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Research Article

Effects of Dietary L-glutamine Supplement on Performance, Egg Quality, Fertility and Some Blood Biochemical Parameters in Guinea Fowls *(Numida meleagris)*

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Abstract

The present study was conducted to investigate the effect of dietary supplementation of L-glutamine and synergistic effects between glutamic acid in wheat and glutamine on productive performance, egg quality characteristics, blood biochemical parameters and fertility traits of guinea fowls fed with corn-soybean meal-wheat based diets. 120 guinea fowls (*Numida meleagris*) were allocated to a completely randomized design with six treatments consisted of four replicates and 5 birds per replicate. Treatments were included: 1) corn-soybean meal based diet (control1), 2) corn-soybean meal-wheat based diet- (control2), 3) control1 containing 0.5% L-glutamine, 4) control1 containing 1% L-glutamine, 5) control2 containing 0.5% L-glutamine, 6) control2 containing 1% L-glutamine. Results showed that diet supplementing with L-glutamine significantly increased egg mass, egg production, egg weight, shell thickness, haugh unit, levels of follicle-stimulating hormone and luteinizing hormone, hatchability and one-day chick weight and also improved feed conversion ratio compared with control1 and 2 diets ($P<0.01$). The best response for fertility traits was achieved in birds fed with control2 containing 1% glutamine. It can be concluded that 1% glutamine has positive effects on performance, some egg quality traits and fertility parameters. Positive synergistic effect between wheat and glutamine on fertility traits can be valuable in guinea fowls.

Keywords: *Guinea fowl, Glutamine, Wheat, Performance, Egg traits, Fertility*

Gine Tavuklarında *(Numida meleagris)* Diyete L-glutamin İlavesinin Yumurta Kalitesi, Fertilite ve Bazı Kan Biyokimyasal Değerleri Üzerine Etkisi

Özet

Bu çalışma mısır-soya fasulyesi-buğday ile beslenen Gine tavuklarında diyete L-glutamin ilavesinin ve buğdaydaki glutamik asit ile glutamin arasındaki sinerjistik etkinin verim performansına, yumurta kalitesi parametrelerine, kan biyokimyasal değerlerine ve fertilite üzerine etkilerini araştırmak amacıyla yapılmıştır. Çalışmada 120 Gine tavuğu (*Numida meleagris*) tamamen rastgele dizaynda olmak üzere dört tekrar ve her tekrarda 5 tavuk olacak şekilde altı çalışma grubuna ayrıldı. Uygulamalar şu şekilde gerçekleştirildi: 1) mısır-soya fasulyesi temelli diyet (kontrol 1), 2) mısır-soya fasulyesi-buğday temelli diyet (kontrol 2), 3) %0.5 L-glutamin içeren kontrol 1, 4) %1 L-glutamin içeren kontrol 1, 5) %0.5 L-glutamin içeren kontrol 2, 6) %1 L-glutamin içeren kontrol 2. Elde edilen sonuçlar, L-glutamin ilave edilen diyet ile beslenen tavuklarda yumurta kütlesinde, yumurta üretiminde, yumurta ağırlığında, kabuk kalınlığında, Haugh biriminde, folikül stimüle edeci hormon ve luteinize edici hormon seviyelerinde, yumurtadan çıkma oranı ve bir günlük civciv ağırlıklarında anlamlı derecelerde artış olduğunu gösterdi ($P<0.01$). Ayrıca yem konversiyon oranı kontrol 1 ve 2 ile karşılaştırıldığında iyileşme göstermekteydi. Fertilite için en iyi değerler %1 glutamin içeren kontrol 2 ile beslenen tavuklarda gözlemlendi. %1 glutaminin performans, bazı yumurta kalitesi özelliklerine ve fertilite parametrelerine pozitif etkisi olduğu kanısına varıldı. Buğday ve glutamin arasındaki pozitif sinerjistik etki Gine tavuklarında fertilite bakımından değerli olabilir.

