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Effect of Dietary Supplementation with Different Levels of Arginine on Some Blood Traits of Laying Hens

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Abstract: This experiment was carried out at the Field of Poultry, Department of Animal Resources, College of Agriculture, University of Baghdad during the period from 1/5/2011 until 5/7/2011 to study the effect of adding arginine to laying hens diet on certain blood traits. A total of 100 Brown Lohmann laying hens chicken, 38 weeks of age were randomly distributed on four treatment groups with 25 hens for each treatment. Treatment groups were: T1: Bird fed diet with no additional arginine (control group); T2, T3 and T4: Birds fed diet supplemented with 0.4, 0.7 and 0.9% respectively. Therefore, the total amounts of arginine in the four treatments (T1, T2, T3 and T4) become 1.1, 1.5, 1.8, 2.0% respectively. Results of this experiment revealed that there were no significant differences ($p \geq 0.05$) between treatment groups regarding the total of Red Blood Cells Count (RBC) as well as with respect to each of the Hemoglobin concentration (Hb) and Packed Cell Volume (PCV). It was also noticed that there were no significant differences ($p \geq 0.05$) between treatment groups concerning each of Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). Moreover, results of this study also denoted that supplementing ration of laying hens with different levels of arginine (T2, T3 and T4) resulted in significant increase ($p \leq 0.05$) in total White Blood Cells Count (WBC) and percentage of Heterophil (H) cells and significant decrease ($p \leq 0.05$) respecting percentages of Lymphocyte (L) cells and monocyte cells and H/L ratio as compared with control group (T1). In conclusion, adding arginine to the diet of laying hens at levels higher than the levels recommended by the NRC did not have a negative effect on physiological performance of birds, as indicated by the non significant differences between treatment groups as regards RBC, PCV, Hb, MCV, MCH and MCHC. However, supplementing arginine to the diet of laying hens resulted in enhancement of immune response as indicated by significant increase in WBC in comparison with control group. On the other hand, adding arginine to laying hens ration didn't cause any stress on birds as indicate by the significant decrease in H/L ratio as compared with control group.

Key words: Arginine, blood traits, laying hens

INTRODUCTION

Arginine is one of amino acids called α-amino acids (2-amono-5-diaminomethylidene amino pentanoic) and its shape is L-form of the most shapes of 20 in his nature. Chemical structure of this amino acid is $C_6H_{14}N_4O_2$ and it is one component in the mRNA composition and enters into the genetic code and share with another 19 amino acid in the interactions of protein synthesis (IUPAC-IUBMB, 2007). Tapiero *et al*. (2002) found that L-Arginine is one of the most essential amino acids and reliable through the different development stages (Wu *et al*., 2004) through the need to give arginine to premature babies as the urgent need to develop. Arginine manufactured in mammals through citrulline interactions, where there is interaction in presence of cytosolic enzymes of reactions to Argininosuccinate Synthetase (ASS) and Argininosuccinate Lyase (ASL), which requires the presence of ATP and it becomes a AMP (IUPAC-IUBMB, 2007). Tamir and Ratner (1963) have been noted the differences in the metabolism of arginine in birds compared to mammals, experiments showed that these enzymes that are found in the liver of mice differ from those found in birds, where L-Arginine is a source of enzymes Nitric Oxide Synthase (NOS) is release to form Nitric Oxide (NO), which has a big role in the way of blood transfusion in the blood vessels (Biliar, 1995) and arginine play important role in cell division and healing of wounds and immune effectiveness and hormones secretion (Tapiero *et al*., 2002; Stechmiller *et al*., 2005). Snyder *et al*. (1956) and Wietlake *et al*. (1954) reported that arginine is not synthesized by the birds' liver, but it should add to the feed. Several nutrition experiments were conducted to add arginine to birds' diets (Abdel-Maksoud *et al*., 2010; Martin *et al*., 2010; Veldkamp *et al*., 2000), but all these experiments are based on the basis of NRC requirements of arginine. Therefore, the present study were conducted to evaluate the effect of dietary supplementation with different levels of arginine which have been above the levels recommended by NRC on certain blood characteristics of laying hens.

