EFFECT OF ADDING SOME ANTIOXIDANTS TO DIET CONTAINING FATTY ACIDS ON PRODUCTIVE AND PHYSIOLOGICAL PARAMETERS OF SILVER MONTAZAH CHICKENS STRAIN. 1. DURING GROWTH PERIOD

A.M.A Bealish¹, A.S.A. Bahakaim², Sahar A. Hamed², I. M. Assaf² and M.M. Soliman¹
¹Dep. of Poult. Breeding Res. Anim.Prod. Res. Instit., Agric. Res. Center, Dokki, Giza.
²Dep. of Poult. Nutrition Res. Anim.Prod. Res. Instit., Agric. Res. Center, Dokki, Giza.

(Received 24/6/2018, accepted 15/8 /2018)

SUMMARY

An experiment was conducted for a period of 9 wks. to investigate the effect of adding some antioxidants to diet contain fatty acids on growth performance, some physiological and hematological parameters as well as evaluating the carcass characteristics of Silver Montazah male chicks. For this purpose 315 five-weeks old Silver Montazah (SM) males were used up to 14 wks. of age. All chicks were randomly divided into seven equal groups (45 chicks, each) with three replicates (15 chicks each). Chicks were wing banded and individually weighed, with nearly similar average initial live body weight of all groups. The 1ˢᵗ group was fed the basal diet that contained Linoleic acid (n-6) and Linolenic acid (n-3) at the ratio of 4:1, respectively and served as control. The 2ⁿᵈ and 3ʳᵈ groups were fed the basal control diet supplemented with 125 and 250g Butylated Hydroxy Toluene/Ton diet, respectively. The 4ᵗʰ and 5ᵗʰ groups were fed the basal control diet supplemented with 100 and 200g vit. E /Ton diet, respectively. The 6ᵗʰ and 7ᵗʰ groups were fed the basal control diet supplemented with 5 and 10kg citric acid /Ton diet, respectively. Results indicated that dietary antioxidants supplementation especially citric acid at the two studied levels and vit. E (200 g/Ton) significantly (P≤0.01) increased live body weight at 9 and 14 wks. of age, body weight gain during the periods of 5-9 and 5-14 wks of age compared to the control group. Moreover, significant increases were obtained for red blood cells (RBC’s) and plasma total protein and globulin concentrations for groups fed diet supplemented with antioxidants compared with the control group. Conversely, significant lower heterophils/ lymphocytes ratio and plasma concentration of total lipid, cholesterol and triglycerides were associated with supplemental antioxidants. Relative weights of liver and spleen of SM males were increased with antioxidants supplementation. In conclusion, for attaining the goal of economic improvement of meat production from local strain of chicken (SM) in Egypt. Dietary supplemental of antioxidants to feed containing fatty acids is practical application especially by 5 and 10kg citric acid and 200g vit. E /Ton diet to SM male chicks, could promoted the growth performance and had beneficial effects on some physiological and hematological parameters, as well as adjusted the carcass characteristics and immune status.

Keywords: Antioxidents, fatty acids, chickens, productive performance, reproductive performance

INTRODUCTION

In avian species, alpha linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) cannot be synthesized in the body and have to be supplied in the diet; therefore they are called essential fatty acids. It is interest to note that the ratio of n-6/n-3 PUFA appears to be more important for fat and cholesterol metabolism and in modulating antibody synthesis than the absolute concentrations of n-3 PUFA in the diet (Sijben et al., 2001). So, there is a need to regulate the intake of n-6 and n-3 PUFA in balanced proportions. Al-Khalifa et al. (2012) suggested that feeding broiler chicks diets rich in n-3 PUFA suppresses some aspects of the immune response that are considered to be important lines of defense against tumor, viral, bacterial, and other
infections. It remains to be determined whether this has an effect on risk of infection, which could have important implications for the poultry industry. Feeding either 4:1 or 6:1 LA to LNA containing diet were effective in increasing live body weight, body weight gain, improving feed conversion, reducing blood total lipids and cholesterol, and enhancing the immune response in Dandarawy chicks (Hassan et al., 2011).

Synthetic antioxidants, such as butylated hydroxytoluene (BHT), can inhibit lipid oxidation in feed but they have toxic properties (Kahl and Kappus, 1993) resulting in strict regulations regarding their use in foods. These findings, together with increased resistance to the use of synthetic additives, have increased interest in the antioxidant properties of naturally occurring substances (Gordon, 1990).

Vitamin E (Vit. E) is a metabolic nutrient that has received a lot of attention with respect to its importance to the immune response in poultry. However, chicken cannot synthesis Vit. E, therefore, its requirements must be given from dietary sources (Chan and Decker, 1994). Also, Vit.E has been reported as a natural antioxidant. It prevents the oxidation of unsaturated lipid materials within the live cell, thus protecting the cell membrane from oxidative damage (Gore and Qureshi, 1997). Furthermore, Vit. E serves as a physiological antioxidant through inactivation free radicals. As well as it was improved growth performance and immune response in Inshas chickens (Alm El-Dein et al., 2013).

Citric acid (CA) is one of the most widely-used as feed additives. It is commonly used as a preservative, acidulant, pH control agent, flavor enhancer, and antioxidant in many foods. Several studies support the statement that the addition of citric acid to broiler rations improved weight gain (Afsharmanesh and Pourreza, 2005), increased feed consumption (Moghadam et al., 2006) and improved feed efficiency (Abdel-Fattah et al., 2008). Adding 2-6 CA% improved BW, BWG and FCR of broiler chicks (Ao et al., 2009 and Ghazalah et al., 2011). Generally, the positive impact of dietary acidifiers on growth performance may be attributed to: (1) a reduction of the pH values in the feed and digestive tract, serving as a barrier against pathogenic microorganisms which are sensitive to low pH values; (2) the direct antimicrobial effect; (3) the reduction in buffering capacity in conjunction with improving digestibility coefficients. Therefore, the objectives of this experiment were to study the effect of adding some antioxidants to feed containing fatty acids on growth performance, some physiological and hematological parameters as well as evaluating the carcass characteristics of Silver Montazah male chicks.