Anahtar sözcükler: *Gine tavuğu, Glutamin, Bığday, Performans, Yumurta özellikleri, Fertilite*

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INTRODUCTION

Guinea fowl are originating from Africa. Asia and Latin America raise semi-domesticated species while in Europe, North America and Australia breed large-scale production of dominated guinea fowl [1]. This topic well shows adaptability guinea fowls to any condition. Guinea fowls have been shown to have resistance against common diseases and also lower requirement to labor and management [2]. Guinea fowl meat has a higher protein content of approximately 28% compared to 20% for domestic fowl [2]. It has been reported that guinea fowl eggs, due to more thickness, have better storage time than chick eggs [3]. These birds have much advantage but those have problems. Guinea fowls only lay in warm season [4]. It has been shown that guinea fowl lays by 100 eggs during 9 months [3] and has low hatchability [4]. It seems feeding strategies can help the birds for improvement in fertility and performance traits.

Wheat is usually applied in some countries as the major energy source in poultry diets. The composition of wheat is commonly more variable than other cereals. Protein level can vary from 10-18%, depending on cultivars and growing conditions. Wheat is contained higher amounts of protein compared to corn, and it provides only slightly less energy. However, there are some potential problems from feeding much more than 30% in a diet. Wheat contains by 5-8% of pentosans, which can create problems such as viscosity which can subsequently lower digestibility [5]. Wheat protein is known to be low in some amino acids which are known as essential for the human diet, especially lysine and threonine, but they are rich in glutamic acid [6].

Glutamine, a semi-essential amino acid or in some condition essential, has mobilizable nitrogenous groups in its structure [7] and also modulates in intestine health of animals [8]. Glutamine can be synthesized form combination glutamic acid and ammoniac by glutamine synthetase, especially in muscle [7]. Glutamine and glutamic acid totally form by 14% of egg proteins [9]. It has been accepted glutamine role as energy source for intestine cells [10], increasing the mucin synthesis [11] and modulating in gene expression [12]. It has been shown glutamine modulation in digestive system can improve absorption and subsequently increase performance and other traits [9]. It has been shown glutamine role in cellular immunity [7] and fertility [13]. Glutamine improved hatchability by decrease in blood urea nitrogen and oxidation activity [13].

As mentioned guinea fowls have lower fertility and performance and on the other hand glutamine has positive role in improvement of performance, fertility and egg traits. In addition, wheat contains much amount glutamic acid which maybe subsequently converted to glutamine. Thus, we hypothesized replacement of part corn by wheat can help to increasing the glutamine and finally improvement in mentioned traits. Thus, this study was conducted to investigate the effect of dietary supplementation of L-glutamine on performance, egg quality characteristics, some blood biochemical parameters and fertility traits of guinea fowls fed with corn-soybean meal-wheat based diets.

MATERIAL and METHODS

Birds

The current study was conducted in East Azarbaijan Research Center for Agriculture and Natural Resources and all the used procedures were approved by standard committee of Research's Science University (Approval date: 04/02/2016; No: 10030). A total number of 150 Guinea fowl, 38 weeks of age with weight mean 1800±50 g, were selected for pre-trial period (two weeks). In this period, birds were fed with corn-soybean meal diets and finally 120 hens were selected. 120 guinea fowls *(Numida meleagris)* were allocated to a completely randomized design with six treatments consisted of four replicates and 5 birds per replicate (4 females and 1 male) for 40 days. Experimental conditions were similar for all birds including; light cycling 16 h light: 8 h dark, similar temperature and free access to feed and water. Birds were fed with corn-soybean meal-wheat based diets containing 0.5 and 1% L-glutamine. Experimental treatments were as follows; 1) corn-soybean meal based diets (control 1), 2) corn-soybean meal-wheat based diets (control 2), 3) control 1 containing 0.5% L-glutamine, 4) control 1 containing 1% L-glutamine, 5) control 2 containing 0.5% L-glutamine, 6) control 2 containing 1% L-glutamine. Glutamine was purchased from Wellife Korean Company. Diets were formulated according to the Guinea-Fowls [1]. The diets composition is presented in *Table 1*. The proximate analyses of diets were performed according to Association of official Analytical Chemists AOAC [14].