Table 1: Ingredients and chemical composition of the diet fed to laying hens

Ingredients (%)	T1 (0%)	T2 (0.4%)	T3 (0.7%)	T4 (0.9%)
Yellow corn	34.0	34.0	34.0	34.0
Wheat	35.0	35.0	35.0	35.0
Soybean meal	17.5	17.5	17.5	17.5
Protein concentrate*	5.0	5.0	5.0	5.0
Lime stone	6.7	6.7	6.7	6.7
Diphosphate calcium	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3
Oil	0.5	0.5	0.5	0.5
Vitamin + minerals***	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0
Calculated content**				
CP	17.6	17.6	17.6	17.6
ME	2756.0	2756.0	2756.0	2756.0
L-Arginine*****	1.1	1.5	1.8	2.0
L-Lysine	1.0	1.0	1.0	1.0

*Antraco Protein concentrate provide per kg: 40% protein, 2000 kcal ME/kg, fat 15%, ash 20%, calcium 5.6%, phosphorus 3.1%, lysine 3.4%, methionine 2.4%, methionine + cystine 3.2%, bag weight is 50 kg produced in Jordan on 9/4/2011.

**Calculated composition was according to NRC (1994).

***Type of a mixture of minerals and vitamins is Collivet, Syrian origin.

*****L-Arginine is Chinese origin, the proportion of purity is (≥99.0%)

MATERIALS AND METHODS

This experiment was conducted the Field of Poultry of the Department of Animal Resources, College of Agriculture, University of Baghdad during the period from 1/5/2011 to 5/7/2011 by using 100 commercial Brown Lohmann laying hens, 38 weeks OF AGE. Birds were housed in individual cages (40 x 40 x 40 cm). Birds were randomly distributed on four treatment groups with 25 hens for each treatment. Treatment groups were: T1: Bird fed diet with no additional arginine (control group); T2, T3 and T4: Birds fed diet supplemented with 0.4, 0.7 and 0.9% respectively. Therefore, the total amounts of arginine in the four treatments (T1, T2, T3 and T4) become 1.1, 1.5, 1.8, 2.0% respectively. Ingredient and chemical composition of diet were shown in Table 1. The birds were allowed free access to food and water. A regime of 16 h constant lighting and continuous ventilation were provided and all birds were kept under uniform management conditions throughout the experimental period.

Blood samples were collected at the end of each week of the experiment (for 8 weeks), from 10 birds selected at random in each treatment groups. Blood was collected from brachial vein into plastic tubes contains Potassium EDTA as anticoagulant to prevent blood clotting.

Blood traits measured

Hemoglobin concentration (Hb): A total of 20 Micro liter of blood samples in tubes containing anticoagulant were withdraw using capillary glass pipette for this purpose and mixed with 5 ml of Drabkin's reagent and left for 5 min and then placed in a centrifuge at 5,000 cycles/minute for 15 min to remove the f nuclei and of red blood cells and then read the optical density of these samples by using a spectrophotometer using the reagent itself. After that the reading of optical densities of samples were dropped on the curve of standard hemoglobin that has been done using several dilutions of standard hemoglobin and read using a spectrophotometer (Varley *et al*., 1980).

Table 2: The components of Natt and Herrick solution

Ingredients	Amount
NaCl	3.88 g
Na_2SO_4	2.50 g
$Na_2HPO_4.12H_2O$	2.91 g
KH_2PO_4	0.25 g
Formalin (37%)	7.50 ml
Methyl violet 2B	0.10 g

These ingredients were added together and then complete them to a total volume of 1000 ml with distilled water, using a volumetric flask