MATERIALS AND METHODS

This study was carried out at Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt.

Chicks and experimental design:

A total number of 315 five-week old Silver Montazah (SM) male chicks (Egyptian local strain) were used in this experiment up to 14 wks. of age. All chicks were randomly divided into seven equal groups (45 chicks, each) with three replicates (15 chicks each). Chicks were wing banded and individually weighed to the nearest gram, with nearly similar average initial live body weight of all groups. The 1st group was fed the basal diet that contained Linoleic acid (n-6) and Linolenic acid (n-3) at the ratio of 4:1, respectively, and served as control. The 2nd and 3rd groups were fed the basal control diet supplemented with 125 and 250g Butylated Hydroxy Toluene/Ton diet, respectively. The 4th and 5th groups were fed the basal control diet supplemented with 100 and 200g vit. E /Ton diet, respectively. The 6th and 7th groups were fed the basal control diet supplemented with 5 and 10kg citric acid /Ton diet, respectively. Birds of each replicate were separately housed in floor pen. All chicks were kept under the same managerial hygienic and environmental conditions. Light was provided 16 hours daily throughout the experimental period, Feed and water were provided ad libitum. The basal experimental diet was formulated to meet the nutrient requirements of SM males during the growth period (from 5-14 wks of age) according to feed composition Tables for animal and poultry feed stuffs used in Egypt (2001). The composition and calculated analysis of the experimental basal diet are shown in (Table 1).
Table (1): The composition of the experimental basal diet.

| Ingredients                      | Starter diet (5 – 8 weeks) | Grower diet (9 – 14 weeks) |
|----------------------------------|----------------------------|----------------------------|
| LA:LNA ratio                     | 4:1                        | 4:1                        |
| Yellow corn                      | 53.3                       | 59.0                       |
| Soyabean meal 44%               | 30.6                       | 14.25                      |
| Wheat bran                       | 9.5                        | 22.0                       |
| Di Calcium Phosphate             | 1.55                       | 1.25                       |
| Limestone                        | 1.40                       | 1.40                       |
| Vitamins & Minerals mixture      | 0.30                       | 0.30                       |
| NaCl (salt)                      | 0.30                       | 0.30                       |
| DL-Methionine                    | 0.05                       | -----                      |
| Linseed oil                      | 0.86                       | 0.77                       |
| Soybean oil                      | 2.14                       | 0.73                       |
| Total                            | 100                        | 100                        |
| Calculated analysis:            |                            |                            |
| Crude protein (%)                | 19.02                      | 14.10                      |
| ME (kcal/kg diet)                | 2845                       | 2706                       |
| Crude fiber (%)                  | 4.50                       | 4.80                       |
| Ether extract (%)                | 5.86                       | 4.80                       |
| Calcium (%)                      | 1.00                       | 0.90                       |
| Available Phosphor (%)           | 0.45                       | 0.40                       |
| Lysine (%)                       | 1.09                       | 0.70                       |
| Methionine (%)                   | 0.38                       | 0.27                       |
| Methionine + Cystine (%)         | 0.70                       | 0.53                       |

*Premix added to the 1 kg of diet including Vit.A 10000 I.U; vit. D3 2000 I.U; vit. E 15 mg; vit. K3 1 µg; vit B1 1mg; vit. B2 5mg; vit. B12 10 µg; vit B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; folic acid 1mg; Biotin 50 mg; choline 300 mg; zinc 50mg; copper 4mg; iodine 0.3 mg; iron 30mg; selenium 0.1mg; manganese 60mg; cobalt 0.1mg and carrier CaCo3 up to 1kg.

** According to feed composition Tables for animal and poultry feedstuffs used in Egypt, (2001).

**Measurements:**

**Productive performance:**

Individual live body weight (LBW) for each replicate within each treatment group was recorded at 5, 9, and 14 wks of age and feed consumption (FC) was recorded at the same ages. Body weight gain (BWG) and feed conversion, FCR (g feed/g gain) were calculated for the periods of 5-9, 9-14 and 5-14 wks of age.

**Carcass traits:**

At the end of the experimental period (14 wks of age) three males from each group were randomly chosen, weighed, slaughtered and after complete bleeding, feathers were removed. The birds were weighed after removing heads, legs and viscera to determine the percentage of carcass weight included wings and necks. The heart, liver, empty gizzard, and lymphoid organs (thymus and spleen) as well as different parts of carcass were separated, weighed and their relative percentages to live body weight were calculated.

**Blood biochemical analysis and hematological picture:**

Blood samples were collected from each slaughtered bird during exsanguinations in two heparinized test tubes. The fresh blood of the first tube was used to evaluate the total count of red and white blood cells, hematocrit value (PCV %), hemoglobin concentration (Hb), and differential counts of leucocytes. The other
blood tube was centrifuged at 3000 rpm for 20 minutes, and plasma was separated and stored at -20°C until assayed for total protein (TP), albumin (A), globulin (G), total lipids (TL), total cholesterol (Tch), high density lipoproteins (HDL), low density lipoproteins (LDL), and alkaline phosphatase activity (Alk P) as well as hepatic enzymes, aspartate amino transaminase (AST) and alanine amino transaminase (ALT), which determined calorimetrically by using commercial diagnostic kits. Globulin (G) was calculated by subtraction of plasma albumin from total protein, and albumin /globulin (A/G) ratio was calculated.

Statistical analysis:

Data were subjected to one-way analysis of variance using SAS (2001). Differences among means were detected by using Duncan's multiple range test (Duncan, 1955). The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis, and then actual means are presented. The following model was used:

\[ Y_{ij} = \mu + T_i + e_{ij} \]

where, \( Y_{ij} \) = observation for each dependent variable; \( \mu \) = General mean; \( T_i \) = Treatment effects (i = 1,2... and 7); \( e_{ij} \) = Random error.