Analysis of Amino Acids

In the present study, corn, wheat and soybean meal ingredients are diets. Thus analyses of amino acid corn, wheat and soybean meal were important. Analysis of amino acids was performed using high performance liquid chromatography (HPLC) as explained by Moral et al.[15] and the data are presented in *Table 2*. The amino acid composition was reported as percentage of protein content (i.e., in g/100 g of protein).

Performance

The number of produced eggs and their weight mean were daily recorded. Feed intake (FI), egg production (EP), egg weight (EW) and egg mass (EM) were calculated each 10 days/once and feed conversion ratio (FCR) was also calculated. EM was calculated as was previously explained by Hou [16], as follows;

$$EM = EP\ (\%) \times EW\ mean$$

Table 1. *Ingredient and nutrient composition of experimental diets*

Ingredients (%)	Control1	Control2	Control1+0.5% Gln	Control1+1% Gln	Control2+0.5% Gln	Control2+1% Gln
Corn	59.35	39.1	59.35	59.35	39.1	39.1
Soybean meal	29.3	27	29.3	29.3	27	27
Wheat	-	22	-	-	22	22
Vegetable oil	2	2.5	2	2	2.5	2.5
Sand	1	1	0.5	-	0.5	-
L-glutamine	-	-	0.5	1	0.5	1
Oyster shell	5	5.1	5	5	5.1	5.1
DCP	2	1.95	2	2	1.95	1.95
Salt	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Met+ Cys	0.7	0.7	0.7	0.7	0.7	0.7
Analysis						
Dry matter (%)	91	90.8	91	91	90.8	90.8
ME (kcal/kg)	2839	2808	2839	2839	2808	2808
Crude protein (%)	18	18	18	18	18	18
Ca (%)	2.5	2.5	2.5	2.5	2.5	2.5
Available P (%)	0.5	0.5	0.5	0.5	0.5	0.5
Met+ Cys (%)	0.8	0.8	0.8	0.8	0.8	0.8
Ether extract (%)	2.80	2.40	2.80	2.80	2.40	2.40
Crude fiber (%)	4.00	4.30	4.00	4.00	4.30	4.30
Ash (%)	6.50	6.80	6.50	6.50	6.80	6.80

DCP: *Di-calcium phosphate;* ***ME:*** *metabolizable energy;* ***CP:*** *crude protein; Available* ***P:*** *Available phosphorous;* ***Lys:*** *Lysine.* [a] *Vitamin premix provided the following per kilogram of supplement: vitamin A, 9.000 IU; vitamin D_3, 2.000 IU; vitamin E, 1.800 IU; nicotinic acid, 30 mg; vitamin B_{12}, 0.015 mg; vitamin K_3, 4 mg; biotin, 0.15 mg; ; folic acid, 1.0 mg; niacin, 30.0 mg; panthotenic acid, 25.0 mg; pyridoxine, 2.9 mg; ; riboflavin, 6.6 mg; thiamin, 1.18 mg.* [b] *Mineral premix supplied the following per kilogram of supplement: manganese oxide, 100 mg; $FeSO_4$. $7H_2O$, 50 mg; zinc oxide, 100 mg; copper, 10 mg; I, 1.0 mg; Se, 0.2 mg*

Table 2. *Amino acid composition (g/100 g of protein) for used wheat, corn and soybean meal*

Ingredients	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	Val
Wheat	3.1	5.1	5.6	3.5	32	4.4	2.8	3.4	5.3	3.3	1.7	4.5	4.3	5.3	6.1	4.1	4.1
Corn	3.5	7.7	12.5	1.5	11.5	5.1	4.2	5.1	5.1	9.2	2.9	8.1	2.4	5.5	5.1	4.2	3.5
Soybean meal	3.9	7.1	11.4	1.6	17.1	4.1	2.6	4.7	7.8	6.5	1.3	5.5	4.5	5	4.4	4	5.1

Egg Quality Traits

Three eggs, close to mean, from each replicate were selected per 10 days/once and then egg weights, albumen percentage, yolk percentage and shell percentage were measured by a digital scale. Shell thickness was measured using micrometers (OSK 13469- Japan). Yolk color was also measured by color indexes (Iran), with numbers from 1 to 15. Haugh unit was calculated as was previously explained by Hou [17], as follows;

