Egypt. Poult. Sci. Vol. (39) (III): (711-730) (2019) **(1903-1013)**

Egyptian Poultry Science Journal

http://www.epsj.journals.ekb.eg/

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

INFLUENCE OF REDUCING CRUDE PROTEIN ON GIMMIZAH CHICKENS PERFORMANCE DURING LATE LAYING PERIOD

2- SUPPLEMENTED WITH AMINO ACIDS, ZINC AND VITAMIN D_3

B.M. Abou-Shehema

Anim. Prod. Res. Inst., Agric. Res. Center, Egypt.

Corresponding Author: Bahaa Abou-Shehema Email:bahaashehema@yahoo.com

Received: 01/08/2019 Accepted: 20 /08/2019

ABSTRACT: The present study aimed to determine the influence of low crude protein diet (LCP, 13%) enriched with methionine (Met) and lysine (Lys) and different zinc levels with or without vitamin D_3 on the productive and reproductive performance of Gimmizah chickens during the late laying period. A total number of 320 Gimmizah chickens (280 hens + 40 cocks) aged 52-week were individually weighed and randomly divided into eight treatment groups with five replicates per each during the experimental period (52 - 64 weeks of age). Birds of the first group were fed the basal diet and served as control. Second one was fed LCP, 13% without any supplementation. Whereas, the third group fed LCP diet enriched with Met (0.6 g/kg diet) and Lys (1.4 g/kg diet), to equalize their corresponding levels of the basal diet. The fourth and fifth groups were fed LCP diet enriched with Met and Lys supplied with zinc (Zn, 50 and 100 mg/kg diet, respectively). The sixth group was fed LCP diet enriched with Met and Lys supplied with vitamin D_3 (Vit D_3, 2000 IU/kg diet). While, the seventh and eighth groups were fed LCP diet enriched with Met and Lys supplemented with Zn (50 mg/kg diet**)** plus Vit D_3 (2000 IU/kg diet), and Zn (100 mg/kg diet**)** plus Vit D_3 (2000 IU/kg diet), respectively. The chickens fed LCP diet represented the worst significant records of egg production, egg weight, egg mass and fed conversion ratio compared with control. While, groups fed LCP diet enriched with Met and Lys alone or supplemented with studied agents similarly restored the previous parameters to control level. Egg shell weight, shell thickness, SWUSA, hatched chick weight and hatchability percentages for aged chickens fed LCP diet enriched with Met and Lys alone or supplemented with both Zn levels were significantly improved compared with control. Birds fed LCP diet enriched with Met and Lys supplemented with Zn levels plus Vit D_3 significantly improved Hgb, PCV, WBCs, lymphocyte, phagocytic activity, HDL, LDL, TAC, SOD, MDA and Ca compared with control and LCP diet groups. Total protein, albumin, globulin, total lipids and triglycerides for birds fed LCP diet enriched with Met and Lys alone or supplied with Zn levels with or without Vit D_3 did not represent any significant differences compared with control.

In conclusion, feeding Gimmizah chickens LCP (13%) diet enriched with Met and Lys alone is adequate for achieving the optimal productive and reproductive performance besides best record of net returns during the late laying period.

Key words: Chicken, Methionine, lysine, Zinc, Vitamin D_3, Productivity.

INTRODUCTION

As hens grow older, the nutrient requirements decrease beside egg production, eggshell quality and hatchability deteriorate (Wu *et al*., 2005; Odabasi *et al*., 2007; Rizk *et al*., 2008; Zita *et al*., 2009). The crucial role of nutritionists is to reduce the cost of feedstuffs whilst ensuring efficiency of utilization of low crude protein diet supplemented with amino acids to meet minimum amino acid standards reported by NRC (1994) especially during late stage of egg production (Alagawany *et al*., 2016). It has been proven that decreasing crude protein level in the diet has many advantages. However, commercial amino acids must be supplemented to meet the requirements of limiting amino acids as the dietary crude protein is decreased (Mousavi *et al*., 2013). In corn soybean meal basal diets for laying hens, methionine and lysine are the first and second limiting amino acids (Gheisar *et al*., 2011). Recent reports indicated that promising results in terms of maintaining bird's performance and maximizing profitability can be obtained by the use of low protein diets with supplementation of amino acid for laying hens (Kashani *et al*., 2014). Andrade (2003) reported that productive performance of laying hens fed low crude protein diet was improved with added synthetic methionine and lysine at the peak of lay. Moreover, Silva *et al*. (2010) reported that supplied different crude protein levels (12, 14, 16, and 18%) with synthetic amino acids improved feed intake, egg weight, egg mass, and albumen percentage linearly with increasing crude protein levels.

Zinc is a very crucial trace element involved in a wide range of metabolic activities such as carbohydrates metabolism and protein synthesis (Underwood and Suttle, 1999). It is directly related to catalysis and co-catalysis of enzymes which control cell process including DNA synthesis, normal growth, bone development, reproductive, normal immune function and activating carbonic anhydrase enzyme which is essential for egg shell formation (Batal *et al*., 2001). Several trials have shown an improvement in production from poultry when Zn was added to the diet. El-Husseiny *et al*. (2008) found an improvement in body weight gain, better egg production and quality in laying hens after Zn supplementation (inorganic zinc oxide). Supplementation of Zn has been reported to increase the overall egg production of laying hens (Gerzilov *et al*., 2015). Similarly, Moustafa *et al*. (2004) indicated that supplementing Egyptian laying hens diet with zinc oxide or Zn – methionine at two levels of 100 or 150 ppm resulted in significantly greater egg production. Increased mineral deposition in the eggs of laying hens has been shown after supplementation of Zn alone, and in combination with other trace minerals like copper, manganese and chromium (Yenice *et al*., 2015). Additionally, Zamani *et al*. (2005) observed that the average of egg production and eggshell thickness were found to be significantly higher in the layers that supplied with 150 mg Zn/Kg of diet than those receiving 0, 50, and 100 mg Zn/kg of diet.

Vitamin D_3 (cholecalciferol) is one of the most essential dietary factors responsible for normal growth, egg production, shell quality and reproduction in fowls (Ameenuddin *et al*., 1982). It is also a required component of the endocrine system of birds and regulates calcium and phosphorus homeostasis and bone mineralization. Frost and Roland (1990) indicated that adding $1,25(OH)_2D_3$ in layer diet increased eggshell percentage, egg breaking strength and shell weight. Also, Abdel-Azeem and El-Shafei (2006) reported that FCR, shell quality and plasma Ca were significantly improved ($P<0.05$) when the Vit D_3 increased from 2000 to 3000 IU/kg in laying quail diet. Moreover, Abd El-Maksoud (2010) reported that increasing dietary levels of Ca up to 4% with a level of 4000 IU/kg diet of Vit D_3 improved eggshell quality without negative effects on productive performance.

The objective of this study was to investigate the influence of low crude protein diet (LCP, 13%) enriched with methionine and lysine and different dietary levels of zinc with or without vitamin D_3 on the productive and reproductive performance and some physiological responses of Gimmizah chickens during late laying period.

MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agriculture Research Center. The experiment was conducted from 15 March to 7 May 2016 to investigate the influence of low crude protein diet (LCP, 13%) enriched with methionine (Met) and lysine (Lys) and different dietary levels of zinc with or without vitamin D_3 on the productive and reproductive performance and some physiological responses of Gimmizah chickens during the late laying period.

Birds, management and experimental design

A total number of 320 Gimmizah chickens (280 hens + 40 cocks) aged 52-week were individually weighed and randomly divided into eight treatment groups. Each treatment groups was represented by five replicates (7 hens + 1 cock) and housed in 40 floor pens (open sided system) until the end of the experiment (64 weeks of age). The first group was fed the basal diet and served as control. Second one was fed the low crude protein diet (LCP, 13%) without any supplementation. Whereas the third was fed the LCP diet enriched with Met (0.6 g/kg diet) and Lys (1.4 g/kg diet), to equalize their corresponding levels of the basal diet. The fourth and fifth groups were fed LCP diet enriched with Met and Lys supplemented with zinc (Zn, 50 and 100 mg/kg diet, respectively).The sixth group was fed LCP diet enriched with Met and Lys supplemented with vitamin D_3 (Vit. D_3, 2000 IU/kg diet). While, seventh and eighth groups were fed LCP diet enriched with Met and Lys supplemented with Zn (50 mg/kg diet**)** plus Vit D_3 (2000 IU/kg diet), and Zn (100 mg/kg diet**)** plus Vit D_3 (2000 IU/kg diet), respectively. Zinc was supplied to the diet in form of zinc oxide 72%. Experimental diets were formulated according to **Feed Composition Table for Animal and Poultry Feedstuffs in Egypt (2001)** as shown in Table 1. Feed and water were provided *ad libitum* throughout the experimental period. Birds were subjected to 16 hrs light and 8 hrs dark during the experimental period. Vaccination and medical care were done according to common veterinary care under veterinarian's supervision.

