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# *Efficacy of Zinc/Creatine Supplementation on Improving Growth Performance of Local Balady Chicks*

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## ABSTRACT

An experiment was designed to study the efficacy of Zinc and or creatine monohydrate (CMH) supplementation, either alone or in combination, on improving growth performance of Balady chicks. A total number of 144 Balady chicks were randomly divided into four equal groups. The first treatment was fed the basal diet and served as control, while groups 2, 3, and 4 were given the basal diet and supplemented drinking water with, 0.6 mg/ml (zinc sulphat); 0.45 mg/ml (CMH); 0.6 mg/ml (zinc sulphat) + 0.45 mg/ml (CMH), respectively. The results indicated that the live body weight, weekly body weight gain and feed efficiency were significantly ($p\leq0.05$) improved in all treated groups compared with the control. Also, significant decrease ($p\leq0.05$) has been recorded in water consumption values of treated groups compared to control. Moreover, significant increase was recorded in carcass yield, kidney, spleen, bursa, thymus and intestine density (weight/length) with treated groups as compared to control group. On the other hand, serum total protein, globulin, total lipids, total antioxidant, thyroxine hormone level were significantly higher in treated groups than the control. Furthermore, Red and white blood cell counts, hemoglobin level, hematocrit values and antibody titres against Newcastle disease virus, were significantly improved in all treated groups. Therefore, it can be concluded that Zn and or CMH supplementation improved the physiological, productive traits of Balady chicks. The best significant results of performance were recorded for group the supplemented with Zn and CMH in combination compared with those of other treatments.

## INTRODUCTION

Poultry production plays the most important role in providing huge amounts and cheap source of animal protein in Egypt. A pure Egyptian breed, referred to as Balady local strains, is an animal resource which is established for both high quality meat and egg production, Taha *et al*. (2010). They are preferred to those produced by exotic hybrids and accepted by all consumers for a number of reasons, foremost being the degree of meat maturity, taste, texture and flavors, wholesome and more nutritious, Aymen *et al.* (2012). The higher premium prices of the Balady chicken are in part due to consumer preferrences and the limited scale of production. (Sonaiya & Swan, 2004).

Unfortunately, Egyptian Balady local strains are of medium size, the adult live body weight averages 1.2-1.7 kg in 60-65 days, Aymen *et al.* (2012). Moreover, the FCR for the native breeds (about 3 kg of feed for each kg of live weight 1:3) is much higher than that for the imported exotic broiler strains (1:1.8) and the time taken by the native breeds to reach the marketing weight (60 to 80 days) is almost double the time required for the exotic strains to reach the market (35 to 42 days), Enaiat *et al.* (2010). In addition, there is limited attention to develop and improve the production performance of local chicks.

The use of feed additives as well as zinc (Zn) and creatine monohydrate (CMH) in broiler diets are becoming increasingly frequent to enhance growth performance. Zn is one of the essential trace elements that stimulate the activity of about 100 enzymes in the body, Bartlett & Smith (2003). Zn is necessary for many physiological functions, including growth, skeletal development, immune function, sexual maturation, protein synthesis, gene expression, fat absorption, antioxidant defense, wound healing, improves appetite, metabolism of carbohydrates, proteins, lipids, skin health, and many essential biochemical processes of chickens, Suttle (2010). Hence, the body can't store Zn, so there may be a need to provide minimum daily intake. The NRC (1994) Zn requirement for broilers is 40 mg/kg. Zhao *et al.* (2014), suggested that appropriate concentrations of Zn resulted in improving the efficiency of feed utilization and growth performance in broilers. We hypothesized that Zn and/or CMH supplementation in drinking water as feed additives would elicit positive effects on growth performance of Egyptian Balady chicks when included in vegetable diet. CMH is another important feed additive in this study. It is certainly considered an excellent source of amino acids (arginine, glycine and methionine), Consequently, it can be used for poultry diets in comparatively small amounts as a main source of protein supplying feedstuff for better overall health status and to improve chicken production. Creatine is a naturally occurring component in the animal's body tissue and is mainly used in muscle tissues and plays an effective role in energy metabolism via phosphocreatine (PCr) system Wyss & Daouk (2000). Hence, with the absence of animal protein free diet, the risk for creatine deficiency increased. It is likely that the requirement for creatine is proportionally superior in growing chicks than in adults due to the high rate of muscle and nerve growth that occurs. Moreover, all the processes of cells involved in growth and metabolism require energy. CMH supplementation is needed for mainly bodily functions concerning maintenance (i.e., ATP regeneration) and may be vital to support optimal growth (i.e., protein synthesis), Carvalho *et al.* (2013). Halle *et al.* (2006) showed that supplementing CMH up to 10g/kg diet can be regarded as a method for elevating production efficiency and meat yield in broiler chickens. Carvalho *et al.* (2013) also, reported that inclusion of CMH in the control diet also exerts an improvement in body weight gain and feed conversion ratio to broiler chicks compared with the diets with no CMH.

Therefore, this study was designed to test the efficacy of Zn and/or CMH supplementation in improving growth performance and carcass characteristics of growing Balady chicks.

