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The Effects of *in ovo* Administration of Glutamine on Hatchability, Subsequent Performance, Digestive Enzyme Activities, Immune Response and some of Blood Parameter in Broiler Chickens

Research Article

M. Salmanzadeh[1], Y. Ebrahimnezhad[1*], H. Aghdam Shahryar[1] and J. Ghiasighaleh Kandi[2]

[1] Departments of Animal Science, Shabestar branch, Islamic Azad University, Shabestar, Iran
[2] Departments of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran

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*Correspondence E-mail: ebrahimnezhad@iaushab.ac.ir

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ABSTRACT

An experiment was conducted to investigate the effects of *in ovo* feeding (IOF) of glutamine (Gln) on hatching traits, growth performance, digestive enzyme activities, immune response and some of blood parameters in broiler chickens. For this study, 2100 eggs were obtained from 43-wk-old hens. L-glutamine (10, 20, 30, 40 or 50 mg dissolved in 0.5 mL of deionized water) was injected into the albumen on the 14th day of incubation. Hatchability, growth performance, digestive enzyme activities (amylase and lipase), immune response and blood parameters (glucose, cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), total protein, alkaline phosphatase (ALP)) were determined during this experiment. Weight of newly-hatched chicks was significantly greater in groups with Gln injection than in control and sham groups. But IOF caused lower hatchability (13.1% than in the control group (not-injection eggs) ($P<0.05$). Chickens from IOF of Gln showed better weight gain and feed conversion ratio (0-42 day of age) when compared to chicks hatched from the control and sham group. The IOF of the Gln significantly increased glucose concentration, number of the lymphocytes and heterophil and the weight of spleen and bursa of fabricius in newly hatched chicks. In addition, immunoglobulins G and M were also markedly increased in chickens treated *in ovo* with 20, 30, 40 and 50 mg of Gln at 26 days of age. It was concluded that the IOF of the Gln in all doses improved the immune response of broilers and the growth performance of broilers.

KEY WORDS gastrointestinal enzyme, glutamine, immune response, *in ovo,* performance, Poultry.

INTRODUCTION

In ovo feeding (IOF) is a method of supplementing exogenous nutrients into the amnion of the avian embryo (Uni and Ferket, 2003), which can improve the performance of chicks (Salmanzadeh *et al.* 2012; Dong *et al.* 2013; Salmanzadeh *et al.* 2014; Salmanzadeh *et al.* 2016).

In previous studies, different nutrients such as amino acid (Al-Murrani, 1982; Ohta *et al.* 1999; Ohta *et al.* 2001; Ohta *et al.* 2004; Salmanzadeh *et al.* 2011), carbohydrate (Tako *et al.* 2004a; Uni *et al.* 2005; Smirnov *et al.* 2006; Salmanzadeh, 2011), vitamin (Nowaczewski *et al.* 2012), mineral (Tako *et al.* 2004b; Salmanzadeh *et al.* 2012) and other nutrients (Salahi *et al.* 2011; Moghaddam *et al.* 2013)

have been injected into embryonated chicken eggs. Glutamine (Gln) is considered as an important amino acid to be utilized as an energy source for the development of the gastrointestinal tract and stimulates immune cells (Cruzat *et al.* 2018).

Gln has been used in stressful conditions such as trauma to improve the gut barrier function, immune cell response and decrease mortality. Generally, it is now widely accepted that Gln is utilized at high rates by the isolated cells of the immune such as lymphocytes, macrophages and neutrophils (Ardawi and Newsholme, 1983; Newsholme *et al.* 1986; Curi *et al.* 1997).

In some studies, IOF of Gln has increased many functional parameters of immune cells such as T-cell proliferation, B-lymphocyte differentiation, macrophage phagocytosis, cytokine production and antigen presentation (Wells *et al.* 1999; Kew *et al.* 1999; Newsholme, 2001; Yeh *et al.* 2001). In addition, Gln is an amino acid to be utilizated as an energy source for stimulating intestinal cell proliferation, leading to the increase in the absorptive capacity of the gastrointestinal mucosa and consequently the bioavailability of nutrients.

Likewise, previous experiments showed that supplementing the diet with Gln increased the height of the intestinal villus and consequently, improved the growth performance in broilers (Yi *et al.* 2005; Bartell and Batal, 2007; Samli *et al.* 2007; Jazideh *et al.* 2014), turkey poults (Yi *et al.* 2001; Salmanzadeh and Shahryar, 2013a) quails (Salmanzadeh and Shahryar, 2013b) and wenling pigs (Kitt *et al.* 2002), compared to the control groups. Bartell and Batal (2007) indicated that the addition of 1% Gln to the diet of broiler chicks improves the growth performance and may stimulate the development of the gastrointestinal tract and humoral immune response. Furthermore, in another study, Chen *et al.* (2009) demonstrated that, the IOF of Gln were improved body weights measured on the 7th-day post-hatching in ducks.