Haugh unit = 100log [albumen height + 7.57- 1.7 $(EW)^{0.37}$]

Blood Biochemical and Hematological Parameters

Three birds from per replicate were selected (each 10 days/once) and two blood samples were taken form wing vein (2 mL /bird). One blood sample was considered for measurement of glucose, triglycerides and cholesterol by using specified-kits (Bionik-Iran). Other part of same blood samples was used for assessment of total protein and albumin using specified-kits (Pars Azmoon-Iran). Blood biochemical parameters were analyzed by mentioned kits and auto analyzer (Hitachi 911-Japan). The levels of thyroid-stimulating hormone (TSH), triiodothyronine (T3), tetraiodothyronine (T4), luteinizing hormone (LH), follicle-stimulating hormone (FSH) were measured using kits (Liaison-Italy). Other sample was transferred to EDTA-containing tubes. Blood smear was prepared and white blood cells were evaluated with Gisma staining and light microscope as described by Thrall et al.[18].

Fertility Parameters

In the end of trial, a number of collected eggs during experiment (600 eggs) were firstly candled and suitable eggs were transferred to hatchery machine. Incubation period was lasted 27 days. Hatchability percentage and one-day chick weight was calculated

Statistical Analyses

The all data of bird's were subjected to statistical analysis (SAS) [19] using analysis of variance (ANOVA) appropriatefor a completely randomized design. When significant effects were detected by ANOVA, treatment means were compared using Duncan's multiple range test. Differences were considered significant at P<0.01. All of parameters were examined as follows:

$$Yij = \mu + Ti + eij$$

Where Yij is the individual observation, μ is the overall mean, Ti is the effect of treatment, and eij shows the random error.

RESULTS

Table 3 shows the effect of experimental diets on productive performance. Replacement of corn with wheat had not significant effect on productive performance (P>0.05; control 1 *vs* control 2). FI was significantly reduced in birds fed with control 1 containing 0.5% glutamine compared with other birds (*P*<0.05). The highest FCR and lowest EM, EP and EW were seen in birds receiving control 1 and 2 diets than those fed with diets containing glutamine (P<0.05); showing positive effects of glutamine on productive performance. In the corn-soybean meal based diets, EM, EP and EW increase and FCR decreases by increasing glutamine levels (P<0.05), while better response for FCR was achieved at level of 0.5% glutamine in corn-soybean meal-wheat based diets compared with level of 1% (P<0.05). Comparing the level of 0.5% in control 1 and 2 has showed a better response for level of 0.5% in diets containing wheat; showing that lower levels of glutamine have better interaction with wheat. However, diet supplementing with wheat had not negative effects on productive performance compared with corn-soybean meal diet (P>0.05).

Egg quality characteristics are presented in *Table 4*. Albumen percentage, shell percentage and albumen: yolk ratio were not influenced by nutritional modulations (P>0.05). Comparing control 1 and 2 did not show significant differences for egg quality traits (except yolk color); showing that adding wheat to diet had not positive or negative effects on egg quality traits. Yolk color was significantly higher in birds fed with control 2 compared with those fed with control 1 (P<0.05). Dietary inclusion

Table 3. *Effect of experimental diets on productive performance*

Treatments	FI (g)	EM (g/hen/d)	FCR (g/g)	EW (g)	EP(%)
Control 1	89.10^{a}	15.24^{c}	5.85^{a}	36.50^{b}	41.75c
Control 1 + 0.5% Gln	81.01^{b}	17.60^{b}	4.65^{b}	39.09^{a}	45.02^{b}
Control 1 + 1% Gln	89.60^{a}	22.03^{a}	4.10^{c}	39.09^{a}	56.35^{a}
Control	86.77^{a}	14.65^{c}	5.95^{a}	36.21^{b}	40.45^{c}
Control 2 + 0.5% Gln	88.02^{a}	21.45^{a}	4.15^{c}	39.04^{a}	54.94^{a}
Control 2 + 1% Gln	86.70^{a}	19.42^{ab}	4.50^{b}	39.22^{a}	49.51^{b}
P-value	0.006	0.004	0.002	0.004	0.005
SEM	1.44	1.29	0.45	0.63	1.12