RESULTS AND DISCUSSION

Productive performance:

**Live body weight (LBW) and body weight gain (BWG):**

Results of LBW at 9 and 14 wks of age and BWG during the periods of 5-9 and 5-14 wks of age showed significant (P≤0.01) differences among treatments (Table 2). The inclusion of antioxidants especially citric acid at the two studied levels (5 and 10 kg/Ton diet) and vit. E (200 g/Ton diet) significantly (P≤0.05) increased LBW values of SM males at 9 and 14 wks of age compared with the control group. The heaviest LBW values were recorded for males of T7 group followed by those of T6 and T5 groups, respectively throughout the experimental period.

In addition, similar trend was obtained for BWG values during the periods of 5-9 and 5-14 wks of age (Table 2). The heaviest BWG values were recorded for males of T5 (200g vit.E/Ton diet) and followed by T6 and T7 groups.

**Feed consumption (FC) and feed conversion ratio (FCR):**

Data concerning FC shows non-significant differences among treatment groups during all studied periods (Table 3). However, the T1 (control group) recorded the lowest amount of FC during the whole experimental period from 5-14 wks of age. On the other hand, FCR shows significant differences among treatment groups during the periods of 5-9 and 5-14 wk of age. Generally, males of T5 and T6 groups showed the best FCR followed by those of T7 group throughout the experimental period compared to males of control group. However, there were no significant differences in FCR among antioxidant treatments each others at all studied periods. The increase in BW and BWG by adding vit. E may be due to this vitamin has antioxidant properties (Halliwell and Gutteridge, 2000) which effective in protecting cell membranes from the toxic effect of oxygen radicals and associated lipid peroxidation which finally reflects on birds growth performance. Antioxidant such as vit.E act throughout the beginning stage, braking the generation of free radicals (Carrillo-Dominguez et al., 2012.), our results are in agreement with the findings of Lin and Chang (2006) who obtained gradually rise in body weight after 9 wks (growing period) with increasing the level of vit.E (0,40,80 and 120 mg/kg diet). In addition, Chung and Boren (1999) reported that supplementing 240 mg vitamin E/kg diet improved FCR of broiler chicks by 2.3% compared to the control group (33 mg VE/kg). Also, Swain et al. (2000) showed that vitamin E supplementation at levels of 150 and 300 IU/kg resulted in significant improvement in BWG and FCR of broiler chicks compared with the control diet at 42 d under normal conditions. Established NRC requirements (NRC, 1994) may be optimal for growth but not necessarily for best health, disease resistance and oxidative stress counteract. Alm El-Dein et al. (2013) reported that the chicks supplemented with the high level of vit.E (150 mg/kg diet) had significantly (P ≤
0.05) higher LBW and BWG compared to those fed the low levels of vit. E (75 and 100 mg/kg diet) and control group.
Table (2): Effect of dietary antioxidants supplementation on live body weight (g) and live body weight gain (g) of Silver Montazah male chicks at different ages.

| Trait                        | Age (wks) | T1       | T2       | T3       | T4       | T5       | T6       | T7       | MSE  |
|------------------------------|-----------|----------|----------|----------|----------|----------|----------|----------|------|
| Live body weight (g)         | 5 wk      | 410.33   | 418.00   | 414.67   | 417.67   | 405.67   | 408.33   | 413.33   | 5.010|
|                             | 9 wk      | 614.67   b| 645.00 ab| 650.00 ab| 653.67 ab| 664.33 a | 665.67 a | 669.67 a | 13.948|
|                             | 14 wk     | 1152.67  b| 1191.00 ab| 1198.00 ab| 1199.33 ab| 1219.00 a| 1221.33 a| 1224.00 a| 18.151|
| Live body weight gain (g)    | 5-9 wk    | 204.33 b | 227.00 ab| 235.33 ab| 236.00 ab| 258.67 a | 257.33 a | 256.33 a | 10.794|
|                             | 9-14 wk   | 538.00   | 546.00   | 548.00   | 545.67   | 554.67   | 555.67   | 554.33   | 9.033 |
|                             | 5-14 wk   | 742.33   b| 773.00 ab| 783.33 ab| 781.67 ab| 813.33 a | 813.00 a | 810.67 a | 14.856|

a, b, c...Means within the same row with different superscripts are significantly differ (P ≤ 0.05).

T1=Control 1 group, T2 and T3=Fed the basal diet + 125 and 250g BHT/Ton diet, respectively, T4 and T5=Fed the basal diet + 100 and 200g vit.E/Ton diet, respectively, T6 and T7= Fed the basal diet +5 and 10kg citric acid/Ton diet, respectively.

Table (3): Effect of dietary antioxidants supplementation on feed consumption (g/male/period) and feed conversion ratio (g feed/g weight gain) of Silver Montazah male chicks at different ages.

| Trait              | Age (wks) | T1       | T2       | T3       | T4       | T5       | T6       | T7       | MSE  |
|--------------------|-----------|----------|----------|----------|----------|----------|----------|----------|------|
| Feed consumption   | 5-9 wk    | 1192.00  | 1202.67  | 1203.33  | 1200.67  | 1204.00  | 1203.67  | 1211.33  | 19.702|
|                    | 9-14 wk   | 1732.33  | 1735.67  | 1744.00  | 1745.67  | 1746.33  | 1748.67  | 1754.33  | 11.493|
|                    | 5-14 wk   | 2924.33  | 2938.34  | 2947.33  | 2946.34  | 2950.33  | 2952.34  | 2965.66  | 30.339|
| Feed conversion    | 5-9 wk    | 5.857 a  | 5.327 ab | 5.163 ab | 5.090 b  | 4.670 b  | 4.677 b  | 4.733 b  | 0.220 |
|                    | 9-14 wk   | 3.220    | 3.180    | 3.180    | 3.203    | 3.147    | 3.150    | 3.167    | 0.048 |
|                    | 5-14 wk   | 3.940 a  | 3.803 b  | 3.767 ab | 3.773 ab | 3.630 b  | 3.630 b  | 3.660 b  | 0.057 |