All these useful actions of Gln make the amino acid deserving scientific and technical attention as IOF in broiler breeder eggs. It was hypothesized that the IOF of Gln can improve the development of the gastrointestinal tract and stimulate the immune cells that consequently improve the growth performance in broiler chickens. None of the previous studies didn't survey the effects of the IOF of the Gln into the albumen on the 14th day of incubation on hatchability, subsequent performance and immune response. Thus, the aims of this research were to investigate the effects of the IOF of the Gln in broiler breeder eggs on hatchability, subsequent performance, digestive enzyme activities, immune response and some of the blood parameter broiler chickens.

MATERIALS AND METHODS

In ovo feeding and incubation

A total of 2100 fertile eggs of approximately similar weights (66±1 g) were obtained from a broiler breeder (Cobb 500) at 43 weeks of age. All hatching eggs were collected, fumigated with formaldehyde gas, and incubated at 37.8 ˚C and 65% RH. On the 13th day of incubation, the eggs were candled, and the infertile ones or those containing only dead embryos were removed. On the 14th day, fertile eggs were randomly allotted to seven treatments with five replicate per treatment and 60 eggs per replicate. The treatments consisted of without injection (control, C), injection of 0.5 mL deionized water (sham, S), injection of 10 mg (10Gln), 20 mg (20Gln), 30 mg (30Gln) 40 mg (40Gln) and 50 mg (50Gln) of Gln dissolved in 0.5 mL of deionized water.

Before injection, the injection hold area was cleared with ethyl alcohol (70%) and the blunt end was punched using a 22 gauge needle. Then, the IOF solutions were injected into albumen using a 22 gauge needle to a depth of about 13 mm (Salmanzadeh *et al.* 2016) to corresponding eggs. After injection, the eggs were sealed by the cellophane tape and transferred to the incubator.

Control eggs were removed from the incubator together with the treated groups and kept in the same environment. The deionized water injections were included as sham controls primarily to rule out a possible negative response caused by the stress of injection and handling. L-Glutamine was supplied from Sigma® Co (anhydrous ≥ 99%, CAS Number: 56-85-9). All of the treatment solutions were prepared in autoclaved water.

Birds and data collection

After hatching, chickens were transferred to an experimental house and reared for 42 days with the same diet according to the requirements of broilers as recommended by the catalog of Cobb 500 broilers (Table 1). According to the treatment group, each chicken was identified by a neck tag and recorded.

The chickens in each treatment were randomly assigned to corresponding pens (0.09 m^2/bird). Each pen was provided with water and a feeder (pellet). The room temperature was maintained at 32 ˚C from 0 to 4 and then gradually reduced from 32 ˚C to 21 ˚C. All experimental protocols and procedures were approved by the Institutional Animal Care of Iran.

Upon hatching, the number of hatchlings was determined to calculate the hatchability of fertile eggs. The weight of newly hatched chickens was determined by weighing every chicken hatched one by one.

Table 1 Ingredients and nutrient composition of the basal diet (as-fed basis)

Ingredient (%)	Starter (0 to 10 d)	Grower (11 to 26 d)	Finisher (27 to 42 d)
Corn	60.36	65.44	66.8
Soybean meal (44% CP)	34.12	28.62	26.33
Vegetable fat	1.23	1.74	2.84
Dicalcium phosphate	1.83	1.8	1.67
Oyster sell	1.22	1.19	1.13
Salt	0.35	0.3	0.3
Sodium bicarbonate	0.11	0.07	0.07
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
DL-methionine	0.17	0.18	0.18
L-lysine mono hydro chloride	0.11	0.16	0.18
Nutrient analysis			
Metabolizable energy (kcal/kg)	2894	2987	3176
Crude protein (%)	20.3	18.3	18
Ca (%)	1	0.96	0.9
Available P (%)	0.50	0.48	0.45
Met (%)	0.46	0.44	0.43
Met + Cys (%)	0.89	0.84	0.82
Lys (%)	1.20	1.10	1.05

[1] Vitamin premix provided the following per kilogram of diet: vitamin A: 11013 IU; vitamin D_3: 3525 IU; vitamin E: 33 IU; vitamin K: 2.75 mg; vitamin B_{12}: 0.028 mg; Riboflavin: 7.7 mg; Pantothenic acid: 17.6 mg; Niacin: 55.1 mg; Choline: 478 mg; Pyridoxine: 5.0 mg; Thiamine: 2.2 mg; Folic acid: 1.1 mg and Biotin: 0.22 mg.
[2] Mineral premix provided the following per kilogram of diet: Manganese: 64 mg; Zinc: 75 mg; Iron: 40 mg; Copper: 10 mg; Iodine: 1.85 mg and selenium: 0.3 mg.

In each pen, the bodyweight of the bird and feed intake were recorded on days 1, 10, 26, and 42 post-hatching and after that, the mean body weight gain, feed intake, and feed conversion ratio were calculated for each pen (replicate) between 0-10, 11-26, 27-42, and 1-42 days. During each time period, the body weight gain was calculated and expressed as grams per bird.