***SEM:** standard error of means. Footnotes (a-c) show significant differences each column (P<0.01)*

Table 4. *Effect of experimental diets on egg quality traits*

Treatments	Albumen (%)	Yolk (%)	Shell (%)	Albumen/Yolk	Shell Thickness (mm)	Haugh Unit	Yolk Index	Yolk Color
Control 1	54.47	30.79^{b}	14.74	1.77	0.47^{c}	85.97^{c}	37.74^{b}	11.62^{c}
Control 1 + 0.5% Gln	53.75	31.42^{ab}	14.83	1.73	0.51^{a}	87.59^{bc}	38.46^{ab}	12.31^{a}
Control 1 + 1% Gln	54.00	31.70^{ab}	14.30	1.70	0.50^{ab}	92.31^{a}	39.42^{ab}	12.31^{a}
Control 2	54.12	31.03^{b}	14.85	1.66	0.47^{c}	87.14^{bc}	39.07^{ab}	11.75^{b}
Control 2 + 0.5% Gln	54.37	31.37^{ab}	14.26	1.74	0.49^{b}	89.21^{b}	40.53^{ab}	12.06^{ab}
Control 2 + 1% Gln	53.80	32.00^{a}	14.20	1.71	0.50^{ab}	91.15^{a}	41.85^{a}	12.00^{ab}
P-value	0.61	0.003	0.55	0.56	0.0005	0.0001	0.004	0.0001
SEM	0.78	0.42	0.19	0.04	0.004	0.84	0.56	0.08

***SEM:** standard error of means. Footnotes (a-c) show significant differences each column (P<0.01)* of glutamine significantly increased haugh unit and shell thickness (P<0.05).

Effects of wheat and glutamine addition to diet on blood biochemical parameters are presented in *Table 5*. The serum concentrations of triglycerides, albumin and T3 were not influenced by dietary modulation (P>0.05). Comparing the control 1 and 2 did not indicate significant differences between both groups for glucose and total protein (P>0.05). The serum levels of cholesterol, FSH, LH and T4 were significantly increased in control 2 (P<0.05); showing that wheat addition to diet increased cholesterol, FSH, LH and T4 (P<0.05). Addition of glutamine to diet caused conflict results, so that the serum concentration of glucose was reduced by increasing glutamine levels, while the serum concentration of glucose was increased in level of 1% compared to 0.5%. The serum concentration of protein was significantly increased in control 2 diet containing 1% glutamine. Dietary inclusion of glutamine increased FSH and LH (P<0.05). Comparing the level of 0.5% in control diets showed better positive in control 2 compared with control 1; showing positive interaction between wheat and glutamine.

There were not significant differences between control 1 and 2 for hatchability (P>0.05), but 1% glutamine in both control groups, significantly increased hatchability (P<0.05). The weight of 1 day old chicks was increased significantly in control 2 compared with control 1 (*Table 6*; P<0.05). Also glutamine showed synergistic interaction effect with wheat for 1-d chick weight; so that 1-d chick weight was significantly higher in diets containing wheat and 1% glutamine compared to corn-soybean meal based diets containing glutamine.

Hematological parameters of guinea fowls are presented in *Table 7*. As it has been shown in *Table 7*, hematocrit percentage, basophile percentage and level of hemoglobin were not influenced by dietary treatments (P>0.05). The addition of wheat to diet was decreased lymphocyte count (control 2 vs control 1; P<0.05). The heterophil: lymphocyte ratio is reduced in control 2 compared with control 1 (P<0.05) and this is a suitable index for confortable in birds nourished with control 2. Dietary inclusion of wheat also increased white blood cells count (control 2 vs control 1; P<0.05); showing improvement in immunity by wheat. Birds receiving the glutamine showed the increased heterophil: lymphocyte ratio and heterophil count and also the reduced lymphocyte count compared with controls (P<0.05). Glutamine linearly increased white blood cells in corn-soybean meal based diets (P<0.05), while there were conflicting results for diets containing wheat, so that higher levels (1%) did not show significant differences with control 2 (P>0.05). However, glutamine increased white blood cells in corn-soybean meal based diets (P<0.05).