Measurements

Daily egg production (EP) and egg weight (EW, g) were detected for each replicate and egg mass (EM, g/h/d) were calculated. Feed intake (FI, g/h/d) was recorded weekly. Egg production % was calculated during the production period, then feed conversion ratio (FCR) was calculated as g of feed required per each g of egg. Eggs were collected for a 7-day period at 62 week of age and incubated in an automatic incubator. Eggs were candled on day 18 of incubation to identify non fertile eggs. Macroscopic fertility was calculated as the number of fertile eggs as relative to the total number of eggs set while hatchability percentages were calculated as the numbers of hatched chicks as relative to the fertile and total eggs set. Eggs laid on two successive days from each treatment at 60 and 64 weeks of age were used for measuring egg quality traits. Egg shell, yolk, yolk dry matter and albumen were weighed to the nearest 0.1 g (egg shells were washed, the inner eggshell membranes were separated and air-dried for 72 h before weighing). Eggshell thickness (mm) without membranes was determined by micrometer to the nearest 0.01 mm. While, yolk index (YI) was measured according to Funk (1948) and Haugh unit score (HU) according to Haugh (1937) and shell weight per unit of surface area (SWUSA) according to Carter and Jones (1970).

Blood analyses

At the end of the experiment, in the morning at 09.00 to 10.00 h, two blood samples (3 ml, each) were collected from the branchial vein, (one into heparinized tube to separate plasma and the other one into unheparinized tube to separate serum) of five birds / treatment. Serum was stored at - 20º C until chemical analysis. Also, fresh blood samples were used for determination of hemoglobin (Hgb), red blood cell count (RBCs), packed cells volume (PCV) and white blood cell counts (WBCs). White blood cell differential was done according to Hawkey and Dennett (1989). Plasma was immediately separated by centrifugation for 10 minutes at 3200 rpm. Some plasma criteria as glucose, total protein, albumin, globulin, total lipids, triglycerides, cholesterol, HDL, LDL, urea, creatinine, calcium, and phosphorus were determined using commercial kits produced by Diamond Diagnostics Company (29 Tahreer St. Dokki Giza Egypt). The activity of serum aspartate amino transferase (AST), and serum alanine amino transferase (ALT), were determined spectrophotometrically using available commercial Kits. Serum total antioxidant capacity (TAC), superoxide dismutase (SOD) and Malondialdehyde (MDA) were colorimetrically determined using commercial Kits. The phagocytic activity (PA) and phagocytic index (PI) were measured as suggested by Leijh *et al*. (1986).

Economical efficiency

The total feed cost at the end of the experiment for each treatment was calculated depending upon the local market prices of the ingredients used for formulating the experimental diet. Economical efficiency (EE) and relative economic efficiency (REE) were calculated according to input-output analysis.

Statistical analysis

Data were statistically analyzed using one way ANOVA of SAS® (SAS Institute, 1996). Differences among treatment means were estimated by Duncan's multiple range test (Duncan, 1955). The following model was used to study the effect of treatments on the parameters investigated as follows:

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where: Y_{ij} = an observation, μ = overall mean, T_i = effect of treatment (i=1,2,3,…..8) and e_{ij} = experimental random error.

RESULTS

Productive and reproductive traits:

Data of Table 2 showed that the initial, final and body weight change did not statistically differ among the experimental groups. Egg production percentage for aged chickens fed LCP diet was significantly decreased compared with the control group. Whilst, birds fed LCP diet enriched with Met and Lys (Met-Lys-LCP) or supplemented with different experimental agents did not represent any statistical change compared with those for control group. Moreover, groups fed Met-Lys-LCP or combined with Vit D_3 recorded significantly the highest value of EP% (64.6 and 65.0%, respectively) compared with those fed LCP diet alone which recorded the lowest value of EP (56.7%). Egg weight, EM and FCR for chickens fed LCP diet were significantly impaired compared with the control group. While, chickens fed Met-Lys-LCP or supplemented with different experimental agents similarly restored them to the corresponding control levels. On the other hand, feed intake did not represent any statistical change among the experimental groups.

Egg quality traits:

Results presented in Table 3 showed that all experimental treatments had no significant influence on egg shape index, specific gravity, yolk weight, albumen weight and yolk index percentages. Shell weight, shell thickness and SWUSA of chickens fed Met-Lys-LCP or supplemented with different studied agents were significantly improved compared with control group. Moreover, respectable to the previous mentioned parameters, birds fed LCP diet did not demonstrate any significant differences compared with control group. Yolk dry matter percentage for birds fed Met-Lys-LCP or supplemented with different studied agents did not represent any statistical change compared with those of control group except that for the group fed Met-Lys-LCP combined with Vit D_3 which significantly improved compared with those for control group. Furthermore, yolk color had been significantly improved for groups of chickens fed LCP diet with or without different experimental agents compared with control group. Haugh unit scores for groups fed Met-Lys-LCP or supplemented with different experimental agents were significantly improved compared with those fed LCP diet alone.

Hatching traits:

Fertility and hatchability percentages were significantly decreased for Gimmizah chickens fed LCP diet alone compared with the control group. While, fertility percentages for chickens fed Met-Lys-LCP supplemented with both levels of zinc or Zn (100 mg/kg diet) plus Vit D_3 were significantly improved compared with the control group (Table 4). Furthermore, fertility percentages of aged birds fed Met-Lys-LCP alone or supplemented with Vit D_3 or Zn (50 mg/kg diet) plus Vit D_3 did not represent any significant differences compared with control group. Hatchability of fertile eggs percentages for aged chickens fed Met-Lys-LCP or supplemented with both levels of zinc were significantly improved compared with the control and LCP diet groups. While, the groups fed Met-Lys-LCP combined with Vit D_3 or both levels of zinc plus Vit D_3 did not statistically differ than control group. Hatchability percentages for groups fed Met-Lys-LCP alone or supplemented with different studied agents were significantly improved compared with the control group except that for group fed Met-Lys-

LCP combined with Vit D_3 which did not represent any statistical change compared with control group. Hatched chick weight percentages for groups fed Met-Lys-LCP or supplemented with Zn (100 mg/kg diet) were significantly improved compared with those for other experimental groups.

Hematological parameters:

Results presented in Table 5 showed that RBCs count, heterophil and heterophil/lymphocyte ratio did not statistically differ among the experimental groups. Hemoglobin concentrations of birds fed Met-Lys-LCP supplemented with Vit D_3 or both levels of Zn plus Vit D_3 were significantly increased compared with others. Phagocytic activity and PCV for groups fed Met-Lys-LCP supplemented with different studied agents were significantly increased compared with that in the rest groups. Aged layers fed Met-Lys-LCP combined with both levels of Zn plus Vit D_3 had significant increase of WBCs and lymphocyte compared with control group. Whilst, birds groups fed LCP diet and Met-Lys-LCP or supplemented with both levels of Zn did not represent statistical change compared with control group. Phagocytic index of chickens fed Met-Lys-LCP supplemented with Zn (50 mg/kg diet) or Zn (50 mg/kg diet) plus Vit D_3 was significantly increased compared with control group. However, the other experimental groups did not represent any significant differences comparing with control group.

Blood biochemical constituents:

Results of Table 6 demonstrated that total protein, albumin and globulin of groups fed Met-Lys-LCP alone or supplemented with different experimental agents did not statistically differ than control group. While, these mentioned parameters were significantly diminished for group fed LCP compared with control. Values of glucose, AST, ALT and Ca/p ratio did not represent any statistical change among the experimental groups. Also, values of total lipids, triglycerides and cholesterol of chickens fed LCP diet were significantly increased compared with the control group. Contrary, lipid profile was significantly improved since the concentrations of total lipids and triglycerides of chickens fed Met-Lys-LCP alone or supplemented with different studied agents did not significantly differ from the control group except that for triglycerides of group fed Met-Lys-LCP combined with Vit D_3 which significantly increased compared with the control group. Moreover, cholesterol concentrations for groups fed Met-Lys-LCP alone or supplemented with 50 mg Zn /kg diet or Vit D_3 or 100 mg Zn /kg diet plus Vit D_3 were significantly decreased compared with those for control and LCP diet groups. In the same line, hens fed Met-Lys-LCP alone or supplemented with different studied agents significantly decreased LDL compared with the control group. The opposite trend was observed for HDL. All experimental groups represented significant decrease of blood urea compared with control. The groups fed Met-Lys-LCP alone or supplemented with both levels of Zn plus Vit D_3 significantly decreased creatinine concentrations compared with the control group. Total antioxidant capacity and SOD for groups fed Met-Lys-LCP supplemented with different agents were significantly improved compared with that in the rest groups. On the other hand, MDA levels of chickens fed Met-Lys-LCP alone or supplemented with different experimental agents represented significant reduction compared with those for control and LCP diet groups. Plasma calcium levels for groups fed Met-Lys-LCP supplemented with different agents were significantly improved compared with the control group. Chickens fed LCP diet with or without different studied agents represented significant increase of phosphorous compared with the control group.