## MATERIALS AND METHODS

### Location, birds and experimental design

This experiment was carried out at the Poultry Production Research unit, belonging to the biological Application Department, Nuclear Research Center, Egyptian Atomic Energy Authority. A total number of 144 Balady chicks, as a local strain at 5 weeks of age and weighing 500 g on average, were randomly divided into four equal groups (36 birds in each group) with three replicates each. Each replicate contained (6 female and 6 cocks). The first treatment was fed the basal diet and served as control, while groups 2, 3, and 4 were given the basal diet and supplemented drinking water with, 0.6 mg/ml (zinc sulphate); 0.45 mg/ml (CMH); 0.6 mg/ml (zinc sulphate) + 0.45 mg/ml (CMH), respectively. Each replicate was reared in cages of 80 cm width × 150 cm length × 50 cm height, to obtain stocking density of (12 bird/1.2m$^2$). This cage was provided with outdoor feeders and water supply equipment by nipple. All groups were kept under the same conditions of room temperature 25±2°C during the whole experimental period (5 weeks). 50±5% relative humidity (RH), with a photoperiod of 14:10 (L: D) hours. The ingredient`s composition and calculated chemical analysis of the basal diet are given in Table 1.

**Table 1** – The ingredient composition and calculated chemical analysis of the basal diet.

| Ingredients composition | % |
|---|---|
| Ground yellow corn 8.5% | 61.5 |
| Soybean meal (44%) | 25 |
| Corn gluten meal (62%) | 6 |
| Corn oil | 3 |
| Dicalcium. phosphate | 1.7 |
| Limestone | 1.5 |
| DL-methionine | 0.05 |
| Sodium chloride (NaCl) | 0.40 |
| L-Lysine-Hcl | 0.05 |
| Vitamin and Min. Mixture * | 0.3 |
| Total | 100 |
| Calculated chemical analysis | |
| Crude protein | 19.95 |
| Crude fiber | 1.7 |
| Crude fat | 2.68 |
| Calcium | 1.08 |
| Available phosphorous | 0.59 |
| Lysine | 0.85 |
| Methionine | 0.45 |
| Met+cystine | 0.34 |
| Metabolizable Energy | 3080.9 Kcal /kg |

*Vitamin and mineral premix (contained per Kgm):- vit A, 1200 IU; vit D 1100 IU; vit E, 12mg; vitB12, 0.02mg; vit B1, 1mg; choline chloride, 0.16 mg; copper, 3mg; iron, 30mg; manganese, 40mg; zinc, 45 mg; and selenium, 3mg.

## Measurements and collected data

*Growth performance parameters*

Growth performance parameters were measured on Balady chicks at 6, 7, 8, 9 and 10 weeks of age. The average body weight gain (BWG), feed consumption (FC), Water consumption (WC) and feed efficiency (FE) were calculated every week on a pen basis for each treatment during the experiment period (5 weeks). Feed conversion ratio (FCR) was also calculated within each replicate as the ratio of feed intake (FI) to BWG (g feed/g gain) at the end of each week.

*Carcass traits and blood analysis*

At the end of the experimental period (5 weeks of age), four birds per experimental unit (i.e., 12 birds per treatment), with body weights close to the group mean, were weighed and slaughtered for carcass analysis. Carcass, liver, heart, proventriculus, gastrointestinal tract, spleen, and bursa of Fabricius for each slaughter bird were determined as a relative percentage of live body weight. In addition, gastrointestinal tract length was determined. Blood samples were collected from slaughtered birds in two tubes, one with heparin and the other without anticoagulant which centrifuged at 3400 × *g* for 6 min to obtain sera, and then were stored at −20°C until further analysis.

The concentrations of serum total proteins, albumin, total antioxidant, triglyceride, and total cholesterol and the activities of alkaline phosphates (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), were measured with a spectrophotometer (Shimadzu UV 1601) using commercial kits produced by Stanbio Company, USA.

Serum Globulin values were calculated by subtracting albumin values from their corresponding total proteins values of the same sample.

Concerning blood hematological parameters, red blood cells (RBCs) and white blood cells (WBCs), counts were performed using hemocytometer and Natt and Herrick solution according to Harrison and Harrison (1986). The packed cell volume (PCV %) was estimated by microhematocrit centrifuge according to Coles (1986). Hemoglobin concentration (Hb) was determined according to Dacie and Lewis (1991). The Thyroxin (T4) hormone concentration was determined using radioimmunoassay (RIA) Commercial Kit produced by IZOTOP Company (INSTITUTE OF ISOTOPES Ltd.) (http://www.izotop.hu), and samples were counted on Packard Gamma Counter.

*Immunological test*

At the end of the experiment, six birds from each group were chosen at random and housed in batteries. Each bird was vaccinated against Newcastle disease with NDV clone 30 (Nobilis ND Clone 30; Intervet) by eye-drop. Blood samples were withdrawn from jugular vein using an insulin syringe at three times 3, 7 and 9 days of post-vaccination. Blood was allowed to clot then centrifuged immediately to separate serum to determine immune response (antibody titer) of the chickens derived from vaccination against Newcastle disease virus by performing Hemagglutination inhibition (HI) test on serum samples according to the method of King & Seal (1998).

## Statistical analysis

The experiment was performed as a completely randomized design with 4 treatments. Statistical analyses were performed using the GLM procedure of JMP SAS (2008). The pen served as the experimental unit for the growth performance parameters (i.e., BWG, FC, FE, WC and FCR) and other parameters (i.e., serum biochemical). An arc-sin transformation was applied to the percentage values (i.e., carcass and some organs) before testing for differences. Tukey's multiple range test was performed to detect differences among the treatments. All differences were considered significant at $p \leq 0.05$.