a, b, c...Means within the same row with different superscripts are significantly differ (P ≤ 0.05).  T1=Control 1 group, T2 and T3=Fed the basal diet + 125 and 250g BHT/Ton diet, respectively, T4 and T5=Fed the basal diet + 100 and 200g vit.E/Ton diet, respectively, T6 and T7= Fed the basal diet +5 and 10kg citric acid/Ton diet, respectively.
Antioxidant properties of BHT rely on its molecular configuration, because, like other synthetic phenolic antioxidants, it possesses a labile hydrogen atom in the hydroxy group that can be donated and reduce the free radicals generated at the initiation step of lipid oxidation. Thus, BHT itself is oxidized and the subsequent derived radical is stabilized by electronic delocalization in the benzene ring. This way, BHT can stop radical oxidation propagation, which retards lipid oxidation and extends food shelf-life (Warner et al. 1986). BHT has been reported to efficiently react against strong oxidizing radicals such as singlet oxygen (O2(1_g)), hydroxyl radicals (OH), and peroxyl radicals (OOR), although the mechanism of quenching reactions may vary, depending on the reactive oxygen species involved and the local environment (Lambert, et al. 1996). The results of CA are in agreement with the results of some previous reports (Boling et al., 2000; Boling-Frankenbach et al., 2001). The improved performance may be due to decreased pH of the gut part contents, the helpful effect on gut size and morphometry as well as increased nutrient digestibility (Nourmohammadi et al., 2012). Nourmohammadi and Khosravinia (2015) reported that the BWG and FI of broiler chicks were significantly (P <0.01) improved by addition of 30g CA/kg of broiler diet. Similar observations were also recorded by Sultan et al. (2015) who obtained better feed conversion with the administration of citric acid in broiler diet compared with control unsupplemented diet. Shen-Huifang et al. (2005) and Abdel-Fattah et al. (2008) reported that the addition of CA to broiler diet had a positive effect on FCR. The results of this study are in accordance with those of Moghadam et al. (2006), Nezhad et al. (2007) and Abdel-Fattah et al. (2008) who indicated that the addition of CA to poultry diets has a positive effect on live weight gain. Shahin et al. (2009) found that addition of CA to a commercial diet at a level of 0.8% increases the weight of broiler birds by 4.9%. Demirel et al. (2012) reported that supplementation citric acid at 3% improved FCR (P < 0.01) compared with un-supplemented diets.

Carcass traits:

Results presented in Table 4 indicated that, at the end of the experimental period (14 wks of age), the overall mean of carcass percentage was significantly (P≤0.05) increased in males that fed CA containing diets compared with the control group. Moreover, antioxidants supplemented diets had significantly (P≤0.05) increased liver relative weight as compared with the control group. Also, its clear from Table 4 that dietary supplementation of antioxidants especially vit. E and CA significantly improved the immune status as reflected by increasing the relative weights of lymphoid organs (within the normal range) as compared with the control group. These findings are consistent with the findings of Sultan et al. (2015) who obtained a significant increase in carcass yield and liver weight by feeding diets containing citric acid over than the control group. In the present study, relative spleen weight significantly increased in broilers fed VE 200g/Ton diet which may indicate an improvement in the immune status. A similar result was reported by Konjufca et al. (2004). They found that vitamin E supplementation as Dl-α-tocopherol did not increase relative organ weight, except for the spleen. They concluded that the increase in spleen weight with supplemental vitamin E was likely to represent an increase in the number of lymphocytes. Results of CA in the present study are justified by Islam et al. (2008) who reported positive effect of citric acid on liver weight in broilers. Also, Ali et al. (2012) found that dietary organic acids significantly increased the weight of liver and gizzard in Japanese quails. In the same line, Viola and Vieira (2007), Ebrahimnezhad et al. (2008) and Nourmohammadi et al. (2012) indicated that addition of CA caused significant increase in carcass yield of broiler chickens. Ebrahimnezhad et al. (2008) and Nourmohammadi et al. (2012) found that dietary CA and phytase supplementation had affected the relative weight of liver, and heart weight of broiler chickens at 42 d. On the other hand, Abdel-Azeem et al. (2000) noted that addition of CA to the diet was associated with higher dressing and lower liver percentages. This supposition may emphasize the hypothesis of Fushimi et al. (2001) who stated that dietary acidification might stimulate glycogenesis by increasing the influx of glucose 6-phosphate (G-6-P) into the glycogen synthesis pathway through the inhibition of glycolysis due to an increase in citrate concentration.

Hemato-biochemical parameters:

Hematological characteristics:

The results of Table (5) showed that dietary antioxidant treated males had significantly (P≤0.05) higher RBC's number comparing with control group.
Table (4): Effect of dietary antioxidants supplementation on relative weights of carcass, some lymphoid and internal organs of Silver Montazah male chicks at 14 wks of age.

| Trait            | Treatments | T1   | T2   | T3   | T4   | T5   | T6   | T7   | MSE  |
|------------------|------------|------|------|------|------|------|------|------|------|
| Carcass weight   |            | 63.55 b | 65.66 ab | 66.43 ab | 66.44 ab | 66.75 ab | 67.01 a | 68.07 a | 0.969 |
| Spleen weight    |            | 0.258 c | 0.292 b | 0.309 ab | 0.312 a | 0.316 a | 0.317 a | 0.324 a | 0.006 |
| Thymus weight    |            | 0.554 b | 0.603 ab | 0.610 ab | 0.617 a | 0.624 a | 0.656 a | 0.659 a | 0.019 |
| Liver weight     |            | 2.164 b | 2.498 a | 2.505 a | 2.516 a | 2.520 a | 2.523 a | 2.535 a | 0.061 |
| Heart weight     |            | 0.512   | 0.517   | 0.519   | 0.520   | 0.521   | 0.526   | 0.536   | 0.013 |
| Gizzard weight   |            | 2.536   | 2.548   | 2.557   | 2.649   | 2.643   | 2.659   | 2.662   | 0.108 |

a, b, c...Means within the same row with different superscripts are significantly differ (P ≤ 0.05). T1=Control 1 group, T2 and T3=Fed the basal diet + 125 and 250g BHT/Ton diet, respectively, T4 and T5=Fed the basal diet + 100 and 200g vit.E/Ton diet, respectively, T6 and T7=Fed the basal diet +5 and 10kg citric acid /Ton diet, respectively.