DISCUSSION

In this study, diet supplementing with wheat had not negative effects on productive performance compared with corn-soybean meal diet. According to our findings, Shakeri et al.[20] did not observe significant differences in growth performance of broiler chicks supplemented with corn based diets than those fed with wheat based diets. As *Table 2* shows, wheat is containing higher levels of glutamic acid which would be laterconverted to glutamine in the body, by glutamine synthetase, and finally it can improve productive performance. Thus, the presence of glutamic acid in wheat can be the major reason for indifference

Table 6. *Effect of experimental diets on fertility traits*

Treatments	Hatchability (%)	1-d Chick Weight (g)
Control 1	49.00^{d}	24.99^{c}
Control 1 + 0.5% Gln	52.00^{bc}	25.02^{c}
Control 1 + 1% Gln	71.00^{a}	25.85^{b}
Control 2	51.00^{c}	25.03^{c}
Control 2 + 0.5% Gln	54.00^{b}	25.83^{b}
Control 2 + 1% Gln	70.00^{a}	26.20^{a}
P-value	0.001	0.0001
SEM	0.86	0.08

SEM: *standard error of means. Footnotes (a-c) show significant differences each column (P<0.01)*

Table 5. *Effect of experimental diets on blood biochemical parameters*

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	TSH (IU/L)	T3 (ng/dL)	T4 (µg/dL)	FSH (IU/L)	LH (IU/mL)
Control1	187.93^{a}	257.25^{b}	359.69	4.21	5.21^{b}	1.00^{a}	101.89	1.85^{d}	0.88^{d}	0.61^{d}
Control 1 + 0.5% Gln	168.31^{bc}	254.55^{b}	361.06	4.40	5.52^{ab}	0.74^{b}	102.95	2.02^{cd}	1.17^{b}	1.00^{b}
Control 1 + 1% Gln	174.62^{b}	280.25^{a}	371.88	4.05	5.28^{b}	0.61^{bc}	105.23	2.31^{b}	1.39^{ab}	1.13^{ab}
Control 2	182.50^{ab}	296.25^{a}	357.94	4.15	5.28^{b}	1.01^{a}	104.31	2.40^{b}	1.03^{c}	0.78^{c}
Control 2 + 0.5% Gln	162.75^{c}	294.75^{a}	411.69	4.47	5.52^{ab}	0.94^{a}	107.07	2.87^{a}	1.56^{a}	1.30^{a}
Control 2 + 1% Gln	188.00^{a}	281.68^{a}	389.56	4.21	5.78^{a}	0.58^{c}	102.04	2.09^{c}	1.31^{ab}	1.19^{ab}
P-value	0.007	0.001	0.13	0.20	0.006	0.0001	0.81	0.0001	0.0001	0.0001
SEM	4.91	6.88	0.485	0.12	0.09	0.04	3.01	0.06	0.003	0.04

SEM: *standard error of means. Footnotes (a-d) show significant differences each column (P<0.01)*

Table 7. *Effect of experimental diets on blood hematological parameters*

Treatments	Hematocrit (%)	Hemoglobin (g/dL)	Heterophil (%)	Lymphocyte (%)	Heterophil/ Lymphocyte	Monocyte (%)	Eosinophil (%)	Basophile (%)	White Blood cellsX10³ cells/μL
Control1	42.16	14.19	61.88[d]	32.38[a]	1.95[d]	3.56[a]	1.78[bc]	0.40	203.73[d]
Control 1 + 0.5% Gln	40.99	14.21	68.18[b]	25.88[cd]	2.70[a]	3.60[a]	1.92[b]	0.42	212.75[c]
Control 1 + 1% Gln	42.85	14.23	70.11[a]	25.08[d]	2.85[a]	2.91[b]	1.50[c]	0.40	225.23[a]
Control2	40.74	13.83	63.96[d]	29.12[b]	2.25[c]	4.04[a]	2.45[a]	0.46	210.19[c]
Control 2 + 0.5% Gln	41.74	13.88	68.97[ab]	25.01[d]	2.80[a]	3.52[a]	2.10[a]	0.40	216.90[b]
Control 2 + 1% Gln	40.11	13.26	66.55[c]	27.46[c]	2.48[b]	3.54[a]	2.03[ab]	0.42	209.28[c]
P-value	0.36	0.25	0.001	0.0001	0.0001	0.002	0.008	0.50	0.0001
SEM	0.93	0.31	0.73	0.70	0.09	0.18	0.12	0.02	1.24