# RESULTS AND DISCUSSION

## Growth Performance Parameters

Effect of Zn and/or CMH supplementation on growth performance and feed efficiency parameters of Balady chicks are presented in Table (2 and 3). It was observed that Zn and/or CMH supplementation improved total gain, feed conversion ratio and feed efficiency over the entire 5-wk experimental period when compared with the control group. Total feed

**Table 2 –** Live body weight gain (g/bird/week) as affected by Zn and or creatine supplementation (mean±se) in Balady chicks

| Live Body weight gain (g/bird/week) | Experimental groups | | | |
|---|---|---|---|---|
| | Control | Zn | Creatine | Zn &Creatine |
| 1st week | 112.5±5.9 $^{d}$ | 120.7±6.7 $^{c}$ | 133.3±6.9 $^{b}$ | 147.7±6.7 $^{a}$ |
| 2 nd week | 100±6.2 $^{c}$ | 106±6.7 $^{b}$ | 110±7.9 $^{b}$ | 134±7.8 $^{a}$ |
| 3 rd week | 86.3±2.8 $^{c}$ | 103.6±4.1 $^{b}$ | 108.2±6.1 $^{b}$ | 126.3±4.1 $^{a}$ |
| 4 th week | 85.7±3.2 $^{d}$ | 95.5±2.1 $^{b}$ | 102.6±3.4 $^{b}$ | 112.6±3.3 $^{a}$ |
| 5 th week | 80.3±5.6 $^{d}$ | 110.3±5.2 $^{c}$ | 117.3±4.9 $^{b}$ | 141.7±4.2 $^{a}$ |

a,b,c means with different superscripts within the same row are significantly different at ($p \leq 0.05$).

**Table 3 –** Growth and feed performance as affected by Zn and or creatine supplementation in Balady chicks (mean±se) at 70 days of age

| Growth performance | sex | Experimental groups | | | |
|---|---|---|---|---|---|
| | | Control | Zn | Creatine | Zn &Creatine |
| Initial live body weight (g) | Male | 678.3±0.5 $^{a}$ | 678.2±0.4 $^{a}$ | 670.5±0.48 $^{a}$ | 671.0±0.5 $^{a}$ |
| | Female | 494.0±0.5 $^{a}$ | 495.8±0.4 $^{a}$ | 502.6±0.5 $^{a}$ | 497.4±0.49 $^{a}$ |
| | Mean | 586.1±0.5 $^{a}$ | 587.2±0.4 $^{a}$ | 586.6±0.5 $^{a}$ | 584.2±0.5 $^{a}$ |
| Final live body weight (g) at 5 $^{th}$ week | Male | 1190±1.45 $^{d}$ | 1270±1.66 $^{c}$ | 1317±0.88 $^{b}$ | 1428±1.66 $^{a}$ |
| | Female | 909±1.66 $^{c}$ | 972±2.08 $^{b}$ | 995±1.85 $^{a}$ | 1062±2.89 $^{a}$ |
| | Mean | 1049.5±7.6 $^{c}$ | 1121±5.2 $^{b}$ | 1156±4.9 $^{b}$ | 1245±4.2 $^{a}$ |
| Daily weight gain (g) | | 13.29±1.5$^{d}$ | 15.32±1.4$^{c}$ | 16.33±1.4$^{b}$ | 18.92±0.8 $^{a}$ |
| Daily Feed intake (g./bird) | | 45.9±1.3$^{a}$ | 44.0±0.9$^{b}$ | 41.6±1.2 $^{c}$ | 39.5±0.9 $^{c}$ |
| Feed consumption(g)/bird | | 1606.5±2.3$^{a}$ | 1540±2.3$^{b}$ | 1456±2.3$^{c}$ | 1382.5±2.3$^{d}$ |
| Feed conversion ratio (feed : gain) | | 3.45±0.1$^{a}$ | 2.87±0.1$^{b}$ | 2.55±0.09$^{c}$ | 2.09±0.09$^{d}$ |
| Feed efficiency ratio (gain : feed) | | 0.29±0.1$^{d}$ | 0.35±0.1$^{c}$ | 0.39±0.09$^{b}$ | 0.48±0.09$^{a}$ |
| Feed intake (g)/bird | | | | | |
| 1$^{st}$ week | | 57.1±0.37 $^{a}$ | 54.9±0.33 $^{b}$ | 52.8±0.32 $^{c}$ | 48.2±0.37 $^{d}$ |
| 2 $^{nd}$ week | | 60.6±0.33 $^{a}$ | 58.3±0.32 $^{b}$ | 54.01±0.49 $^{c}$ | 50.3±0.33 $^{d}$ |
| 3 $^{rd}$ week | | 64.9±0.39 $^{a}$ | 61.74±0.33 $^{b}$ | 59.22±0.49 $^{c}$ | 55.7 ±0.53 $^{d}$ |
| 4 $^{th}$ week | | 67.4±0.37 $^{a}$ | 64.67±0.19 $^{b}$ | 62.77±0.18 $^{c}$ | 59.1±0.32 $^{d}$ |
| 5 $^{th}$ week | | 71.3±0.48 $^{a}$ | 68.3±0.38 $^{b}$ | 63.7±0.32 $^{c}$ | 63.3±0.74 $^{c}$ |

a,b,c means with different superscripts within the same row are significantly different at ($p \leq 0.05$).