Total red blood cells count: Special pipette was used to estimate the number of red blood cells contain a small chamber, blood withdrawal of to the mark 0.5 and then diluted to 200 times using a solution Natt and Herrick (Table 2) since withdrawn from this solution until the access to the mark 101 in the pipette and then shacked quietly for two minutes to mix the blood with the solution inside the chamber and was eliminated from the first three drops out of the pipette solution, which represent the only dilution was then placing a drop of blood and the solution mix. On a special glass slide for the purposes of counting (Hemocytometer) under the glass cover and spread the solution automatically under the glass cover and wait a few minutes until the silence of cells for movement and then calculate the number of cells using optical microscope as shown red blood cells with transparent cytoplasm and pale tint nucleus and in this slide there is special square to counting red cells, which contains inside a 25 square each

square contains 16 smaller square so the red cells counted within 5 squares of the 25 square as the count in the four squares at the four corners of the large square and square in the center to represent this sample all the squares and by the count of total number of red blood cells following the equation, according to the method referred by Natt and Herrick (1952):

$$\text{The number of red blood cells} = \frac{N}{5} \times 25 \times 200 \times 10$$

That is:

N = Total number of red blood cells, calculated in 5 large squares (80 small squares)
5 = Number of large squares that have been counting inside.
25 = The total number of large squares.
200 = Times number of blood dilution
10 = The resulting number multiply by this number represents the number of red blood cells in 1 mm^3 of blood, as the total area of the special square to count red blood cells = 1 mm^2 (Large square contains 25 squares) and thus the volume of blood diluted inside the square = 1 mm^2 x 0.1 mm (height of solution above the square) = 0.1 mm^3 and this represents the volume of blood that is red blood cell count for it to multiply the output x 10 to represents the number of red blood cells in 1 mm^3.

Packed Cells Volume (PCV): Used in this test accurate capillary tubes open sides and filling the capillary tube by the same blood samples that were collected as mentioned above and horizontally to help blood flow by capillarity until a full two thirds the length of the tube with blood and then close the tube directly after collecting the blood from the same side that has been bringing from to use artificial mud. Then tubes were placed in horizontal on the Micro-hematocrit centrifuge for this purpose for 10 min and then were measured by the percentage of the volume of packed blood cells using a special ruler by using the method of Archer (1965).

Total White Blood Cells Count (WBC): WBC count was calculated in the same way used for counting the number of RBC and diluted with using the same solution. Used special pipette to calculate the number of white blood cells and through which the withdrawal of blood to the mark 0.5 and then complete the volume to the mark 11 using a solution of the dilution that was used in the calculation of the number of red blood cells and this dilute blood 20 times and there are 4 squares especially counting white blood cells on a slide and each one of which contains 16 small square was counting white blood cells in all these squares as cells appear dark blue color and can appear granular form. Used the following equation to calculate the total number of cells:

$$\text{The number of white blood cells} = \frac{N}{4} \times 20 \times 10$$

In 1 ml^3 of blood
As the:

N = Total number of white cells in the four squares.
4 = Number of large squares that have been counting inside.
20 = Number of times in dilution.
10 = Used for the total number in 1 ml^3 of blood as the blood volume in each of the squares of the four rate of 0.1 ml^3.

The percentage was calculated for each of the heterophil, lymphocyte, basophil, monocyte and eosinophil depending on the forms and its presence in the slides.

Calculation of MCV, MCH, MCHC: MCV, MCH, MCHC were calculated depending on the following equations (Al-Daraji *et al*., 2008):

$$\text{MCV (fl)} = (\text{PCV/RBCs}) \times 10$$

$$\text{MCH (pg)} = (\text{Hb/RBCs}) \times 10$$

$$\text{MCHC (g/dl)} = (\text{Hb/PCV}) \times 100$$

Statistical analysis: Data were statistically analyzed using the General Linear Model procedure of SAS (2000). Test of significance for the different between means of each classification was done by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Table 3 exhibit the results of the effect of adding different levels of L-Arginine to the diet of laying hens on the RBC, PCV, Hb, MCV, MCH and MCHC. It was noticed from this table that there were no significant differences between different treatment groups ($p \geq 0.05$) as regards these traits. These results clearly indicated that there are no negative effects of adding arginine to laying hens diet on physiological performance of birds. In addition, it was noticed that in spite of that there were no significant differences between treatment groups with respect to RBC, PCV, Hb, MCV, MCH, MCHC but there was clearly trend for these traits to be higher in arginine groups (T2, T3 and T4) as compared to control group (T1). These results are in agreement with those of Emadi *et al*. (2010) and Atakisi *et al*. (2009) who found that increasing the arginine level in the diet had improved some blood traits and did not have a negative impact on those characters.