Table (5): Effect of dietary antioxidant supplementation on hematological parameters of Silver Montazah male chicks at 14 wks of age.

| Trait                   | Treatments | T1   | T2   | T3   | T4   | T5   | T6   | T7   | MSE  |
|-------------------------|------------|------|------|------|------|------|------|------|------|
| RBCs x (10^6/mm³)       |            | 2.060 b | 2.419 a | 2.466 a | 2.450 a | 2.477 a | 2.443 a | 2.447 a | 0.093 |
| Hb (g/dl)                |            | 15.663 b | 17.295 ab | 17.519 a | 17.239 ab | 17.246 ab | 17.212 ab | 17.115 ab | 0.496 |
| WBCs x (10^3/mm³)       |            | 4.363 b | 4.637 ab | 4.677 a | 4.578 ab | 4.636 ab | 4.574 ab | 4.526 ab | 0.088 |
| Differential leucocyte count (%) |   |   |   |   |   |   |   |   |   |
| Heterophils (H) %       |            | 24.732 a | 20.332 b | 20.809 b | 19.877 b | 20.863 b | 20.010 b | 20.325 b | 0.656 |
| Lymphocytes (L) %       |            | 61.285   | 60.952   | 60.612   | 60.736   | 60.448   | 60.780   | 61.115   | 0.483 |
| H/L ratio               |            | 0.404 a  | 0.334 b  | 0.343 b  | 0.328 b  | 0.345 b  | 0.329 b  | 0.332 b  | 0.011 |

a, b, c...Means within the same row with different superscripts are significantly differ (P ≤ 0.05). T1=Control 1 group, T2 and T3=Fed the basal diet + 125 and 250g BHT/Ton diet, respectively, T4 and T5=Fed the basal diet + 100 and 200g vit.E/Ton diet, respectively, T6 and T7=Fed the basal diet +5 and 10kg citric acid /Ton diet, respectively.
On the other hand, both heterophils percentage and heterophils/ lymphocytes ratio were significantly \((P \leq 0.05)\) lower due to feeding antioxidant additives compared with control group. However, adding 250 g BHT/Ton diet significantly \((P \leq 0.05)\) increased hemoglobin and WBC’s total count compared with control group. Whereas, there no significant differences in lymphocytes percentages were detected between antioxidant treatments each others and control group.

**Biochemical characteristics:**

Data of some plasma blood constituent of Silver Montazah males as affected by feeding diet containing varying types and levels of antioxidant are presented in (Table 6). It is clear that dietary antioxidant supplementation was significantly decreased plasma total lipids, cholesterol and triglycerides concentrations. Meanwhile, plasma total protein and globulin concentrations were significantly increased as compared with control group. However, it could be observed that the highest concentration of plasma HDL was exhibited by feeding 125 g BHT/Ton diet (T2 group), while the lowest concentration was recorded for birds of the control group, with no significant differences between antioxidant treatments each other. On the other hand, birds of T7 group \((10 \text{ kg CA/Ton diet})\) had recorded the lowest concentration of plasma LDL while the highest value was recorded for birds of control group. Where, the rest of treatments took intermediate place between T7 and control groups (Table 6). There were no significant differences in plasma albumin, albumin/globulin ratio, alkaline phosphatase concentrations and hepatic enzymes activities \(\text{aspartate amino transaminase (AST) and alanine amino transaminase (ALT) enzymes}\) due to dietary addition of antioxidants comparable to control group. The present findings are agreement with the reports of many studies using different doses of BHT (Farag et al., 2006; El- Anany and Ali, 2013; Panicker et al., 2014). Vitamin E can donate its phenolic hydrogen to scavenge free radicals (Gülçin, 2012); protect membrane fatty acids and plasma Low density lipoproteins (LDL), High density lipoproteins (HDL) against lipid oxidation (Ricciarelli, et al 2000). The results of this study were supported by those of Gursu et al. (2003) who found that serum activities of AST and ALT were not influenced by dietary Vit.E supplementation. But through its known properties as an intra-membrane antioxidant, Vit. E may protect tissue membrane from lipid peroxidation caused by free radicals attack. It could therefore reduce the associated loss of integrity of function of cell membranes and associated increased cellular permeability. Younis (2014) and Mobarak et al. (2013) revealed a significant increase in total RBC count and Hb concentration, and a significant improving in triglycerides concentration due to supplementation of broiler chickens ration with Vit. E. Growing ducks fed diet with 2 and 3% citric acid increased RBCs, hemoglobin, WBCs and lymphocyte and serum total protein and globulin concentrations and decreased serum total lipids, triglycerides, cholesterol and LDL as compared to control group (Asmaa ELnaggar and Hayam Abo EL-Maaty, 2017). The previous results match with those obtained in broiler chicks (Ghazalah et al., 2011), who reported that dietary organic acids exhibited relatively noticeable by higher concentration of total protein and globulin as compared to the control birds, indicating that the immune response improved by addition of organic acids which might indicate that broiler chicks fed acidifiers-supplemented diets had better immune response and disease resistance. Also, the previous results indicated that supplemental organic acid may improve the immune response, as globulin level has been used as an indicator of immune responses and source of antibody (Kamal and Ragaa, 2014). The findings of serum lipid profile are in agreement with Kamal and Ragaa (2014) who reported that blood total lipids, triglycerides and cholesterol decreased significantly by dietary acidifiers. The beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH (Abdel-Fattah et al., 2008). Attia et al. (2017) found that Vit. E significantly \((P \leq 0.05)\) increased RBC and WBC, serum total protein, albumin, and globulin.