intake of Balady chicks was reduced with Zn and/or CMH supplementation resulting in a 4.1%, 9.3% and 13.94% respectively lower feed intake compared with the control one. Moreover, combination of Zn and CMH showed better results of the tested parameters than CMH or Zn supplementation each alone, respectively. These findings are in agreement with several reports demonstrating that CMH supplemented up to 10g/kg diet of the broilers improved the body weight gains compared with the same diet without the use of the additive, Carvalho *et al.* (2013). Similarly, Fawzy *et al.* (2016) showed also that body weight gain of the birds fed with the diet supplemented with Zn (2 g /10 kg ration) were significantly higher than the control group and the feed conversion ratio was better. On the other hand, consistent with these results, a number of researchers documented that growth rate and feed efficiency were improved by dietary Zn supplementation in broiler chicks Zhao *et al.* (2014) & Fazilati (2013). Fathi *et al.* (2016) also, showed that nano-ZnO supplemented 20 mg /kg diet of the broilers improved the body weight gains and decreased feed intake compared with the control group. In addition, Bahakaim *et al.* (2014) noted a significant decrease in feed intake with the two levels 50 and 150 mg supplemental Zn /kg diet of chicken. The improvement in the Growth Performance Parameters of birds supplemented Zn and/or CMH could be attributed to the mandatory role of Zn as cofactor for more than 300 enzymes, which is essential in many digestive, metabolic and physiological processes in the body which reflect on growth, protein and carbohydrate metabolism. In this regard, the positive effect of Zn supplementation in drinking water on FCR may be due to the improvement in nutrient digestibility and efficiency of its use and therefore, decreased FC and improved FCR. Moreover, Zn plays a protective role on pancreatic tissue against oxidative damage, it may help the pancreas to function properly including secretions of digestive enzymes, thus improving digestibility of nutrients and consequently, performance Park *et al.* (2004).

Zn is a known essentially as a micronutrient for the growth of broilers. In the present study it was observed that appropriate levels of Zn (0.6 mg/ml), improved body weight gain and presented a better feed conversion ratio compared with other groups. The significant reduction observed in FC of this study may be due to the role of Zn which involves control of appetite. Sahraei *et al.* (2013), reported that Zn deficiency results in reduced appetite and depressed growth.

CMH, also, a compound based on three amino acids (arginine, glycine, and methionine), may be involved in this subject because it is a critical precursor in the production of muscle energy. The results of using 0.45 mg/ml (CMH) were in line with those of Zn supplementation, where BWG and FCR were improved in such supplement compared with an unsupplemented group. CMH may enhance muscle performance, growth and protein synthesis, due to an increase in the amount of energy stored as PCr in muscle. Earlier research has suggested that CMH can help the body quickly provide adenosine triphosphate (ATP) through the phosphocreatine. In addition, CMH loaded muscle has the capacity to maintain normal physiological function and to delay the onset of muscle fatigue, Lindahl *et al.* (2006). These observations provide clear and direct evidence of CMH efficacy in terms of growth performance and muscle phosphagen status in growing Balady chicks fed vegetable diets.

## Water consumption

The water consumption data was summarized in Table 4. This study showed that, Zn and/or CMH supplementation decreased water consumption when compared with the control group. Moreover, Zn and CMH supplementation together, showed lower results of the water consumption than other groups. It seems reasonable because of the reduction of caloric consumption per kg of BWG in groups supplemented with Zn and /or CMH. Lemme *et al.* (2010) showed that Zn and/or CMH play central roles in energy metabolism. Mousavi *et al.* (2013) also, showed that CMH supplementation decreased caloric intake per kilogram of BW gain and per kilogram of carcass weight. CMH supplementation is likely alleviating the muscle concentrations of creatine related metabolites as found by Guimaraes (2014). In this way, PCr concentrations increase the overall potential for muscle energy homeostasis as illustrated by Guimaraes (2014), thereby allowing for ATP to be consumed at a higher rate in support of improved metabolic function, this phenomenon has been purported to increase the rate of muscle protein synthesis Dilger *et al.* (2013) which might be beneficial for both skeletal muscle growth and for contractile activity of supply organs, such as the heart. Another benefit might arise from the fact that intramuscular PCr promotes water retention, it can attract water into the muscle cell and increase the cell volume as found by Guimaraes (2014) and this effect of super hydrated muscle may trigger protein synthesis, minimize protein breakdown, and increase glycogen synthesis in the muscle, this process may also support muscle growth as well, and may be illustrated the anabolic effects of creatine supplementation in birds. Unfortunately, the muscle volume and water content were not analyzed in this study. The current experiment's results concluded that Zn and/or CMH supplementation has the potential to improve the FCR and energy efficiency of growing Balady chicks.

**Table 4 –** Water consumption (ml)/bird as affected by Zn and or creatine supplementation in Balady chicks (mean±se)

| Water consumption ml/bird | Experimental groups | | | |
|---|---|---|---|---|
| | Control | Zn | Creatine | Zn &Creatine |
| 1st week | 137.03±1.85 $^{a}$ | 135.2±1.85 $^{a}$ | 125.9±1.85 $^{b}$ | 111.1±5.5 $^{c}$ |
| 2 nd week | 140.7±1.85 $^{a}$ | 135.2±1.85 $^{a}$ | 137.03±1.85 $^{a}$ | 120.4±1.85 $^{b}$ |
| 3 rd week | 175±1.61 $^{a}$ | 163.3±1.7 $^{b}$ | 146.1±3.47 $^{c}$ | 157.4±3.7 $^{b}$ |
| 4 th week | 196.3±1.85 $^{a}$ | 168.5±0.98 $^{b}$ | 168.5±1.85 $^{b}$ | 153.7±1.85 $^{c}$ |
| 5 th week | 218.5±3.7 $^{a}$ | 201.8±1.85 $^{b}$ | 207.5±3.7 $^{b}$ | 177.7±3.2 $^{c}$ |

a, b, c, means with different superscripts within the same row are significantly different at ($p \leq 0.05$).