SEM: *standard error of means. Footnotes (a-d) show significant differences each column (P<0.01)*

between control 1 and control 2. The role of anti-nutrient substances in wheat is well accepted, although their effects are well demonstrated in higher levels (more than 30%) [5]. Thus, lower levels (20% wheat) might be also a reason for indifference between control 1 and 2.

Glutamine improved productive performance, except FI, in guinea fowls. Similar to our observations, the other studies showed that diet supplementing with glutamine increased growth performance of birds [21,22]. Positive effects of glutamine on performance may be explained by several reasons. Firstly, glutamine improved performance by growth and development of digestive system [21]. An increase in gene expression and secreted enzymes activity [12] and increase in mucin synthesis [11] by glutamine can be another reasons for improvement of productive performance of guinea fowls. It is essential to mention that sexuality significantly affects FI and male birds usually consume more FI because of higher requirement [23]. Sexual ratio in all replicates was similar and it could not affect FI. The beneficial effects of glutamine on intestine microflorabeen reported by Francis and Griffiths [7] which can indirectly improve productive performance. A study has been shown the positive correlation between levels of FSH and LH and EP, EW and finally productive performance in laying hens [24]. They believed that these hormones improve productive performance by growth of oviduct and other productive system. On the basis of Ying et al.[24] findings, it can be strongly claimed that FSH and LH are responsible for improvement in productive performance, since their levels were significantly increased in groups containing glutamine *(Table 5)*. It can be concluded that glutamine improves productive performance by mentioned mechanisms but the expected synergistic effect between wheat and glutamine on performance was not seen.

In relation to egg quality, yolk color difference in wheat group compared with corn is not known. Oxycarotenoids are known as responsible for the pigmentation of egg yolk. Corn, wheat and barley are major feed components which can create significant variations in egg yolk pigmentation [25]. Corn and its by-products are known as the most important oxycarotenoid source for layers. A study showed that corn containing feed caused to a fan value of 10, while wheat and barley showed yolk color with a fan value of 4. Differences were explained by oxycarotenoid contents in the raw materials [25]. It seems to be more oxycarotenoid contents in the used wheat cultivar which is causedto increase the yolk color in comparison with corn. Rafuse et al.[26] did not observe significant differences among treatments for albumen height and eggshell in laying hens consuming wheat-based diets. Studies have been tried to increase egg quality and improve in wheat-based diets by adding enzymes. Mirzaie et al.[27] reported that dietary inclusion of xylanase had not any significant effect on egg quality traits. Çiftci et al.[28] reported that substitution 30% corn by wheat did not affect eggshell thickness of laying hens from 27 to 43 wk of age. Dietary inclusion of glutamine increased haugh unit and shell thickness. In contrast to our observations, Ying et al.[24] showed that glutamine addition to diet had not significant effect on haugh unit and shell thickness in laying hens. This difference between our findings and others may be explained by bird type, bird age, hygiene level, etc. Exact mechanism of glutamine in improvement of shell thickness and haugh unit would be needed more investigations.