Economical evaluation:

Data of Table 7 indicated that the group fed LCP diet enriched with Met and Lys alone recorded the best values of economical efficiency (0.78) and relative economical efficiency (114.6%) compared with the control group.

DISCUSSION

Several recent studies have focused on the development of dietary formulations aimed to reducing feed costs while maintaining hen production performance and improved eggshell quality. One of these strategies is based on formulating diets on an ideal protein basis while eliminating or reducing crude protein. The goal is to provide ideal levels of the essential amino acids for optimizing hen performance while minimizing excess amino acids. This is accomplished by reducing the crude protein content of the diet while supplementing any limiting essential amino acids with crystalline amino acids (Mousavi *et al*., 2013; Kashani *et al*., 2014). The results obtained herein indicated that final BW and BW change did not represent any statistical change among the experimental groups (Table 2). These results are in agreement with the results of Bunchasak *et al*. (2005) and Junqueira *et al*. (2006) who reported that change of dietary CP levels (14, 16, 18 and 20%) did not significantly affect BW change of commercial laying hens.

Lowering CP level in aged chicken diet, from 15 to 13% CP, significantly decreased EP, EW and EM by 8.99, 4.84 and 13.43%, respectively. Also, decreasing CP level resulted in the worst FCR (4.51 g feed/g egg) compared with the control group (3.92 g feed/g egg). However, supplied LCP diet with Met-Lys or with Met-Lys plus Vit D_3 recovery the reduction of previous mentioned traits compared with the group fed LCP diet by 13.9, 8.22, 23.1 and 21.1 and 14.6, 5.48, 21.0 and 15.5%, respectively, besides there were no statistical change compared with control group. Moreover, respectable to the previous mentioned parameters, birds fed Met-Lys-LCP combined with both levels of Zn with or without Vit D_3 did not represent any significant differences compared with the control group (Table 2). However, feed intake did not represent any statistical change among the experimental groups. These results are keeping with previous results reported by Bunchasak *et al*. (2005) who stated that poor records of EP, EW and EM were detected for birds fed 14% CP compared with those fed 16 or 18% CP groups through peak period for laying hens. Also, Novak *et al*. (2008) indicated that EW was decreased for layer fed lowering CP during the experimental period (18-60 week-old). Moreover, Bouyeh and Gevorgian (2011) found that FI was insignificantly affected by dietary CP levels in diets of laying hens. Furthermore, Khajali *et al*. (2008) demonstrated that the reduction of protein diets by 1.5 % for long-term feeding (20-72 wk) impaired EP and FCR of Hy-line laying hens in the late stage of production. On the other hand, Novak *et al*. (2006) reported that reducing protein intake from 18.9 to 17.0 g/hen per day (20 to 43 wk of age) and from 16.3 to14.6

g/ hen per day (43 to 63 wk of age) had not changing in EP.

The results of productive performance (EP, EW, EM and FCR) enhancement in the current study due to crystalline Met and Lys supplementation are consistent with those for the previous reports. Silva *et al*. (2010) reported that supplying different crude protein levels (12, 14, 16, and 18%) with synthetic amino acids improved FI, EW and EM linearly with increasing crude protein levels. Also, Tavernari *et al*. (2013) and Lelis *et al*. (2014) demonstrated the possibility of reducing dietary protein levels when crystalline amino acids are added to feed. The improvement due to supplementing poultry diets with lysine or methionine (Schutte *et al.*, 1994) has been reported to increase the efficiency of protein utilization. Also, Mousavi *et al*. (2013) reported that a reduction in dietary protein level (from 18.5% to 15.5% for Hy-line laying hens during 25: 33 wk of age), without any alteration in digestible TSAA and Thr:Lys ratio, led to an inferior EM and FCR during peak production period. Zeweil *et al*. (2011) suggested that Baheij laying hens may be fed low crud protein diet combined with DL-Methionine without adverse effect on productive performance.

Regarding the current results, improving productive performance due to Zn supplementation confirmed those of different authors, as they reported the improvement of EP in laying hens after Zn supplementation (Gerzilov *et al*., 2015; Yenice *et al*., 2015). Moreover, Bahakaim *et al.* (2014) reported that EM was improved after Zn supplementation in layer diet. The improvements in EP, EW, EM and FCR herein could be due to the role of Zn addition to the diet of aged birds as confirmed by Bedwal and Bahuguna (1994) who reported that Zn supplementation had an important role in synthesis and secretion of luteinizing hormone and follicle-stimulating hormone. Also, Gerzilov *et al*. (2015) reported that Zn had an anti-stress and antioxidative properties. Moreover, Zinc is a very important trace element that is involved in a wide range of metabolic activities such as protein synthesis (Underwood and Suttle, 1999). The improvements in feed efficiency of birds given Zn above NRC recommendation were attributed to the role of Zn in various enzymatic activities by enhancing digestibility and nutrient absorption (Kucuk *et al*., 2003). Banerjee (1988) mentioned that the enzymes activity of amylase, lipase, trypsinogen, chemotrypsinogen and some peptidases are related to dietary Zn supplementation. Also, Cole and Lifshitz (2008) reported that Zn deficiency in animals is claimed to result in anorexia, poor food efficiency, growth impairment.

With respect to Vit D_3 supplementation as shown in our results, Ameenuddin *et al*. (1982) reported that Vit D_3 (cholecalciferol) is one of the most important dietary factors responsible for normal egg production in fowls. Moreover, supplementation of Vit D_3 to the deficient diets alleviated the decline in productivity (Newman and Leeson, 1997). Abdel-Azeem and El-Shafei (2006) reported that FCR was significantly improved when the Vit D_3 increased from 2000 to 3000 IU/kg in laying quail diet. While, Panda *et al*. (2006) reported that increasing dietary Vit D_3 in laying diet had no effect on FI of white leghorn breeders during 72 to 88 wks of age. The previous results declare that enriching the LCP diet with Met and Lys could be adequate for optimizing the productive performance of aged chicken without any benefit due to extra addition of Zn and Vit D_3.

Results of the significant improvement of shell weight, shell thickness, SWUSA and yolk color due to supplementing diet with Zn are confirmed by Mabe *et al*. (2003) who suggested that trace elements as Zn, manganese and copper could affect mechanical properties of eggshell by effecting calcite crystal formation and modifying crystallographic structure of eggshell. Also, Zamani *et al*. (2005) mentioned that basal diet supplementation with 50 mg Zn/kg diet besides extra amounts of Zn had beneficial effect on the thickness of egg shell. Guo *et al*. (2002) found that 80 mg dietary Zn/kg is required to improve eggshell strength in older birds at 55 and 59 weeks of age. The importance of improving eggshell structure due to Zn supplementation is explained by Hunton (2005) who mentioned that egg shell is an important structure for embryonic development through the mechanical protection and gas exchange surrounding the eggs. Whereas, Stevenson (1985) reported that high dietary levels of Zn (100 or 200 mg/kg) had no beneficial influence on egg quality measured as eggshell thickness.

Results of egg quality improvement due to Vit D_3 supplementation in the current results are in accordance with those reported by previous authors. Ameenuddin *et al*. (1982) stated that Vit D_3 is important as dietary factor responsible for egg shell quality. However, supplementation of Vit D_3 to the deficient diets alleviated the decline in productivity and shell quality (Newman and Leeson, 1997). Some research findings have demonstrated that diet supplementation with an active metabolite of Vit D_3, i.e. 25-OH-D_3, can positively affect eggshell quality (Bar *et al*., 1988). The beneficial effects of added dietary 25-OH-D_3 can be significantly more pronounced in the second part of the laying cycle (Koreleski and Swiatkiewicz, 2005). Furthermore, Bar (2008) reported that more Vit D_3 content could improve the eggshell quality by increasing the active form of Vit D_3 (1,25-dihydroxycholecalciferol; 1,25$(OH)_2D_3$) production in the kidney as 1,25 $(OH)_2D_3$ stimulated the synthesis of calcium-binding protein which is essential for transportation of calcium across the intestinal membrane and may be essential for transportation of calcium for eggshell formation in the shell gland. Also, Abd El-Maksoud (2010) reported that increasing dietary levels of Ca up to 4% with a level of 4000 IU/kg diet of Vit D_3 improved eggshell quality. Whereas, Panda *et al*. (2006) reported that increasing dietary Vit D_3 in laying diet had no effect on shell quality of white leghorn breeders during 72 to 88 wks of age.