## Carcass traits and relative organ weights

The results of carcass traits and relative organ weights are given in Table 5. The relative weight of carcass was significantly increased in chicks treated

**Table 5 –** Relative weight of carcass and some organs as affected by Zn and or creatine supplementation in Balady chicks (mean±se) at 70 days of age

| Relative weight of carcass and some organs(g) | Experimental groups | | | |
|---|---|---|---|---|
| | Control | Zn | Creatine | Zn &Creatine |
| Live body weight | 1049±1.45 $^{d}$ | 1121±1.66 $^{c}$ | 1156±0.88 $^{b}$ | 1245±1.66 $^{a}$ |
| Carcass | 62.12±1.6 $^{b}$ | 66.47±0.25 $^{a}$ | 65.99±0.65 $^{a}$ | 66.47±1.05 $^{a}$ |
| Liver | 2.1±0.16 $^{a}$ | 2.1±0.14 $^{a}$ | 1.9±0.07 $^{a}$ | 1.9±0.07 $^{a}$ |
| Gizzard | 2.1±0.21 $^{a}$ | 1.9±0.18 $^{a}$ | 2±0.15 $^{a}$ | 1.9±0.16 $^{a}$ |
| Kidney | 0.64±0.08 $^{b}$ | 0.73±0.01 $^{a}$ | 0.71±0.03 $^{a}$ | 0.61±0.06 $^{b}$ |
| Proventriculus | 0.4±0.06 $^{a}$ | 0.41±0.05 $^{a}$ | 0.42±0.04 $^{a}$ | 0.41±0.02 $^{a}$ |
| Spleen | 0.22±0.03 $^{b}$ | 0.26±0.03 $^{a}$ | 0.24±0.01 $^{a}$ | 0.25±0.03 $^{a}$ |
| Bursa | 0.33±0.1 $^{b}$ | 0.38±0.05 $^{a}$ | 0.37± 0.02 $^{a}$ | 0.33±0.06 $^{b}$ |
| Thymus | 0.4±0.07 $^{a}$ | 0.335±0.07 $^{a}$ | 0.37±0.04 $^{a}$ | 0.33±0.045 $^{a}$ |
| Heart | 0.48±0.01 $^{b}$ | 0.52±0.02 $^{a}$ | 0.475±0.007 $^{b}$ | 0.445±0.01 $^{c}$ |
| Small intestine density (weight/ length) | 1.6±0.03 $^{b}$ | 1.8 ± 0.02 $^{a}$ | 1.6±0.03 $^{b}$ | 1.9±0.05 $^{a}$ |

a, b, c, means with different superscripts within the same row are statistically different at ($p \leq 0.05$).

with Zn and CMH either alone or together as compared to the control. Meanwhile, no significant differences were observed in the relative weight of liver, gizzard and proventriculus among the treatment groups. However, Mousavi *et al.* (2013) showed that, the addition of 0.06% CMH reduced the percentage of liver significantly. Moreover, kidney relative weight was significantly increased in chicks treated with Zn or CMH each alone as compared to the other groups. The above result indicated that Zn or CMH supplementation did not induced hepatic and renal malfunction.

In the same trend, heart relative weight was increased in chicks treated with Zn as compared to the other groups. However, Zn and CMH in combination showed the lowest result of the relative weight of heart among groups. The results proved that Zn had significant influence on bird's antioxidant status, hematopoietic system responsibility and growth performance, birds with Zn supplementation in drinking water maintained their antioxidant systems in the heart muscle more effectively, with enhanced antioxidant defense.

The relative weights of lymphoid organs as (spleen and bursa of Fabricius) were increased by the supplemented Zn and or CMH with no significant effects on the relative weights of thymus. The significant increase in the relative weight of the bursa of Fabricius may be attributed to the increase of the number of immune cells, which is due to the effect of Zn and or CMH on the functional activities of the immune system responses which led to the increase in the number of lymphocytes in the primary lymphoid organs. Immune organ weight estimation is a common method for immune status evaluation of birds Heckert *et al.* (2002). Such related organs include thymus, bursa of Fabricius, liver and spleen. Good development of these organs is crucial for optimal Ig synthesis. Therefore, beneficial effects of Zn and or CMH supplementation in the gastrointestinal tract could result in an improvement of overall health, performance and immune response of layer chicks. These findings were similar to those observed by Sunder *et al.* (2008) and disagreed with Osman & Ragab (2007), who reported that supplementation of broiler chicks with Zn did not affect the relative weights of bursa, spleen and thymus. In poultry, Zn is responsible for the activation of the antioxidant status Prasad (2008) and immune system of the bird as a result of increasing the natural killer cell, thymocytes and peripheral T cell counts. Also it stimulates neutrophils and antibodies production. Moreover, Park *et al.* (2004) showed the role of Zn in supporting superior humoral, cell mediated responses and improving the macrophage functions.

The small intestine density (weight/ length), as shown in Table 5, significantly increased in chicks treated with Zn either alone or in combination with CMH as compared to the other groups. This is in line with observations by Lemme *et al.* (2010) and might be due to improving the cell energy metabolism because supplemental CMH increased the muscle creatine content and some metabolites correlated with the energy homeostasis such as PCr and ATP. Such changes might improve the utilization of nutrients for muscle accretion and growth and thus feed utilization. Mousavi *et al.* (2013) reported that the weight of the small intestine was reduced in the low-energy (90%) diets supplemented with CMH. Supplementation with CMH decreased caloric intake per kilogram of BW gain and per kilogram of carcass weight.

