td>97.576 ± 9.96 a</td>
<td>98.863 ± 10.76 a</td>
</tr>
<tr>
<td>T4</td>
<td>10.847 ± 1.27 a</td>
<td>10.737 ± 1.25 ab</td>
<td>2.904 ± 0.23 a</td>
<td>2.912 ± 0.24 b</td>
<td>97.710 ± 9.13 a</td>
<td>97.584 ± 9.72 a</td>
</tr>
<tr>
<td>T5</td>
<td>10.905 ± 1.17 a</td>
<td>10.717 ± 1.34 ab</td>
<td>2.917 ± 0.45 a</td>
<td>2.891 ± 0.20 bc</td>
<td>97.725 ± 10.7 a</td>
<td>98.819 ± 11.70 a</td>
</tr>
<tr>
<td>T6</td>
<td>10.834 ± 1.25 a</td>
<td>10.683 ± 1.26 ab</td>
<td>2.920 ± 0.57 a</td>
<td>2.911 ± 0.27 b</td>
<td>97.738 ± 9.60 a</td>
<td>98.421 ± 9.89 a</td>
</tr>
<tr>
<td>T7</td>
<td>10.826 ± 1.27 a</td>
<td>10.664 ± 1.24 ab</td>
<td>2.899 ± 0.53 a</td>
<td>2.899 ± 0.17 bc</td>
<td>98.662 ± 9.32 a</td>
<td>98.169 ± 9.37 a</td>
</tr>
<tr>
<td>T8</td>
<td>10.891 ± 1.3 a</td>
<td>10.618 ± 1.33 b</td>
<td>2.903 ± 0.49 a</td>
<td>2.865 ± 0.25</td>
<td>99.010 ± 9.36 a</td>
<td>97.634 ± 9.99 a</td>
</tr>
</tbody>
</table>

C: control group; T1 T2 adding Vitamin C to the drinking water of broiler chicken at levels of 50 mg/L and 100 mg/L respectively. T3 and T4 adding ASA (1 gm/L and 1.5 gm/L) respectively, T5 and T6 adding (50 mg Vit. C and 1 gm of ASA/L) and (50 mg Vit. C and 1.5 gm of ASA/L) respectively. T7 and T8 adding (100 mg Vit. C and 1 gm of ASA/L) and (100 mg Vit. C and 1.5 gm of ASA/L) respectively.

**Discussion**

In the current experiment, treated groups recorded a low level of glucose, albumin and globulin; However, these groups showed no significant differences in total protein value compared to the control group which may be refers to the role of ASA and Vit C in alleviating the impact of stress.

Initially exposed to stress, the bird’s neurogenic system is activated by releasing epinephrine, catecholamines and norepinephrine (Siegel, 1980). It seems that epinephrine has the main role in changing metabolism (Assenmacher,
1973). Epinephrine connects to receptors on the cell membrane called P-adrenergic (Bern and Levy, 1990). This results in activations of enzyme; activation of certain protein kinases is most notable which signal the body to activate gluconeogenesis and glycoegenolysis (Guyton, 1991). During stress, the main function of norepinephrine is to enact non-shivering thermogenesis to provide energy to skeletal muscles from adipose tissue, this is due to devoting the majority of energy from glucose to other tissues. (Bern and Levy, 1990; Guyton, 1991).

A study of Abdel-Fattah (2006) confirmed that under heat stress conditions, Japanese quail in comparison to the control group has shown significant decreased plasma of albumin, glucose, total protein, and Albumin to Globulin ration concentration after supplementation of 500 mg/kg ASA. It is worthy to note that plasma globulin was not affected.

Results from experiment shows that supplementation ASA and Vit C significantly lowered calcium levels in broiler blood due to their role in in alleviating the impact of stress, because the pituitary gland sends signals to the internal tissue to release Glucocorticoids GCs in response to stress. These are released into the blood and lead to many cellular events that boost alteration in the cells and tissues to adapt to stressful stimuli, (Chrousos, 2009). Franchini et al., (1993) reported that mineralization of the skeleton of young poultry was enhanced by Vitamin C supplement. Recently it was reported that administration of ASA inhibited hypercalcemic effect of a-hydroxyvitamin D3 (Ueno et al., 1980) and decreases serum levels of total calcium (Kawashima et al.,1980).

Based on the results it is confirmed that, compare to a control group, CK, AST and ALT activity is significantly decreased by supplementing broilers with ASA and Vit C. This reduction of activity of these two enzymes in blood plasma may be a result of ASA and Vit C’s important role in decreasing the impacts of stress. Moreover, Vitamin C could partially stop certain types of damages in the hepatic cells (McDowell, 1989; Parola et al. 1992; Sies and Stahl, 1992; Burtis and Ashwood, 1994; Netke et al. 1997).

Since one of the commonly used indicators of damages in skeletal muscles is CK activity (Clarkson et al., 1992; Morton and Carter, 1992; Komulainen et al., 1995; Sorichter et al., 1997; Clarkson and Sayers, 1999), and its suitability to be used in birds is dependent on the number of studies (Franson et al., 1985; Bollinger et al., 1989; Dabbert and Powell, 1993; George and John, 1993; Knuth and • Chaplin, 1994; Totzke et al., 1999). In addition, AST and ALT enzymes are mainly found in liver, however also found in heart cells, red blood cells, muscle tissue and other organs such as pancreas and kidneys. When there is a disease or damage to a body tissue or an organ, more AST and ALT are released resulting in a higher level of the enzyme in the bloodstream (Al - Daraji et al., 2008), Thus, the level of AST and ALS is proportional to the extent of the damage to the tissue.

In the present study, stress of transportation resulted in changes of T3 and T4 at deferent levels in control groups. These results may be due to the action nature of this hormone. Since thyroid gland activity can be affected by intensity of heat and its secretion is changed due to changes happening in the histology of the gland. (Rachid et al. 2001).

In our study, opposite to the low level of T3 in non-supplemented group, the concentration of T4 was higher in supplemented group. This result may be explained by increasing the albumin concentration in plasma and the binding affinity of albumin to T4. Another reason of increasing T4 concentration in supplemented group is of a declining rate of conversion of T4 to T3 (Bueno et al., 2017), consequently T4 concentration elevated due to lower deterioration and longer half-life of T4 in circulation.

CONCLUSION

Vitamin C and ASA via drinking water can be used to reduce the negative impact of transportation stress of broiler. The best result was obtained from adding 100mg/L vitamin C with 1.5 gm/L salicylic acid.

REFERENCES


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