As can be seen from data of Table 4 that hatchability and hatched chick weight percentages for aged Gimmizah chickens fed Met-Lys-LCP alone or combined with both levels of Zn were significantly increased compared with control and LCP groups. Moreover, fertility percentages for groups fed Met-Lys-LCP supplemented with both levels of Zn were significantly increased compared with others. Therefore, the aforementioned results indicate supplementing the diets with Zn is essential in aged chicken diets to achieve best results of hatching success. Supporting to our results regarding the improvement of hatchability due to Zn supplementation, Badawy *et al*. (1987) observed that hatchability increase is correlated with the increase of Zn supplementation and this increase is primarily due to decrease in the incidence of late embryonic mortality. Moreover, Swiatkiewicz and Koreleski (2008) reported that normal reproductive performance could be realized with Zn supplementation and inadequate Zn status of hen may reduce egg shell quality, hatchability, embryonic development and poor chicks' quality. The improvement of hatching output in our data of this research could be related to the improvement of eggshell quality due to the experimental supplementations of Met, Lys and Zn in the LCP diet.

Results reported herein indicate that supplementation of Vit D_3 or different levels of Zn plus Vit D_3 to Meth-Lys-LCP significantly increased Hgb concentration and PCV%. These results refer to the beneficial effect of combining ability of Zn and Vit D_3 on some hematological parameters. Concerning the increase in Hgb concentration after Vit D_3 supplementation may be due to the influence of Vit D_3 on Hgb through a direct effect on erythropoiesis where it has a synergistic action with erythropoietin; it also increases the storage and retention of Fe and reduction of pro-inflammatory cytokines (Meguro *et al*., 2011). Also, Vit D_3 regulates the process of erythropoiesis by stimulating erythroid progenitor cells in a synergistic fashion with other hormones and cytokines, including erythropoietin and it has been reported that Vit D_3 metabolites are important for normal red blood cell production (Lucisano *et al*., 2014).

Lowering CP level in layer diet (from 15 to 13%) significantly reduced total protein, albumin and globulin concentrations compared with control group. Supplied Meth-Lys-LCP diet with both levels of Zn with or without Vit D_3 significantly improved total protein, albumin and globulin concentrations compared with the group fed LCP diet. Our results regarding the lowering crude protein in the diet are generally in harmony with the results of Bunchasak *et al*. (2005) who reported that increasing CP levels from 12-14 and 14-16% increased plasma total protein and globulin in layer hens. On the other hand, plasma albumin was not significantly affected by the different CP diets (Zeweil *et al*., 2011). Also, Bahakaim *et al.* (2014) reported that globulin and total protein improved after Zn supplementation in layer diet.

Reducing CP in layer diet significantly increased lipid profile (total lipids, triglycerides and cholesterol) compared with the control group as presented in Table 6. Similar results were reported by Ghasemi *et al*. (2014) who demonstrated that blood triglycerides concentration was increased ($p<0.05$) for the group contained 13.9% CP compared to groups contained 15.4% CP in Lohmann laying hens. Also, Torki *et al*. (2015) found that triglycerides was higher ($p<0.05$) in layers received 12 and 10.5% CP diets compared with those of control (16.5% CP). Whereas, Saki *et al*. (2015) reported that blood total cholesterol and low density lipoprotein were not affected ($p>0.05$) by different levels of CP.

Generally, the lipid profile was significantly improved for groups fed LCP enriched with Met and Lys or supplied with 100 mg Zn/kg diet or with 100 mg Zn/kg diet plus Vit D_3. These results are compatible with previously obtained by Herzig *et al*. (2009) who reported that there was a significant decrease of plasma cholesterol when broilers fed high amounts of Zn in diet.

Furthermore, Aksu *et al*. (2010) reported that supplementing the diet with organic complex of zinc, copper and manganese decreased plasma LDL cholesterol, combined with the increase in HDL cholesterol in chickens. In addition to, Tomas and Eva (2011) who found the positive impact of zinc on lipid metabolism indices in cocks. On the other hand, Kucuk *et al*. (2008) did not confirm any significant changes in the concentrations of total cholesterol and triglycerides due to supplementing the diet with 30 mg of Zn per 1 kg of a feed mixture.

Blood urea concentrations as an indicator of kidney function were significantly improved for all groups fed LCP diet alone or supplied with different experimental agents compared with the control group. Several studies were done and proved the beneficial effect of reducing the dietary crude protein on some parameters of kidney function (Hsu *et al*., 1998; Ghasemi *et al*., 2014), while others did not find any significant influence by dietary treatments varying in protein content (Ji *et al*., 2014).

All immunity indices, antioxidant and lipid peroxidation status detected herein (phagocytic activity and index, MDA, TAC and SOD) were significantly improved for all groups fed Meth-Lys-LCP diet combined with Vit D_3 or both levels of Zn with or without Vit D_3 compared with the other experimental groups. Numerous studies have been conducted and revealed the potential role of supplementing the diet with Zn on some of the mentioned parameters as Baum *et al*. (2000) who mentioned that Zn is a necessary part of superoxide dismutase (SOD) enzyme that plays an antioxidant defense system. Furthermore, Atakisi *et al*. (2009) found that zinc supplementation improved TAC and reduced MDA concentrations in Japanese quails. Besides, Bao *et al*. (2013) stated that Zn increases the activation of antioxidant proteins, molecules, and enzymes such as glutathione (GSH), catalase, and SOD. The apparent increase of phagocytic activity and phagocytic index in the current results could plays a vital role in improving the immune status of birds as confirmed by Solomons (1998) who stated that the improvement of the immunity function may be due to the playing role of Zn in humeral immune system. Also, Ibs and Lothar (2003) reported that Zn enhances proliferation ability of B-cells, pre B-cell and immature B-cell.

Concerning the blood calcium and phosphorus concentrations, they are significantly increased for birds fed LCP enriched with Met and Lys or supplied with Vit D_3 or both levels of Zn with or without Vit D_3 compared with the control group. As previously indicated, there is little collaborative data on the response of blood Ca and P in the blood of aged chickens due to amino acids or Zn supplementation as Abdel-Azeem and El-Shafei (2006) reported that plasma calcium was significantly improved when the Vit D_3 increased from 2000 to 3000 IU/kg in laying quail diet.

IN CONCLUSION,

feeding Gimmizah chickens LCP (13%) diet enriched with Met (0.6 g/kg diet) and Lys (1.4 g/kg diet) alone is adequate for achieving the optimal productive and reproductive performance besides best record of net returns during the late laying period.

Table (1): Ingredient and calculated composition of the experimental diets for Gimmizah chickens

Ingredients	Basal diet Kg/ton (D1)	LCP diet Kg/ton (D2)	Enriched LCP diet Kg/ton (D3)	Calculated composition*			
					D1	D2	D3
Yellow corn,	670.0	704.0	704.0	ME, kcal/Kg	2750	2750	2750
Soybean meal, (44%)	215.0	154.0	154.0	CP, %	15.0	13.0	13.0
Wheat Bran	8.0	34.0	31.8	Lysine, %	0.73	0.64	0.73
Limestone	81.0	81.27	81.5	Methionine, %	0.33	0.28	0.36
Dicalcium phosphate	18.0	18.5	18.7	TSAA,%	0.59	0.51	0.59
Premix**	3.0	3.0	3.0	Calcium, %	3.18	3.19	3.19
NaCl,	3.0	3.0	3.0	Avail. Phos. %	0.46	0.46	0.46
DL.Methionine	1.0	0.78	1.6	Zinc, mg/kg	71	71	71
L.Lysine HCl	0.0	0.45	1.4				
Choline Chloride	1.0	1.0	1.0				
Total	1000	1000	1000				

*Calculated values were according to NRC (1994) text book values for feedstuffs.