## Hematological Responses

The effect of Zn and or CMH supplementation on the hematological parameters of Balady chicks are presented in Table 6. These results indicated that, Zn and or CMH supplementation have significant effect on the level of Hb, PCV, WBCs and RBC's count in treated groups when compared with the control group. Our results parallel to the study conducted by Sajadifar (2012), who showed that Zn supplementation up to 200 mg/kg diet significantly increased the total WBCs of broiler chicks in compare with control group. Also, several researchers observed better immune response with increasing the level of Zn supplementation, Ezzati *et al.* (2013). However, this finding disagreed with Donmez *et al.* (2001), who reported that Zn

**Table 6 –** Blood hematology levels (mean±se) as affected by Zn and or creatine supplementation in Balady chicks at 70 days of age

| Trails | Experimental groups | | | |
|---|---|---|---|---|
| | Control | Zn | Creatine | Zn &Creatine |
| RBCs Count×$10^6$ | 4.13±0.06 $^c$ | 4.3±0.07 $^b$ | 4.67±0.05 $^a$ | 4.45±0.03 $^b$ |
| Hb (g/dl) | 22.4±0.3 $^c$ | 23±0.1 $^b$ | 24±0.15 $^a$ | 23±0.1 $^b$ |
| PCV % | 83.6±0.4 $^b$ | 85.9±0.2 $^a$ | 85.5±0.1 $^a$ | 85.6±0.5 $^a$ |
| WBCs Count×$10^3$ | 279.9±7.4 $^b$ | 349.9±6.3 $^a$ | 344.7±4.9 $^a$ | 347.5±5.8 $^a$ |

a, b, c, means with different superscripts within the same row are significantly different at ($p \leq 0.05$).

supplementation did not affect peripheral blood leukocyte counts. It seems that Zn supplementation of broilers increased lymphocyte and proliferation in visceral blood, Yang *et al.* (2011). Moreover, Zn plays an important role in immunomodulation by increasing the counts of thymocytes, peripheral T cells and enhancing the interferon production. Zn increases the T and B lymphocytes and enhances the immune responses of birds, Hudson *et al.* (2004). Our leukogram results, Table 6, are identical to the theory that Zn has an effective role in optimizing immune response, innate and acquired immune systems in chicks. Moreover, it has a role in increasing the antioxidant enzymes activities in the present study. Furthermore, the addition of Zn to poultry ration increased the hematopoietic system activity due to the increase of serum Zn level which stabilizes and regulates the cell membranes functions and protects it from lipid peroxidation Gruber & Rink (2013). CMH also increased the hematological parameters of Balady chicks because CMH is involved in energy production from the inner mitochondria to the cytosol. CMH supplementation has been shown to be beneficial in diseases in which there is mitochondrial dysfunction such as encephalopathy, Parkinson's, myopathy and lactic acidosis, Persky & Brazeau (2001). These results would be explained as the supplementation of Zn and or CMH affecting positively blood- cell forming processes. Moreover, increased blood WBCs count might be related to the production of more immune cells that play an important role in defending the biological system against different diseases.

### Immune response

In the present study, attempts were made to evaluate the use of Zn and or CAM as feed supplements for antimicrobials and antiviruses in terms of their ability to improve disease resistance, and enhance overall health and production in poultry.

Serum antibody titers against Newcastle disease virus-based on HI test in treated chicks with Zn and or CAM supplementation showed a higher response ($p \leq 0.05$) than those of chicks in control group on days 3, 7 and 9 post vaccination, Table 7. Moreover, chicks treated with Zn alone and in combination with CAM were similar and significantly increased the antibody titer against NDV, higher than chicks treated with CAM alone. These findings are in agreement with several studies. Sajadifar (2012) showed that Zn supplementation up to 200 mg/kg diet significantly increased the antibody titer against NDV and total WBCs of broiler chicks in comparison to the control group. Bartlett and Smith (2003) reported that the broilers receiving up to 181mg Zn had a higher response for total, IgM, and IgG antibodies. Feng *et al.* (2010) and Hudson *et al.* (2004) observed higher cellular immune response and antibody titers against Newcastle disease in broiler fed diets supplemented with 90 mg Zn/kg as Zn-gly compared to 160 mg Zn/kg as ZnSO4.

**Table 7 –** Antibody titres to Newcastle disease virus as affected by Zn and or creatine supplementation in Balady chicken (mean±se) at 70 days of age

| Days of treatment | Antibody titres against Newcastle disease virus | | | |
|---|---|---|---|---|
| | Experimental groups | | | |
| | Control | Zn | Creatine | Zn &Creatine |
| 3rd day | $3.75 \pm 0.25^{b}$ | $5.25 \pm 0.25^{a}$ | $4.5 \pm 0.29^{ab}$ | $5.25 \pm 0.25^{a}$ |
| 7th day | $5.25 \pm 0.25^{c}$ | $8.25 \pm 0.25^{a}$ | $7.25 \pm 0.25^{b}$ | $8.25 \pm 0.25^{a}$ |
| 9th day | $4.25 \pm 0.25^{b}$ | $6.75 \pm 0.475^{a}$ | $5.75 \pm 0.25^{a}$ | $6.75 \pm 0.475^{a}$ |

a, b, c, Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

Zn is necessary to maintain the activity of natural killer cells and phagocytosis of macrophages, Gruber & Rink (2013). The overall serological data of this study showed that both Zn and or CAM supplementation seems to increase antibody titer against NDV and total WBCs. Thus, supplementing Zn and or CAM in broiler's drinking water could be considered as a natural promoter of immune system against diseases and increase of poultry's resistance.