Results showing that wheat addition to diet increased cholesterol, FSH, LH and T4. Smits et al.[29] showed that the increase in non-starch polysaccharide content, enough in wheat, lowered cholesterol absorption and plasma cholesterol concentration in broiler chicks. Other study also showed that triglyceride and total cholesterol concentrations were numerically decreased in wheat-based diet, although they were not significantly different [30]. It is believed that NSP can bind to bile salts, lipids and cholesterol which finally reduce cholesterol concentration [30]. It was expected to reduction the serum concentration of cholesterol by wheat, due to viscosity substances, but such result was not found and it may be explained because of using low levels of wheat in diet. Similarly Luo et al.[31] showed that wheat-based diets did not have significant effects on level of T4. They suggested more investigations for the relationship between wheat and hormone levels, because of complexity. Mirzaie et al.[32] did not found significant effects of xylanase on thyroid hormones in wheat-based diets. The serum concentration of glucose was increased in level of 1% compared with 0.5%. This result is confusing and the mechanism of action is not known. Iwashita et al.[33] recommended glucose during exercise and post-exercise, due to lowering the glucose level. Another study has been shown glutamine as improving the serum concentration of cholesterol, albumin and total protein [34]. Several studies have indicated that glutamine supplementation could stimulate protein synthesis [35,36]. Studies have been shown that glutamine supplementing increased fat-free mass in athletes [37]. Dietary inclusion of glutamine increased FSH and LH. Ying et al.[24] showed that dietary inclusion of glutamine (0.4-0.8%) increases the level of FSH and LH in laying hens. They showed that glutamine improves gonadal hormone levels in animal body for a better genital system growth. Human studies have been shown to increase testosterone hormone after supplementing the glutamine [37]. It has been reported that glutamate biosynthesis from glutamine by binding to N-methyl- D-aspartate receptors was an event contributing to the pubertal activation of luteinizing hormone-releasing hormone [38] and pulsatile gonadotropin-releasing hormone secretion [39]. The relation between glutamine and T4 is not known.

An increase in 1-d chick weight can be explained by synergistic interaction effects between glutamic acid in wheat and glutamine supplement. Our claim was confirmed by Oliaei et al.[40] who indicated that *in ovo* administration of glutamine increased 1-d chick weight by 3.6%. Glutamine increased the hatchability in guinea fowls. Suchner et al.[13] suggested that glutamine improved hatchability by decreasing urea nitrogen and oxidation activity in blood. However, improved fertility traits in guinea fowls are valuable and this study suggests glutamine asa factor for improving the fertility traits in guinea fowls.

Heterophils are phagocytizing cells and their counts will increase against bacterial, microbial and chemical infections. Lymphocytes are the most leucocytes in normal condition and heterophil: lymphocyte ratio is an important index for evaluating stress conditions. Dietary inclusion of wheat increased white blood cells count (control 2). Also El-Katcha et al.[41] reported that dietary inclusion of wheat to diet increased humoral immunity in broiler chickens. The role of wheat for improving the immune system is not known, but it may be related to wheat components. Wheat is containing high amount of glutamic acid which can then converts to glutamine. The role of glutamine for improving the immune system function would be discussed; in this study Glutamine increased white blood cells. In agreement with our findings some researchers has been reported the glutamine role for improving the immune system under normal and stress conditions [20,22]. Glutamine is used at high levels in immune cells such as, lymphocytes, macrophages and neutrophils [42]; suggesting that glutamine involves in immune system. Also its role has been accepted in other immune cells, i.e. T-cell proliferation, B-lymphocyte differentiation, macrophage phagocytosis, antigen presentation and cytokine production [43] and also in anti-oxidant system against ischemia-reperfusion injury in rats [44].

The present study was conducted to investigate the synergistic effects between glutamine supplementation and wheat on performance, egg traits, blood and fertility parameters in guinea fowls, since wheat contains high amount of glutamic acid which can be subsequently converted to glutamine for the first time. The results showed that diet supplementing with L-glutamine significantly increased egg mass, egg weight, shell thickness, haugh unit, levels of follicle-stimulating hormone and luteinizing hormone, hatchability percentage and one-day chick weight and also lowered feed conversion ratio compared with control 1 and 2 diets. The synergistic effect between wheat and glutamine was observed in 1-d chick weight. Guinea fowls have low fertility so that the combination of glutamine and wheat can help to improve their fertility. Therefore glutamine can be suggested as an effective factor for improving the performance, some blood and hematological parameters and also fertility parameters in birds.

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