**CONCLUSION**

In conclusion for attaining the goal of economic improvement of meat production from local strain of Silver Montazah males in Egypt. Dietary supplemental of antioxidants to feed containing fatty acids is a practical application to promote the growth performance and had beneficial effects on some physiological and hematological parameters, as well as improving the carcass yield and immune status.
**Table (6): Effect of dietary antioxidant supplementation on some plasma biochemical parameters of Silver Montazah male chicks at 14 wks of age.**

| Trait                  | T1     | T2     | T3     | T4     | T5     | T6     | T7     | MSE  |
|------------------------|--------|--------|--------|--------|--------|--------|--------|------|
| Total lipids (mg/dl)   | 268.32 | 241.28 | 237.16 | 233.98 | 238.68 | 235.95 | 239.65 | 6.842|
| Cholesterol (mg/dl)    | 160.95 | 130.46 | 138.98 | 134.77 | 134.91 | 131.96 | 132.20 | 7.198|
| Triglycerides (mg/dl)  | 137.57 | 109.81 | 111.55 | 115.18 | 113.65 | 113.32 | 116.47 | 5.817|
| HDL (mg/dl)            | 45.09  | 54.68  | 51.38  | 52.05  | 51.05  | 52.24  | 53.27  | 2.788|
| LDL (mg/dl)            | 88.19  | 80.06  | 81.41  | 82.83  | 80.81  | 80.98  | 79.80  | 2.404|
| Total protein (g/dl)   | 4.507  | 5.023  | 5.053  | 4.953  | 5.027  | 4.993  | 5.037  | 0.139|
| Albumin (A) (g/dl)     | 1.687  | 1.697  | 1.683  | 1.720  | 1.653  | 1.687  | 1.633  | 0.103|
| Globulin (G) (g/dl)    | 2.820  | 3.327  | 3.387  | 3.233  | 3.373  | 3.307  | 3.403  | 0.137|
| A/G (Ratio)            | 0.600  | 0.513  | 0.503  | 0.530  | 0.493  | 0.517  | 0.480  | 0.044|
| AST (U/L)              | 55.37  | 52.40  | 54.24  | 53.19  | 53.74  | 52.06  | 54.50  | 2.110|
| ALT (U/L)              | 28.11  | 27.80  | 27.64  | 28.46  | 28.01  | 28.09  | 28.13  | 0.785|
| Alkaline phosphatase   | 174.33 | 173.33 | 166.00 | 185.00 | 167.00 | 181.33 | 174.67 | 14.358|

*a, b, c...Means within the same row with different superscripts are significantly differ (P ≤ 0.05). T1=Control 1 group, T2 and T3=Fed the basal diet + 125 and 250g BHT/Ton diet, respectively, T4 and T5=Fed the basal diet + 100 and 200g vit.E/Ton diet, respectively, T6 and T7=Fed the basal diet +5 and 10kg citric acid /Ton diet, respectively.*
REFERENCES

Abdel-Azeem, F., Y.M. El-Hommosany and Nematallah, G.M.A. 2000. Effect of citric acid in diets with different starch and fiber levels on productive performance and some physiological traits of growing rabbits. Egypt. J. Rabbit Sci., 10: 121-145.

Abdel-Fattah, S. A., Sanhoury, M. H. E., Mednay, N. M. E. and Abdul-Azeem, F. 2008. Thyroid activity of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci. 7: 215222.

Afsharmanesh, M., and Pourreza, J. 2005. Effect of calcium, citric acid, ascorbic acid, vitamin D3 on the efficacy of microbial phytase in broiler starters fed wheat-based diets on performance, bone mineralization and ileal digestibility. Int. J. Poult. Sci. 4:418–424.

Ali, A.S., Reza, N.H., Enayat R. and Jalal, S. 2012. Herbal additives and organics as antibiotic alternatives in broiler chicken diet for organic production. Afr. J. Biotech., 11:2139-2145.

Al-Khalifa, H., I D. I. Givens, C. Rymer and Yaqoob. P. 2012. Effect of n-3 fatty acids on immune function in broiler chickens. Poult. Sci., 91:74–88.

Alm El-Dein A.K.; Soliman M.M. and Abd El-AziZ, S. 2013. Effect of dietary Vitamin E supplementation on some productive, reproductive and immunological traits of Inshas chickens strain. Egypt. Poult. Sci. Vol (33) (IV): (939-955).

Ao, T.; Cantor A.H.; Pescatore A. J.; Ford M. J.; Pierce J. L.; and Dawson, K. 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult. Sci., 88: 111-117.

Asmaa Sh. ELnaggar1 and Hayam M. A. Abo EL-Maaty 2017. Impact of using organic acids on growth performance, blood biochemical and hematological traits and immune response of Ducks (Cairina Moschata). Egypt. Poult. Sci. 37: 907–925.

Attia Y.A., Al-Harthi M.A., El-Shafey A.S., Rehab Y.A. and Kyun Kim, Woo. 2017. Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics, Annals of Animal Science, DOI: 10.1515/aas-2017-0012.

Boling-Frankenbach, S. D., Snow, J. L., Parsons, C. M., and Baker, D. H. 2001. The effect of citric acid on the calcium and phosphorus requirements of chicks fed corn-soybean meal diets, Poultry Sci., 80, 783–788.

Boling-Frankenbach, S. D., Webel, D. M., Mavromichalis, I., Parsons, C. M., and Baker, D. H. 2000. The effects of citric acid on phytate-phosphorus utilization in young chicks and pigs, J. Anim. Sci., 78, 682–68.