### Biochemical and hormonal parameters

The effects of Zn and or CAM supplementation on some plasma biochemical and hormonal parameters (Means ±SE) are presented in Table 8. The data clearly showed that protein profile: plasma total protein, albumin and globulin were significantly increased in treated groups when compared with the control.

Protein profile in Balady chicks supplemented with Zn and or CMH showed an elevation in total proteins, albumin, and globulin levels. Similarly, plasma total protein was increased with Zn dietary supplementation in broiler. Feng *et al.* (2010) and Fawzy *et al.* (2016), found an elevation in total protein, albumin, and globulin levels in broilers chicks supplemented with Zn

**Table 8 –** Serum constituents and hormonal levels as affected by Zn and or creatine supplementation in Balady chicken (mean±se) at 70 days of age

| Parameters | Experimental groups | | | |
|---|---|---|---|---|
| | Control | Zn | Creatine | Zn &Creatine |
| Total protein(g/dl) | 4.2±0.05$^{b}$ | 4.57±0.03$^{a}$ | 4.56±0.04$^{a}$ | 4.4±0.04$^{a}$ |
| Albumin(g/dl) | 2.83±0.04$^{b}$ | 3.04±0.04$^{a}$ | 3.04±0.04$^{a}$ | 3.0±0.03$^{a}$ |
| Globulin(g/dl) | 1.35±0.07$^{c}$ | 1.53±0.07$^{a}$ | 1.52±0.09$^{a}$ | 1.4±0.09$^{b}$ |
| T. lipids (mg/dl) | 667±8.9$^{c}$ | 729±7.02$^{a}$ | 683.06±10.63$^{b}$ | 748.2±15.35$^{a}$ |
| Triglyceride(mg/dl) | 157.7±2.86$^{c}$ | 181.3±6.9$^{b}$ | 171.45±3.14$^{b}$ | 188±7.8$^{a}$ |
| Cholesterol(mg/dl) | 154.7±0.39$^{c}$ | 162±1.0$^{b}$ | 161.5±0.$^{b}$ | 165±0.5$^{a}$ |
| ALT (u/ml) | 51.85±0.8$^{c}$ | 57.03±0.8$^{b}$ | 53.3±0.2$^{c}$ | 58.91±0.8$^{a}$ |
| AST (u/ml) | 104.5±1.02$^{c}$ | 116.8±1.4$^{b}$ | 107.5±1.28$^{c}$ | 124.7±0.33$^{a}$ |
| Alk. Phosphatase (IU/L) | 96.8±0.57$^{c}$ | 102.2±0.53$^{b}$ | 98.6±0.3$^{b}$ | 116.6±0.62$^{a}$ |
| T.antioxidant(mM / L) | 1.22±0.05$^{d}$ | 1.74±0.02$^{a}$ | 1.4±0.02$^{c}$ | 1.61±0.02$^{b}$ |
| T4(nmol/L) | 52.78±0.87$^{d}$ | 87.15±0.9$^{b}$ | 68.13±0.8$^{c}$ | 97.45±0.6$^{a}$ |

a, b, c, means with different superscripts within the same row are statistically different at ($p \leq 0.05$).

oxide 2 g /10 kg ration. The increases in total proteins, albumin, and globulin levels may result from increased muscle mass by exogenous CAM and or Zn. CAM serves as a stimulus of protein synthesis and muscle hypertrophy or reduced protein catabolism. Studies by Dilger *et al.* (2013) support this theory that CAM is an end-product of contraction; its' supplementation enhanced muscle fiber size and increased lean body mass. On the other hand, Zn is necessary for many physiological functions, including growth and protein synthesis; its' supplementation enhanced fat absorption, improved appetite, metabolism of carbohydrates, proteins, lipids, and many essential biochemical processes of chickens, Suttle (2010).

Related to lipids profile: plasma total lipids, cholesterol and triglycerides showed an elevation in all treated groups compared with the control. Zn and CMH in combination, was significantly ($p \leq 0.05$) higher in plasma lipid profile compared to all other groups. These results disagree with Uyanik *et al.* (2001), who reported that Zn decreased cholesterol, triglycerides. CAM supplementation has also been shown to lower total plasma cholesterol and triglycerides, Earnest *et al.* (1996). The elevation in lipid profile may result from increased fat absorption as shown by Bartlett & Smith (2003). In addition, the major role of CMH is in energy metabolism through PCr, as shown by Wyss & Daouk (2000).