**Provided the following per kg of diet:Vit. A, 1200 IU; Vit. D. 2000 IU; Vit. E, 100 IU; Vit. C, 3 mg; Vit. K, 4 mg; VitB1, 3 mg; Vit B2, 3 mg; Vit B6, 5 mg; Vit B12, 0.03 mg; Bantothinic acid, 15 mg; Folic acid, 2 mg; Biotin, 0.20 mg; Cobalt, 0.05 mg; Copper, 10 mg; Iodin, 50 mg; Manganese, 90 mg; Selenium, 0.20 mg and Zinc, 50 mg.

Table (2): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 on productive performance of Gimmizah chickens during late laying period

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine						SEM	P Value
			Only	+Zn 50 mg /kg diet	+Zn 100 mg /kg diet	+Vit D_3 2000 IU /kg diet	+Zn50mg + Vit D_3 2000 IU /kg diet	+Zn100mg + Vit D_3 2000 IU /kg diet		
Initial BW (52 WK),g	1851	1827	1877	1836	1866	1878	1848	1854	88.5	0.6798
Final BW (64 WK),g	1953	1922	1979	1940	1977	1992	1957	1962	95.6	0.5236
Change of BW (52-64 WK),g	102	95	102	104	110	114	109	108	47.2	0.9726
Egg production %	62.3^{ab}	56.7^{c}	64.6^{a}	59.6^{bc}	59.4^{bc}	65.0^{a}	58.7^{bc}	60.7^{b}	2.49	0.0236
Egg weight, g	53.7^{a}	51.1^{b}	55.3^{a}	54.6^{a}	55.3^{a}	53.9^{a}	54.1^{a}	54.5^{a}	0.837	0.0006
Egg mass, g/h/d	33.5^{ab}	29.0^{c}	35.7^{a}	32.5^{ab}	32.8^{ab}	35.1^{ab}	31.8^{bc}	33.0^{ab}	1.55	0.0060
Feed intake, g/h/d	126	131	127	131	130	132	128	129	3.98	0.8009
FCR, g feed/g egg	3.92^{b}	4.51^{a}	3.56^{b}	4.03^{ab}	3.96^{b}	3.81^{b}	4.05^{ab}	3.93^{b}	0.223	0.0179

^{a,b,c,d} means having different superscripts in the same row are significantly different ($P<0.05$).

SEM: standard error of means, P value: probability level, CP: crude protein, LCP: Low crude protein, BW: body weight, FCR: feed conversion ratio.

Table (3): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 on egg quality of Gimmizah chickens during late laying period

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine						SEM	P Value
			Only	+Zn 50mg /kg diet	+Zn 100mg /kg diet	+Vit D_3 2000 IU /kg diet	+Zn50mg + Vit D_3 2000 IU /kg diet	+Zn100mg + Vit D_3 2000 IU /kg diet		
Egg shape index	78.2	75.8	77.0	77.9	76.6	75.1	77.4	75.7	1.663	0.5160
Specific gravity, g/cm^3	1.083	1.077	1.081	1.084	1.083	1.089	1.082	1.084	0.0032	0.0627
Shell weight %	8.88^c	9.00^c	9.25^b	10.51^a	10.03^{ab}	10.36^a	9.97^{ab}	10.22^a	0.4309	0.0013
Shell thickness, mm	0.331^d	0.348^{cd}	0.367^{abc}	0.363^{abc}	0.355^{bc}	0.385^a	0.387^a	0.379^{ab}	0.0271	0.0001
SWUSA*, mg/cm^2	72.7^c	74.0^c	76.4^b	87.9^a	82.6^{ab}	84.5^a	81.8^{ab}	83.9^a	3.230	0.0002
Yolk weight %	36.0	34.1	37.7	36.9	37.5	37.8	37.9	37.7	1.722	0.3715
Albumen weight %	55.1	56.9	53.0	52.6	52.5	51.8	52.2	52.1	1.897	0.1283
Yolk dry matter %	16.9^{bc}	15.2^c	17.9^{ab}	17.4^{ab}	18.0^{ab}	19.3^a	17.8^{ab}	18.3^{ab}	1.020	0.0217
Yolk index	52.5	47.7	50.5	50.4	49.1	50.7	50.8	51.2	1.497	0.1224
Yolk color	5.33^b	6.67^a	6.50^a	6.33^a	6.50^a	6.67^a	6.33^a	6.33^a	0.3890	0.0393
Haugh unit score	74.7^{ab}	68.2^b	82.4^a	83.7^a	83.0^a	80.6^a	80.4^a	82.1^a	4.722	0.0316

^{a,b,c,d} means having different superscripts in the same row are significantly different (P<0.05).
SEM: standard error of means, P value: probability level, CP: crude protein, LCP: Low crude protein.
*SWUSA: shell weight per unit of surface area.

Table (4): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 on hatching traits of Gimmizah chickens during late laying period

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine						SEM	P Value
			Only	+Zn 50mg /kg diet	+Zn 100mg /kg diet	+Vit D_3 2000IU /kg diet	+Zn50mg + Vit D_3 2000 IU /kg diet	+Zn100mg + Vit D_3 2000 IU /kg diet		
Fertility %	88.0^c	83.0^d	89.6^{bc}	92.1^a	91.9^a	88.5^{bc}	88.2^c	89.9^b	0.7563	0.0001
Hatchability of fertile eggs %	90.4^b	87.7^c	92.8^a	93.3^a	93.0^a	91.3^{ab}	91.4^{ab}	92.4^{ab}	0.9695	0.0001
Hatchability of total eggs %	79.5^d	72.7^e	81.8^{bc}	84.2^a	84.9^a	80.8^{cd}	82.4^{bc}	83.5^{ab}	0.8438	0.0001
Hatched chick weight %	66.0^{cd}	65.0^d	69.5^a	68.7^{ab}	69.5^a	67.6^{bc}	67.5^{bc}	65.9^{cd}	0.7457	0.0001

^{a,b,c,d,e} means having different superscripts in the same row are significantly different (P<0.05).
SEM: standard error of means, P value: probability level, CP: crude protein, LCP: Low crude protein.

Table (5): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 on hematological parameters and some immunological traits of Gimmizah chickens during late laying period

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine						SEM	P Value
			Only	+Zn 50mg /kg diet	+Zn 100mg /kg diet	+Vit D_3 2000IU /kg diet	+Zn50mg + Vit D_3 2000 IU /kg diet	+Zn100mg + Vit D_3 2000 IU /kg diet		
RBCs, $x10^6/mm^3$	2.40	2.35	2.42	2.38	2.39	2.70	2.56	2.69	0.1401	0.0768
Hgb, g/dl	10.66^b	10.84^b	10.30^b	10.92^b	10.54^b	12.36^a	12.13^a	12.30^a	0.4290	0.0001
PCV %	27.13^c	26.80^c	28.06^c	31.67^b	30.72^b	35.20^a	34.72^a	35.00^a	1.205	0.0001
WBCs, $x10^3/mm^3$	23.46^{cd}	22.92^d	24.03^{bcd}	24.86^{bc}	25.20^{abc}	24.93^{bc}	25.50^{ab}	26.80^a	0.7899	0.0009
Lymphocyte %	41.2^{cd}	39.4^d	41.2^{cd}	44.0^{bc}	43.2^{bc}	45.0^{ab}	46.2^{ab}	48.0^a	1.605	0.0001
Heterophil %	24.46	24.73	25.03	26.62	26.06	26.10	26.76	27.66	1.378	0.2848
H/L ratio	60.80	62.82	60.78	60.36	60.88	57.84	58.28	58.10	4.569	0.9522
Phagocytic Activity %	17.0^d	16.3^d	18.0^{cd}	21.7^{ab}	21.0^{ab}	20.0^b	22.3^a	20.7^{ab}	1.008	0.0001
Phagocytic Index %	1.41^{cd}	1.39^d	1.45^{bcd}	1.70^a	1.61^{abc}	1.59^{abc}	1.68^{ab}	1.63^{abc}	0.1039	0.0223

^{a,b,c,d} means having different superscripts in the same row are significantly different ($P<0.05$).
SEM: standard error of means, P value: probability level, CP: crude protein, LCP: Low crude protein.