Carrillo-Domínguez S, Avila G E, Vásquez P C, Fuente B, Calvo C C, Carranco J M E and Pérez-Gil Romo, F. 2012. Effects of adding vitamin E to diets supplemented with sardine oil on the production of laying hens and fatty-egg acid composition African Journal of Food Science, Vol. 6, No. 1, pp.12-19.

Chan, K. M. and Decker, E.A. 1994. Endogenous skeletal antioxidants Crit. Rev. Food. Sci. Nutr., 34:403-426.

Chung, T.K. and Boren, B. 1999. Vitamin E use in commercial flocks examined. Feedstuffs 6, 11-14.

Demirel G., Pekel A. Y., Alp M., and Kocabağl, N. 2012. Effects of dietary supplementation of citric acid, copper, and microbial phytase on growth performance and mineral retention in broiler chickens fed a low available phosphorus diet. J. Appl. Poult. Res. 21:335-347.

Duncan, D. B. 1955. Multiple range and F. test. Biometrics 11:1-42.
Ebrahimnezhad, Y., M. Shivazad, R. Taherkhani and Nazeradl, K. 2008. Effects of citric acid and microbial phytase supplementation on performance and phytate phosphorus utilization in broiler chicks. J. Poult. Sci., 45: 20-24.

El-Anany, A. and Ali, R. 2013. Biochemical and histopathological effects of administration various levels of Pomposia (Syzygium cumini) fruit juice as natural antioxidant on rat health. Journal of Food Science and Technology, 50, 487–495.

Farag, R. S., E. A. Mahmoud, A. M. Basuny and Ali, R. F. 2006. Influence of crude olive leaf juice on rat liver and kidney functions. International Journal of Food Science and Technology, 41, 790–798

Feed Composition Tables for Animal and Poultry Feedstuffs Used in Egypt 2001. Technical Bulletin No.1, Central lab for Feed and Food; Ministry of Agriculture, Egypt.

Fushimi, T., K. Tayama, M. Fukaya, K. Kitakoshi and Nakai, N. 2001. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. J. Nutr., 131: 1973-1977.

Ghazalah, A. A.; Atta, A.M.; Elkloub, K.; Moustafa, M. EL. and Riry, Shata, F.H. 2011. Effect of dietary supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. Int. J. Poult. Sci., 10: 176-184.

Gordon, M. H. 1990. The mechanism of antioxidant action in vitro. In: BJF Hudson (Ed.), Food antioxidants Elsevier Applied Science, London, 1–18.

Gore, A. B. and Qureshi, M. A. 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure.Poult Sci., 76: 984-991.

Gülçin, İ. 2012. Antioxidant activity of food constituents: An overview. Arch.

Gursu, M.F.; Sahin, N. and Kucuk, O. 2003. Effects of vitamin E and selenium on thyroid status, adrenocorticotropic hormone and blood serum metabolite and mineral concentrations of Japanese quails reared under heat stress (34°C).Trace Elem-Exp. Med.16: 95-104.

Halliwell, B. and Gutteridge, J.M.C. 2000. Free Radicals in Biology and Medicine, 3rd ed. (New York, Oxford University Press).

Hassan, M.S.H., Nadia L. Radwan, Abdel Khalek, A.M. and Abd El-Samad, M.H. 2011. Effects of different dietary linoleic acid to linolenic acid ratios on some productive, immunological and physiological traits of Dandarawy chicks. Egypt. Poult. Sci., 31:149-160.

Islam, M.Z., Khandaker, Z.H., Chowdhury, S.D. and Islam, K.M.S. 2008. Effect of citric acid and acetic acid on the performance of broilers. Journal of Bangladesh Agricultural University 6: 315-320.

Kahl, R., and Kappus, H. 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Zeitschrift Fur Lebensmittel-Untersuchung Und -Forschung. 196:329–338.

Kamal, Azza M.; and Ragaa, Naela, M. 2014. Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. Nat. Sci. 12 (2): 38-45.

Konjufca, V.K., Bottje, W.G., Bersi, T.K. and Erf, G.F. 2004. Influence of dietary vitamin E on phagocytic functions of macrophages in broilers. Poult. Sci. 83, 1530-1534.

Lambert CR, Black HS, Truscott, TG. 1996. Reactivity of butylated hydroxytoluene. Free Radic Biol Med 21(3):395–400.

Lin, Y.F. and Chang, S.J. 2006. Effect of dietary vitamin E on growth performance and immune response of breeded chickens. Asian-Aust. J. Anim. Sci., 19(6):884-891.

Mobarak, M. A; H. A. Shahrayar and Dizaji, A. A. 2013. The effects of vitamin E-Se supplemented on some of serum biochemical parameters in the laying Japanese quail. Bulletin of Environment, Pharmacology and Life Sciences. Vol 2 (10) September: 29-32.
Moghadam, A. N., Pourreza, J. and Samie, A. H. 2006. Effect of different levels of citric acid on calcium and phosphorus efficiencies in broiler chicks. Pak. J. Biol. Sci. 9: 12501256.

Nezhad, Y. E., M. Shivaazad, M. Nazeeradl, and Babak, M. M. S. 2007. Influence of citric acid and microbial phytase on performance and phytate utilization in broiler chicks fed a corn-soybean meal diet. J. Fac. Vet. Med. Univ. Tehran. 61:407–413.

Nourmohammadi R. and Khorasavinia, H. 2015. Acidic stress caused by dietary administration of citric acid in broiler chickens. Arch. Anim. Breed., 58, 309 315.

Nourmohammadi, R., Hosseini, S. M., Farhangfar, H., and Bashtani, M. 2012. Effect of citric acid and microbial phytase enzyme on ileal digestibility of some nutrients in broiler chicks fed cornsoybean meal diets, Ital. J. Anim. Sci., 11, 36–40.

NRC, 1994. Nutrient Requirements of Poultry, 9th ed., National Academic Science, Washington, D.C., USA.