The results of plasma ALT, AST and alkaline phosphates (ALP) enzyme activities revealed significant increase in all treated groups compared to the control group and the increment was more effective and elevated when Zn was combined with CMH in the drinking water of chicks. This finding is inconsistent with Fazilati (2013) who reported that nano-ZnO, up to 200 mg/ kg diet showed significant increase in ALT and AST activities in male rats. Similarly, Al-Daraji and Amen (2011) reported an increase in ALP activity with Zn supplementation. Nagalakshmi *et al.* (2015) showed that the ALP activity increased linearly with gradual increase in organic Zn supplementation higher than Zn supplemented from the inorganic source. Zn being an integral component of ALP, higher bioavailability of organic source might have resulted in the increased ALP activity. Fathi *et al.* (2016) showed that nano-ZnO supplemented 20 mg /kg diet of the broilers, increased ALP activity with no significant effect on ALT and AST activates compared with the control group. These findings are in disagreement with those mentioned by Persky and Brazeau (2001) who reported that CMH supplementation had no effect on hepatic function as indicated by no changes in blood liver enzymes (i.e., ALT and AST). Zn also was shown to reduce both of these enzymes to the range of normal level, which represent the non-pathological metabolism of the liver and heart. Ahmadi *et al.* (2014), reported that nano-ZnO had no significant effects on ALT and AST activities in serum of broilers. Although the values of serum levels of ALT, AST and ALP activity in chicks treated with Zn and CMH each alone were different, they were all within the normal range value and can be correlated with better function of the liver and appear non-pathological metabolism of the liver and heart which reflect better health of local Balady chicks subjected to this study. The changes in the serum transaminases level may depend on the rate of protein metabolism, Guyton & Hall (2006). In addition, increased ALP activity may be a result of increased plasma concentrations of cholesterol in treated groups, Table 8. On the other hand, the higher levels of ALT, AST and ALP activity recorded in the serum of the chicks treated with Zn and CMH combined, among treatment groups, act as a hepatocellular damage indicator Bloom (2002). Any abnormal increase in serum levels of ALT, AST and ALP activity may imply in liver damage. Therefore, the relatively stable levels of these enzymes may be associated with hepatoprotective effects. Furthermore, the activities of serum ALP, ALT and AST enzymes could be important in the diagnosis of diseases as well as in the investigation and thorough assessment of feed, drugs and extracts used in the treatment as these could give indications of progressive toxicity long before the actual manifestation of the toxic effects, Bloom (2002). Moreover, increased serum ALP, ALT and AST activities have all been associated with physiological stressful condition.

AST and ALT usually appear in serum when there is damage to the liver and muscle tissues caused by excessive stress, Uyanik *et al.* (2001). Concerning to plasma total antioxidant capacity (T-AOC), our result revealed that there was significant elevation in plasma T-AOC in treated groups than the control. Zn group was significantly ($p \leq 0.05$) higher in plasma total antioxidant than all other groups. These results totally coincided with the observations of Zhao *et al.* (2104) and Fawzy *et al.* (2016) who reported that serum T-AOC increased_significantly in Zn oxide 2 g /10 kg diet supplemented broilers as compared with the control. Fathi *et al.* (2016) showed that nano-ZnO supplemented 20 mg /kg diet of the broilers improved the bird's antioxidant status compared with the control group. Liu et al. [47] showed also that Zn Supplemental 0.9 g/kg diet resulted in promoting antioxidant ability and activities of broilers.

The elevation in plasma T-AOC of chicks treated with CMH are in disagreement with those mentioned by Wang *et al.* (2015), who showed that CMH supplementation 0.6-1.2 g/kg diet does not offer any significant protection via directly reducing free radicals, muscle lipid peroxidation or increased antioxidant capacity of transported broilers in summer. Michiels *et al.* (2012) also showed that plasma total antioxidant capacity, was reduced by CMH supplementing 0.6-1.2 g/kg broilers diet. Zhang *et al.* (2014) reported that the protective effect of CMH supplementation for maintaining meat quality in transported broilers is mainly related to its beneficial effect for producing more muscle PCr to produce ATP, and thus reduce the muscle glycolysis, rather than its antioxidant activity.

The total antioxidant capacity in the body related mainly to the dynamic balance of active oxygen, where T-AOC is an integrative factor reflecting the status of all the antioxidants in serum and body fluids. Hepatic damage may lead to a reduction in T-AOC Zhao et al. (2014). Moreover, Zn is considered a component of more than 300 enzymes and can manipulate oxidative processes. Researcher Niles *et al.* (2008) showed that Zn acts as an antioxidant to reduce cell membrane damage due to free radicals.

The elevation in plasma T-AOC of the chicks in the group treated with Zn higher than the CMH group, may be a result from Zn being considered a major part of the antioxidant enzyme superoxide dismutase, which protects the body from harmful effects of 'reactive oxygen species' as reported by Niles *et al.* (2008). Zn serves as a part of various enzyme systems within the avian body, Park *et al.* (2004). It is believed that Zn supplementation induces antioxidant effects to prevent oxidative DNA damage and mutagenesis, suppress oxidative stress such as toxicity of certain drugs and ethanol toxicity by the stimulation of certain substances which have antioxidant properties. Therefore, it can be concluded that Zn supplementation positively correlates with the bird's antioxidant status and may play a vital role in the maintenance of superior health.

Plasma concentration of thyroxin ($T_4$) in treated chicks with Zn and or CAM supplementation was significantly higher ($p \leq 0.05$) than those of chicks in the control group. Similar result was obtained by Michiels *et al.* (2012) who showed significant increase in plasma level of thyroid hormones with CMH supplementing 0.6-1.2 g/kg diet of broiler chickens. Depending on the previous results, we are not forgetting that Zn and or CAM also improved the growth rate of treated chicks which could be logically related to the increased plasma concentrations of the active form of thyroid hormone. The elevation in concentration of thyroxin ($T_4$) in treated chicks may result from Zn and or CAM supplementation affecting a number of physiological and metabolic processes and the positive correlation between thyroxin and body weight. Persky & Brazeau (2001) reported that thyroid hormones increased creatine content into skeletal muscle up to 3-fold relative to controls. Zhao *et al.* (2014) observed that Zn deficiency leads to failure of growth and poor development of gonadal functions. Dönmez *et al.* (2001) observed that serum free thyroxin levels increased significantly during adequate Zn period.

## CONCLUSION

Zn and CMH, alone or in combination in the drinking water had improved the physiological, productive traits of Balady chicks. The best significant results of performance were recorded for the group supplemented with Zn and CMH combined, compared with those of other treatments. Moreover, serological data from the present study showed the effectiveness of "Zn and or CAM" supplementation, they stimulate a protective immune response which sufficiently and positively correlate with the bird's antioxidant status.

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