Table (6): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 some blood biochemical constituents of Gimmizah chickens during late laying period

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine						SEM	P Value
			Only	+Zn 50mg /kg diet	+Zn 100mg /kg diet	+Vit D_3 2000IU /kg diet	+Zn50mg + Vit D_3 2000 IU /kg diet	+Zn100mg + Vit D_3 2000 IU /kg diet		
Glucose, (mg/dl)	187	190	194	190	186	188	185	184	37.5	0.1888
Total protein, (g/dl)	4.66^a	3.58^b	4.59^a	4.62^a	4.52^a	4.61^a	4.65^a	4.49^a	0.503	0.0001
Albumin, (g/dl)	2.71^a	2.12^b	2.65^a	2.61^a	2.59^a	2.63^a	2.60^a	2.62^a	0.118	0.0008
Globulin, (g/dl)	1.96^{ab}	1.46^c	1.94^{ab}	2.01^{ab}	1.93^{ab}	1.98^{ab}	2.04^a	1.87^b	0.069	0.0001
Total lipids, mg/dl	392^b	455^a	405^b	381^b	391^b	405^b	412^{ab}	393^b	7.67	0.0435
Triglycerides, mg/dl	142^{cd}	163^a	146^{bcd}	142^{cd}	138^d	153^b	150^{bc}	148^{bcd}	4.58	0.0002
Cholesterol, mg/dl	138^b	148^a	128^c	128^c	132^{bc}	127^c	134^{bc}	130^c	3.25	0.0001
HDL, mg/dl	49.72^c	50.23^{bc}	55.87^a	53.66^{ab}	54.94^a	54.02^{ab}	56.11^a	57.70^a	1.79	0.0007
LDL, mg/dl	59.59^a	64.86^a	42.79^b	46.29^b	49.61^b	42.09^b	48.06^b	43.09^b	4.26	0.0001
AST, U/L	34.7	33.6	32.5	33.7	34.8	35.1	33.9	32.7	1.25	0.3467
ALT, U/ L	15.92	15.5	14.1	15.02	14.82	14.3	15.7	14.82	0.660	0.0999
Urea, mg/dl	27.37^a	25.27^b	24.87^b	23.53^c	24.77^b	21.60^d	20.30^e	20.73^{de}	0.493	0.0001
Creatinine, mg/dl	0.926^a	0.893^{ab}	0.843^{bc}	0.848^{bc}	0.863^{abc}	0.869^{abc}	0.807^c	0.820^{bc}	0.035	0.0297
MDA, Mmol/dL	1.180^a	1.117^a	0.919^b	0.778^c	0.800^c	0.849^{bc}	0.766^c	0.752^c	0.053	0.0001
TAC, Mmol/dL	396^b	405^b	413^b	460^a	458^a	449^a	464^a	461^a	11.61	0.0001
SOD, U/dl	221^b	218^b	225^b	264^a	258^a	255^a	269^a	267^a	9.06	0.0001
Calcium, mg /dl	22.51^d	23.32^{cd}	23.89^{bc}	24.49^b	24.53^b	25.85^a	25.61^a	26.01^a	0.484	0.0001
Phosphorous, mg/dl	6.40^b	6.85^a	7.00^a	7.08^a	6.84^a	7.28^a	7.11^a	7.28^a	0.199	0.0023

^{a,b,c,d} means having different superscripts in the same row are significantly different ($P<0.05$). SEM: standard error of means, P value: probability level, CP: crude protein, LCP: Low crude protein.

Table (7): Influence of reduced crude protein enriched with amino acids and different dietary levels of zinc with or without vitamin D_3 on economical efficiency (EE) and relative economical efficiency (REE) of experimental treatments

Criteria	Control CP (15%)	LCP (13%)	LCP (13%) diet enriched with methionine and lysine					
			Only	+Zn 50mg /kg diet	+Zn 100mg /kg diet	+Vit D_3 2000IU /kg diet	+Zn50mg +Vit D_3 2000 IU /kg diet	+Zn100mg +Vit D_3 2000 IU /kg diet
Egg production %	62.3	56.7	64.6	59.6	59.4	65	58.7	60.7
Total egg produced (EP% X 84d)[1]	52.3	47.6	54.3	50.1	49.9	54.6	49.3	51.0
Egg price (LE)[2]	68.0	61.9	70.5	65.1	64.9	71.0	64.1	66.3
FI /day/ hen (g)[3]	126	131	127	131	130	132	128	129
Total FI /hen Kg(3X84day)[4]	10.6	11.0	10.7	11.0	10.9	11.1	10.8	10.8
Price of kg diet (LE)[5]	3.82	3.7	3.71	3.71	3.72	3.72	3.73	3.74
Total feed cost/hen(4X5, LE)[6]	40.4	40.7	39.6	40.8	40.6	41.2	40.1	40.5
Net Revenue (2-6, LE)	27.6	21.2	31.0	24.3	24.2	29.7	24.0	25.8
Economical efficiency, EE	0.68	0.52	0.78	0.59	0.60	0.72	0.60	0.64
Relative economical efficiency, REE	100	76.3	114.6	87.0	87.4	105.6	87.6	93.1

[2]Price of 30 eggs = 38 LE, Economical efficiency (E.E) = (Net Revenue / Total feed cost) *100
Relative economical efficiency (REE), assuming control treatment = 100 %.

REFERENCES

Abd El-Maksoud, A. 2010. Effect of dietary calcium and vitamin D_3 levels on egg production and egg shell quality of hy-line brown - egg type laying hens. Egypt. Poult. Sci. Vol (30) (IV): (1097-1120).

Abdel-Azeem, F.A.; and El-Shafei, A.A. 2006. Effect of different levels of cholecalciferol (vitamin D_3) on performance and skeletal bone formation of laying Japanese quail reared under hot climate region. Egypt Poult. Sci., 26: 221-234.

Aksu, D.S.; Aksu, T.; and Ozsoy, B. 2010. The effects of lower supplementation levels of organically complexed minerals (zinc, copper and manganese) versus inorganic forms on hematological and biochemical parameters in broilers. Kafkas Universitesi Veteriner Fakultesi Dergisi., 16: 553-559.

Alagawany, M.; Abd El-Hack, M.E.; Farag, M.R.; Tiwari, R.; Sachan, S.; Karthik, K.; and Dhama, K. 2016. Positive and negative impacts of dietary protein levels in laying hens. Asian Journal of Animal Science., 10: 165 – 174.

Ameenuddin, S.; Sunde, M.; DeLuca, H.F.; Ikekawa, N.; and Kobayashi, Y. 1982. 24-hydroxylation of 25-hydroxyvitamin D_3 is it required for embryonic development in chicks. Poult. Sci., 217: 451-452.

Andrade, L. 2003. Performance and egg quality of laying hens in the first and second production cycles fed different levels of crude protein and amino acids in the [masters] ration. Goiânia (GO): Federal University of Goiás.

Atakisi, O.; Atakisi, E.; and Kart, A. 2009. Effects of dietary zinc and L-arginine supplementation on total antioxidants capacity, lipid peroxidation, nitric oxide, egg weight and blood biochemical values in Japanese quails. Biol. Trace. Elem. Res., 132: 136-143.

Badawy, E.M.; Edrise, B.M.; and Al-Wakeel, A.M. 1987. The relationship between quality, egg constituents and hatchability of the eggs of Hubbard broiler breeders, a field study. Journal of Veterinary Medicine., 35 (1):105-115.

Bahakaim, A.S.A.; Abdel Magied, H.A.; Osman, S.M.H.; Omar, A.S.; Abdelmalak, N.Y.; and Ramadan, N.A. 2014. Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment .Egyptian Poultry Science Journal., 34 (I): 39-56

Banerjee, G.C. 1988. Feeds and Principles of Animal Nutrition. 2nd Edn., Oxford and IBH Publishing Co. PVT Ltd., New Delhi..

Bao,Y.M.; Romero, L.F.; and Cowieson, A.J. 2013. Functional patterns of exogenous enzymes in different feed ingredients. World's Poultry Science Journal., 12: 759-773.

Bar, A. 2008. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds Comp Biochem Physiol., 151(4):477-490.

Bar, A.; Striem, S.; Rosenberg, J.; and Hurwitz, S. 1988. Egg shell quality and cholecalciferol metabolism in aged laying hens. J. Nutr., 18: 1018-1023.

Batal, A. B.; Parr, T. M.; and Baker, D. H. 2001. Zinc bioavailability in tetra basic zinc chloride and the dietary zinc requirement of young chicks fed soy concentrate diet. Poult. Sci., 80:87-90.

Baum, M.K.; Shor-Posner, G.; and Campa, A. 2000. Zinc Status In Human Immunodeficiency Virus Infection. The American Society for Nutritional Science. 9pp.

Bedwal, R. S.; and Bahuguna, A. 1994. Zinc, copper and selenium in reproduction. Experientia., 50: 626–640.

Bouyeh, M.; and Gevorgian, O.X. 2011. Influence of different levels of lysine, methionine and protein on the performance of laying hens after peak. J. Anim. Vet. Adv., 10: 532-537.