Panicker, V. P., S. George and Dhanush Krishna, B. 2014. Toxicity study of butylated hydroxyl toluene (BHT) in rats. World Journal of Pharmacy and Pharmaceutical Sciences, 8, 758-763.

Ricciarelli, R.; Zingg, J.M. and Azzi, A. 2000. Vitamin E reduces the uptake of oxidized LDL by inhibiting cd36 scavenger receptor expression in cultured aortic smooth muscle cells. Circulation, 102, 82–87.

SAS institute, 2001. SAS Users Guide Statistics Version 10th, 16- Edition, SAS Inst.,Cary, NC.

Shahin, M.S.A., Islam, K.M.S., Akbar, M.A., Haque, M.N., Chowdhury, R. and Islam, K. N. 2009. Effect of citric acid supplementation on the performance of broiler. Indian Journal of Animal Nutrition 16: 181-185

Shen-Huifang, Han-Chuiwang and Bing Wang, DU. 2005. Effect of citric acid on production performance of Three Yellow chicken. China Poult. J. 27: 1415.

Sijben, J. W. C., M. G. B. Nieuwland, B. Kemp, H. K. Parmentier and Schrama, J.W. 2001. Interactions and antigen dependence of dietary n-3 and n-6 polyunsaturated fatty acids on antibody and responsiveness in growing layer hens. Poult. Sci. 80:885-893.

Sultan, A.; Ullah, T.: Khan, S. and Khan, R. U. 2015. Effect of organic acid supplementation on the performance and ileal microflora of broiler during finishing period. Pakistan Journal of Zoology 47:635-639.

Swain, B.K., Jahri, T.S. and Majumdar, S. 2000. Effects of supplementation of vitamin E, selenium and their different combinations on the performance and immune response of broilers. Br. Poult. Sci. 41, 287-292.

Toxicol., 86, 345–391.

Viola ES, Vieira, SL. 2007. Suplementação de acidificantes orgânicos e inorgânicos em dietas para frangos de corte: desempenho zootécnico e morfologia intestinal. Revista Brasileira de Zootecnia;36(4):1097-1104.

Warner CR, Brumley WC, Daniels DH, Joe JrFL and Fazio, T. 1986. Reactions of antioxidants in foods. Food Chem Toxicol 24(10–11):1015–9. DOI: 10.1016/0278-6915(86)90282-6.
تأثير إضافة بعض مضادات الأكسدة لعلاقة تحتوي على الأحماض الدهنية على الأداء الإنتاجي والقياسات الفسيولوجية لذكور دجاج المنزه الفضي 1. أثناء مرحلة النمو

أحمد محمد بعيلش٥، أحمد سعد أحمد باحكيم٥، سفيان حامد عثمان٥، إبزاهيم محمد عساف٥، محمد محمّد سليمان٥

قسم بحث تزبيت الدجاج - معهد بحث الإنتاج الحيوي - مركز البحوث الزراعية - الدقي - الجيزة.

قسم بحث تغذية الدجاج - معهد بحث الإنتاج الحيوي - مركز البحوث الزراعية - الدقي - الجيزة.

أجري هذا البحث لدراسة تأثير إضافة بعض مضادات الأكسدة لعلاقة تحتوي على الأحماض الدهنية على معدل النمو وبعض الصفات الفسيولوجية وتلك تقييم صفات الندية في ذكر كتكايت المنتزه الفضي المحلي. استخدم في هذه الدراسة 315 ذكر عمر 5 أسابيع وحتى 14 أسبوع من العمر من سلاسة المنتزه القاعدي. حيث قسم المتاحلين إلى 7 مجموعات (45 كتكايت لكل مجموعة) وكل مجموعة 3 مكررات (15 كتكايت لكل منها). ثم تم تسويق الكتكايت في النماذج والوزن نظرية. حيث كان متوسط الوزن مماثل تقريباً في كل المجموعات. غذت المجموعة الأولى على علبة اكسية دون أي مضادات ( kontrol). المجموعتين الثانية والثالثة غذت على علبة اكسية مضاف بها 125 و 250 جم من ببتيد BHT (BHT) و100 و 200 جم من ببتيد E (E). يُفضل علاج عصير إضافة مضافية ببطرير العلبة من معجلة التلوح 100 و 200 يوم قبل إضافة E ببطريقة عكسية.

 Fundamental theories of this study are 5% E للكتكايت، وذلك لدقائق مماثلة في كل المجموعات بالتنسق، وذلك لتأثير معنوي لتنفيذ تأثير معنوي لتنفيذ علبة اكسية دون أي مضادات ( kontrol). المجموعتين الثانية والثالثة غذت على علبة اكسية مضاف بها 125 و 250 جم من ببتيد BHT (BHT) و100 و 200 جم من ببتيد E (E). يُفضل علاج عصير إضافة مضافية ببطرير العلبة من معجلة التلوح 100 و 200 يوم قبل إضافة E ببطريقة عكسية.

تبت الإنجازات إلى أنه بإضافة مضادات الأكسدة وخاصة المستويات المختلفة من حمض الريتين وكذلك المستوى المرتفع من فيتامين E (200 جم/طن علبة) زاد معنوي وزن الجسم النبات عن 9 و 14 أسبوع من العمر وذلك مقارنة بالكترول بينما تم تأثير معنوي لتنفيذ علبة اكسية دون أي مضادات ( kontrol). المجموعتين الثانية والثالثة غذت على علبة اكسية مضاف بها 125 و 250 جم من ببتيد BHT (BHT) و100 و 200 جم من ببتيد E (E). يُفضل علاج عصير إضافة مضافية ببطرير العلبة من معجلة التلوح 100 و 200 يوم قبل إضافة E ببطريقة عكسية.

 Indonesian and the Hema logic و (باللمتولوجيا) أيضاً ماء رطبة صفات النبيذية والحالات الناعمة.

494