Table 3: Effect of adding different levels of L-Arginine to the diet of laying hens on RBC, PCV, Hb, MCV, MCH, MCHC (Mean±SE)

	Treatments			
Traits	T1	T2	T3	T4
RBC (x10^6/mm^3)	2.11±00.36a	2.18±00.27a	2.16±00.20a	2.20±00.36a
PCV (%)	35.00±13.39a	36.60±15.54a	35.40±14.70a	35.90±12.93a
Hb (g/100 ml)	12.56±01.00a	14.10±01.22a	13.60±01.70a	13.70±01.04a
MCV (fl)	162.17±39.07a	167.88±17.72a	163.88±54.53a	163.18±22.62a
MCH (pg)	59.24±21.90a	64.67±06.77a	64.76±18.92a	60.52±07.49a
MCHC (g/100 ml)	35.88±09.31a	39.16±07.27a	38.41±07.64a	38.16±09.99a

T1, T2, T3, T4 represent supplementing the diet of hens with 0, 0.4, 0.7 and 0.9%, respectively. Similar letters within a row indicate that there were no significant differences ($p \geq 0.05$) between treatment groups

Table 4: Effect of adding different levels of L-Arginine to the diet of laying hens on WBC, the percentage of Heterophil (H), Lymphocyte (L), monocyte, basophil, eosinophil and H/L ratio (Mean±SE)

	Treatments			
Traits	T1	T2	T3	T4
WBC (x10^6/mm^3)	18.85±2.15b	20.50±3.35a	22.10±2.32a	23.20±3.40a
Heterophil (H) (%)	23.40±2.05b	25.00±1.54a	26.40±4.16a	26.60±2.32a
Lymphocyte (L) (%)	63.20±3.72a	65.40±1.67b	66.70±4.20b	66.90±2.33b
H/L ratio	0.35±0.05a	0.31±0.03b	0.33±0.15b	0.32±0.04b
Monocyte (%)	9.00±2.15a	5.50±2.48b	2.90±1.74c	2.50±3.47c
Basophil (%)	1.60±0.25a	1.50±0.20a	1.40±0.25a	1.50±0.00a
Eosinophil (%)	2.80±7.31a	2.60±1.02a	2.60±0.24a	2.50±0.60a

T1, T2, T3, T4 represent supplementing the diet of hens with 0, 0.4, 0.7 and 0.9%, respectively. Different letters within a row indicate that there were no significant differences ($p \leq 0.05$) between treatment groups

It was shown from Table 4 that dietary supplementation with different levels of arginine (T2, T3 and T4) resulted in significant increase ($p \leq 0.05$) in WBC and percentage of heterophil cells and significant decrease ($p \leq 0.05$) in percentages of lymphocyte and monocyte cells as compared with control group (T1). Whereas, there were no significant differences between treatment groups concerning percentages of eosinophil and basophil cells. This increase in WBC are in agreement of Grazi *et al*. (1975) who reported that the addition of arginine in the diet led to increase in activity of enzymes in the liver, including Nitric Oxide Synthase enzymes (NOS). Knowles and Moncada (1994) showed that arginine has a very important role in physiological terms, including defense and stimulate the immune system. This was confirmed by Kawk *et al*. (1999) who noticed that increased L-arginine by 1% in the broiler diet resulted in significant increase in weights of thymus and bursa gland and spleen. Emadi *et al*. (2010) reported that the increase in arginine level in the diet to 3% caused significant enhancement in immune response. However, the increase in arginine: lysine ratio during stress resulted in significant increase in resistance of bird to stress as indicated by significant decrease in H/L ratio in these birds in comparison with control group (Brake *et al*., 1994; Mendes *et al*., 1997).

In conclusion, adding arginine to hens' diet at levels higher than that recommended by NRC have no negative effect on physiological performance of these birds. However, supplementary arginine caused significant enhancement in immune response and resistance to stress. Therefore, supplementary arginine could be used as beneficial tool for enhance general physiological status of laying hens.

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