Bunchasak, C.; Poosuwan, K.; Nukraew, R.; Markvichitr, K.; and Choothesa, A. 2005. Effect of dietary protein on egg production and immunity responses of laying hens during peak production period. Int. J. Poult. Sci., 4: 701-708.

Carter, T.C.; and Jones, R.M. 1970. The hen's egg shell shape parameters and their interrelations. Br. Poult. Sci., 11: 179-187.

Cole, C.R.; and Lifshitz, F. 2008. Zinc nutrition and growth retardation. Paediatric Endocrinology Reviews, 5(4), 889-96.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics, 11:1–42.

El-Husseiny, O.M.; Abdallah A.G.; and Abdel-Latif, K.O. 2008. The influence of biological feed additives on broiler performance. Int. J. Poult. Sci., 7: 862-871.

Feed Composition for Animal and Poultry Feedstuffs in Egypt 2001. Technical bulletin. Central lab. For Feed and food; Ministry of Agriculture.

Frost, J.J.; and Roland, D.A.S.R. 1990. Influence of vitamin D_3, 1α-Hydroxyvitamin D3 and 1,25-dihydroxyvitamin D_3 on egg shell quality, Tibia strength, and various production in commercial laying hens. Poultry Sci., 69: 2008 – 2016.

Funk, E.M. 1948. The relation of the yolk index determined in natural position to the yolk index as determined after separating the yolk from the albumen. Poult. Sci., 27: 367.

Gerzilov, V.; Nikolov, A.; Petrov, P.; Bozakova, N.; Penchev, G.; Bochukov, A. 2015. Effect of a dietary herbal mixture supplement on the growth performance, egg production and health status in chickens. Journal of Central European Agriculture.,16(2).

Ghasemi, R.; Torki, M.; and Ghasemi, H.A. 2014. Effects of dietary crude protein and electrolyte balance on production parameters and blood biochemicals of laying hens under tropical summer condition. Trop. Anim. Health Prod., 46: 717-723.

Gheisar, M.M.; Foroudi, F.; and Shad, A.G. 2011. Effect of using L-Threonine and reducing dietary levels of crude protein on egg production in layers. Iranian Journal of Applied Animal Science., 1(1), 65-68.

Guo, Y.M.; Yang, R.; Yuan, J.; Ward, T.L.; and Fakler, T.M. 2002. Effect of Availa Zn and $ZnSO_4$ on laying hen performance and egg quality. Poultry Science, 81 (Suppl.), 40.

Haugh, R.R. 1937. The Haugh units for measuring egg quality. Poult. Magazine, 43: 552-575.

Hawkey, C.M.; and Dennett, T.B. 1989. A color atlas of comparative veterinary hematology. Wolf Publishing limited, London, England.

Herzig, J.; Navratilova, M.; Totusek, J.; Suchy, P.; Vecerek, V.; Blahova, J.; and Zraly, Z. 2009. The effect of humic acid on zinc accumulation in chicken broiler tissues. Czech Journal of Animal Science, 54, 121–127.

Hsu, J.C.; Lin, C.Y.; and Chiou, P.W. 1998. Effects of ambient temperature and methionine supplementation of a low protein diet on the performance of laying hens. Animal Feed Science and Technology., 74: 289-299.

Hunton, P. 2005. Research on eggshell structure and quality: an historical overview. Braz. J. Poult. Sci., 7:67-71.

Ibs, K.H.; and Lothar, R. 2003. Zinc-altered immune function. J. Nutr., 133:1452S–1456S.

Ji, F.; Fu, S.Y.; Ren, B.; Wu, S.G.; and Zhang, H.J. 2014. Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. J. Applied Poult. Res., 23: 384-392.

Junqueira, O.M.; de Laurentiz, A.C.; da Silva Filardi, R.; Rodrigues, E.A.; and Casartelli, E.M.C. 2006. Effects of energy and protein levels on egg quality and performance of laying hens at early second production cycle. J. Applied Poult. Res., 15: 110-115.

Kashani, S.; Mohebbifar, A.; Habibian, M.; and Torki, M. 2014. Effects of phytase supplementation of low protein diets on performance, egg quality traits and blood biochemical parameters of laying hens. Annual Research and Review in Biology.,4(4), 684-698.

Khajali, F.; Khoshouie, E.A.; Dehkordi, S.K.; and Hematian, M. 2008. Production performance and egg quality of Hy-line W36 laying hens fed reduced-protein diets at a constant total sulfur amino acid: Lysine ratio. J. Applied Poult. Res., 17: 390-397.

Koreleski, J.; and Swiatkiewicz, S. 2005. Efficacy of different levels of a cholecalciferol 25-OH-derivative in diets with two limestone in laying hen nutrition. Journal of Animal and Feed Sci., 14: 305-315.

Kucuk, O.; Kahraman, A.; Kurt, I.; Yildiz, N.; and Onmaz, A. C. 2008. A combination of zinc and pyridoxine supplementation to the diet of laying hens improves performance and egg quality. Biol. Trace Elem. Res., 126:165–175.

Kucuk, O.; Sahin, N.; and Sahin, K. 2003. Supplemental zinc and vitamin A can alleviate negative effects of heat stress in broiler chickens. Biol. Trace Elem. Res., 94:225–235.

Leijh, P.C.; Van Furth, R.; and Van Zwet, T.L. 1986. In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In: Handbook of Experimental Immunology, Weir M.D., Herzenberg L.A., Blackwell C. (eds). Oxford, Blackwell Scientific Publications, pp. 1–21.

Lelis, G.R.; Albino, L.F.T.; Tavernari, F.C.; Calderano, A.A.; Rostagno,

H.S.; Barros, V.R.S.M. and Maia, R.C. 2014. Digestible valine to digestible lysine ratios in brown commercial layer diets. J Appl Poult Res., 23: 683-690.

Lucisano, S.; Di Mauro, E.; Montalto, G.; Cernaro, V.; Buemi, M.; and Santoro, D. 2014. Vitamin D and anemia. J Ren Nutr., 24(1):61–62.

Mabe, I.; Rapp, C.; Bain, M.M.; and Nys, Y. 2003. Supplementation of corn soybean meal diet with manganese, copper and zinc from organic or inorganic sources improves egg shell quality in aged laying hens. Poult. Sci., 82: 1903-1913.

Meguro, S.; Tomita, M.; and Katsuki, T. 2011. Plasma 25-hydroxyvitamin D is independently associated with hemoglobin concentration in male subjects with type 2 diabetes mellitus. Int J Endocrinol.

Mousavi, S.N.; Khalaji, S.; Ghasemi-Jirdehi, A.; and Foroudi, F. 2013. Investigation on the effects of dietary protein reduction with constant ratio of digestible sulfur amino acids and threonine to lysine on performance, egg quality and protein retention in two strains of laying hens. Italian J. Animal Sci., 12: e2; 9-15.

Moustafa.; Kout El-Kloub.; Hassan, R.A.; El.Ganzory, E.H.; and El-Abd, E.A. 2004. Effect of different sources and levels of zinc on the performance of local laying hens. Egypt. Poult. sci., 24 (ii): 369-385.

Newman, S.; and Leeson, S. 1997. Skeletal integrity in layersat the completion of egg production. World's Poult. Sci. J., 53:265–277.

Novak, C. L.; Yakout, H. M.; and Remus, J. 2008. Response to varying dietary energy and protein with or without enzyme supplementation on leghorn performance and economics. 2. Laying period. J. Appl. Poult. Res. 17:17-33.

Novak, C.; Yakout, H.M.; and Schideler, E. 2006. The effect of dietary protein level and total amino acid:lysine ratio on egg production parameters and egg yield in Hy-Line W-98 hens. Poult. Sci., 85:2195-2206.

NRC. National Research Council. 1994. Nutrient requirements of poultry (9th ed.).National Academy Press Washington, D. C.

Odabasi, A.Z.; Miles, R.D.; Balaban, M.O.; and Portier, K.M. 2007. Changes in brown eggshell color as the hen ages. Poult. Sci., 86: 356-363.

Panda, A.K.; Rama Rao, S.V.; Raju, M.V.L.N.; Niranjan, M.; and Reddy, B.L.N. 2006. Influence of Supplemental Vitamin D_3 on Production Performance of Aged White Leghorn Layer Breeders and Their Progeny. Asian-Aust. J. Anim. Sci.,19:1638 – 1642.

Rizk, R.E.; Nadia A. EL-Sayed; Shahein, E.H.A.; and Hedaia, M.S. 2008. Relationship between eggshell membranes and embryonic development through different egg production periods in two developed chicken strains. Egy. Poult.Sci., 28:535-551.

Saki, A.A.; Zamani, P.; Haghi, M.; Aliarabi, H.; and Malecky, M.; *et al.*, 2015. Molecular aspects, blood parameters, bone mechanical properties and performance of laying hens in response to various levels of methionine and protein. Revue Medecine Veterinaire., 166: 47-53.

SAS Institute, 1996. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC

Schutte, J. B.; De Jong, J.; and Bertram, H.L. 1994. Requirement of the laying hen for sulfur amino acids. Poult. Sci., 73:274–280

Silva, M.F.R; Faria, D.M.; Rizzoli, P.W.; Santos, A.L.; Sakamoto, M.I.; and Souza, H.R.B. 2010. Performance, egg quality and nitrogen balance of commercial laying hens fed diets containing different levels of crude protein and lysine. Brazilian Journal of Animal Science., 39(6):1280-1285.

Solomons, N.W. 1998. Mild human zinc deficiency produces an imbalance between cell-mediated and humoral immunity. Nutr Rev., 56: 27-8.

Stevenson, M.H. 1985. Effect of added cassava root meal, ZnO and their interaction on the production and quality of eggs from laying hens. J. Sci. Food Agric.36:909–914.

Swiatkiewicz, S.; and Koreleski, J. 2008. The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. Vet. Med.53: 555-563.

Tavernari, F.C.; Lelis, G.R.; Vieira, R.A.; Rostagno, H.S.; Albino, L.F.T. and Neto, A.R.O. 2013. Valine needs in starting and growing Cobb (500) broilers. Poult Sci, 92: 151-157.

Tomas Parak.; and Eva Strakova., 2011. Zinc as a feed supplement and its impact on plasma cholesterol concentrations in breeding cocks. Acta Vet. Brno., 80: 281-285.

Torki, M.; Mohebbifar, A.; Ghasemi, H.A.; and Zardast, A. 2015. Response of laying hens to feeding low-protein amino acid-supplemented diets under high ambient temperature: Performance, egg quality, leukocyte profile, blood lipids and excreta pH. Int. J. Biometeorol., 59: 575-584.

Underwood, E.J.; and Suttle, N.F. 1999. In: The Mineral Nutrition of Livestock 3rd Ed. CABI Publishing, CAB International, Wallingford, Oxon, UK.

Wu, G.; Liu, Z.; and Roland, D.A.S.R. 2005. Performance comparison and nutritional requirements of five commercial layers strains in phase V. Int. J. Poult. Sci., 4: 182-186.

Yenice, E.; Mizrak, C.; Gultekin, M.; Atik, Z.; and Tunca, M. 2015. Effects of organic and inorganic forms of manganese, zinc, copper, and chromium on bioavailability of these minerals and calcium in late-phase laying hens. Biological Trace Element Research: DOI 10.1007/s12011-015-0313-8

Zamani, A.; Rahmani, H.R.; and Pourreza, H.R. 2005. Eggshell quality is improved by excessive dietary zinc and manganese. In: Proceedings 15 th European Symposium on Poultry Nutrition, 25–29 September, Balatonfured, Hungary., 520–522.

Zeweil, H.S.; Abdalah, A.A.; Ahmed, M.H.; and Marwa, R.S.A. 2011. Effect of different levels of protein and methionine on performance of baheij laying hens and environmental pollution. Egypt. Poult. Sci., 31: 621-639.

Zita, L.; Tůmová, E.; and Štolc, L. 2009. Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. Acta Veterinaria Brno., 78: 85–91.

الملخص العربى

"تأثير خفض مستوى البروتين على أداء دجاج الجميزة خلال المرحلة الاخيرة من الانتاج."
2- بـإضـافـة الاحمـاض الامنيـــة والزنك وفيتامين د3

بـهاء محمـد السيـد أبـوشحيمـة
معهد بحوث الإنتاج الحيواني – مركز البحوث الزراعية – مصر

أجريت هذة التجربـة لدراسـة تـأثير خفض مستوى البروتين واستخدام مستويات مختلفة من الزنك وفيتامين د3 بالعلائق على أداء الدجاج البياض خلال المرحلة الاخيرة من الانتاج. استخدم فى هذة التجربة عدد 320 طائر (280 دجاجة و 40 ديك) عمر 52 اسبوع من سلالة الجميزة. تم وزن الطيور فرديا وقسمت عشوائيا الى ثمانية مجموعات كل مجموعة تتكون من خمس مكررات فى عنبر يعمل بالنظام المفتوح (7 دجاجات وديك لكل مكررة) حتى نهاية التجربة عند 64 اسبوع. أستخدمت المجموعة الأولي كمجموعة مقارنة (كنترول) وتم تغذيتها علي العليقة الأساسية (15 % بروتين خام) ، غذيت المجموعة الثانية علي عليقة منخفضة فى محتواها من البروتين الخام (13 % بروتين خام)، غذيت المجموعة الثالثة على العليقة المقدمة للمجموعة الثانية مع اضافة المثيونين (0,6 جم/كجم علف) والليسين (1,4 جم/كجم علف) ، غذيت المجموعتين الرابعة والخامسه على نفس العليقة المقدمة للمجموعة الثالثة مضافا إليها الزنك بمعدل 50 ، 100 مليجرام/كجم علف علي الترتيب، كذلك غذيت المجموعة السادسة على نفس العليقة المقدمة للمجموعةالثالثة مضافا إليها فيتامين د3 بمعدل 2000 وحدة دولية/كجم علف ، بينما غذيت المجموعتين السابعة والثامنه على نفس العليقة المقدمة للمجموعة الثالثة مضافا إليها الزنك بمعدل 50 مليجرام/كجم علف مع فيتامين د3 بمعدل 2000 وحدة دولية/كجم علف ' الزنك بمعدل 100 مليجرام/كجم علف مع فيتامين د3 بمعدل 2000 وحدة دولية/كجم علف على الترتيب. ويمكن إيجاز النتائج المتحصل عليها فيما يلى :

أدى تغذية الطيور على عليقة منخفضة بالبروتين إلى إنخفاض معنوى فى كلا من إنتاج و وزن و كتلة البيض وتدهور فى الكفاءة التحويلية للعلف مقارنة بالمجموعة المقارنة. بينما أدي تغذية الطيورعلى العليقة المنخفضة فى البروتين والغنية فى محتواها من المثيونين و الليسين فقط أو مضاف إليها فيتامين د3 أو الزنك مع او بدون فيتامين د3 الى تحسن معنوي فى تلك الصفات سالفة الذكر لدرجة انها لم تختلف معنويا عن مجموعة المقارنة. أظهرت نتائج وزن وسمك القشرة ووزن القشرة بالنسبة لوحدة المساحة وكذلك النسب المئوية لوزن الكتاكيت والتفريخ للدجاج المغذى على العليقة المنخفضة من البروتين والغنية فى محتواها من المثيونين و الليسين فقط أو مضاف اليها الزنك بمعدلية تحسنا معنويا مقارنة بالمجموعة المقارنة. كذلك أظهرت الدجاجات المغذاة على العليقة المنخفضة فى البروتين والغنية فى محتواها من المثيونين و الليسين ومضافا اليها الزنك مع فيتامين د3 تحسنا معنويا فى تركيز الهيموجلوبين وحجم كرات الدم وعدد كرات الدم البيضاء والخلايا الليمفاويه وكذلك النشاط البلعمى والكولستيرول منخفض الكثافة والكولستيرول عالى الكثافة وكفاءة مضادات الاكسدة وانزيم سوبر اوكسيد دسميوتيز ودليل أكسدة الدهون (المالونالدهيد) والكالسيوم مقارنة بمجموعة المقارنة أو المجموعة المغذاة على عليقة منخفضة فى محتواها من البروتين فقط. من ناحية اخرى فان مستوى البروتين، الجلوبيولين، الالبيومين، الدهون الكلية ، الجلسيريدات الثلاثية بالنسبة للدجاجات المغذاة على علائق منخفضة فى محتواها من البروتين والغنيه فى محتواها من المثيونين والليسين فقط او مضافا اليها الزنك بمعدلية مع أو بدون فيتامين د3 لم يختلف معنويا عن مجموعة المقارنة .

الخلاصة:- تغذية دجاج الجميزة البياض خلال الفترة الاخيرة من انتاج البيض على علائق منخفضة فى محتواها من البروتين الخام (13% بروتين خام) مضافا اليها المثيونين والليسين فقط يمكن أن يكون كافيا للحصول على أفضل اداء انتاجى وتناسلى وكذلك أعلي كفاءة اقتصادية .