The feed intake (g of feed intake/bird) over the entire rearing period was calculated by totaling feed consumption in each time interval between each bird sampling. The feed conversion ratio (g feed/g weight gain) was calculated by dividing the total food intake by the total weight gain in each pen.

Digestive enzyme activities assay

After thawing, all of vacuum-packed were opened and then using a sensitive scale, 0.05 gram of the mucosal layer of small intestine (15 birds per treatment) was weighed and along with 10 mL liter of phosphate buffer saline (pH=7) was formed into a homogenized solution using the sonic Vibracell Sonics (VCX 130 TE USA) device. The enzymic activities of amylase and lipase were measured according to the procedure (calorimetric method). For the detection of the enzymic activity measuring the total protein throughout the use of the Pirogallol (calorimetric) method was needed. The level of the activity of the enzymes of each sample is divided into the amount of its total protein so that the activity level of the enzyme can be calculated according to the IU in liter/gram protein (Teshfam, 1984).

Determination of blood biochemistry parameters and immune tissue weight

For this purpose, the broilers fasted for approximately 12 hours. Then, the blood samples were collected in non-heparinized blood from the wing vein of the broiler on days 0 and 10 of the experiment. After clotting for 4 hours at room temperature (18-22 ˚C), samples were centrifuged at 3200 g for 5 min at room temperature and the serums were transferred into vials and stored at the temperature of -20 ˚C until used. The serum biochemistry parameters (glucose, cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, total protein, alkaline phosphatase) were measured by the spectrophotometric method using commercial kits of pars azmun® Co, Tehran, Iran (Rifai *et al.* 1999; Salmanzadeh *et al.* 2012).

Broiler chickens were then individually weighed, killed by cervical dislocation, defeathered and eviscerated. The weight of each spleen, thymus, and bursa of Fabricius was recorded and the corresponding percentages (% of live body weight) were calculated. The thymus weight was determined as the five lobes located bilaterally on the sides of the esophagus.

Leukocyte profile determination

The blood samples were collected in the non-heparinized blood from the wing vein of the broiler on days 0 and 10 of the experiment. Immediately, 3 thin smears were prepared from each blood sample on clean microscope slides and allowed to air-dry.

The smears were stained with a modified Wright's stain, then cover-slipped, and a total of 100 heterophils and lymphocytes were identified and counted under 100 × oil microscope objective lens. Other types of leukocytes were not counted or used in calculations. The heterophil:lymphocyte ratios (H:L ratio) was calculated by dividing the number of heterophils through the number of lymphocytes.

Sheep red blood cells (SRBC) immunization and hemagglutination assay

The birds were immunized by SRBC as previously described by Gross (1978). In short, 30 mL of blood was collected from a sheep. The antigen was prepared by washing the sheep red blood cells and suspending them in a saline concentration of 0.05%. On day 19, a 0.05% SRBC solution was administered intravenously into 3 chicks per pen (15 birds per treatment) at dose of 0.1 mL in the brachial vein of each bird (Bartell, 2006). On day 26 (7 days after antigen challenge), 1.0 mL sample of blood was collected from the brachial vein of each SRBC challenged bird and placed in a heparinized microfuge tube. The blood samples were centrifuged at 10000 rpm for 2 min and the plasma fractions were removed. The hemagglutination assay was run as previously described by Gross (1978). In short, 50 µL of saline was added to each well of a 96 well microtiter plate. Fifty µL of the plasma sample was added to the appropriately labelled well in the top row. The diluted plasma samples were carefully mixed and 50 µL was transferred to the well directly below and this dilution procedure was repeated for all the wells in a column. Fifty µL of the 0.05% SRBC solution was added to each well of the microtiter plate.

The contents of each well were mixed and the plate was incubated overnight at 37 ˚C. The following day, the antibody titer was determined by identifying the first "negative" well (the first well in which a distinct button of red blood cells forms at the bottom of the well). The hemagglutination antibody titers were expressed as log2 of the reciprocal of the lowest dilution which produced the agglutination by the assay. The mean of the titers were determined for each treatment group.

Analysis of Ig in serum

The serum samples for all treatment and age groups were analyzed for IgG and IgM at the same time to avoid variation that may occur with analyses done at different times. Serum IgG and IgM were determined using ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX 77356). The absorbance was measured at 450 nm. The absorbance of the control wells were adjusted to zero prior to measuring absorbance in the samples. Because the absorbance units are linearly related to the logarithm of the Ig concentration (Piquer *et al.* 1991), we considered that the absorbance measurements obtained could be used as estimates of Ig concentrations. Therefore, no standard 198 curve was used to calculate the Ig.

Statistical analysis

The experimental design was a completely randomized design. The average of the data was calculated as the least squares mean by using of the general linear models (GLM) procedure. Samples size was determined by Roberts *et al.* (2002). The Normality test confirmed with Kolmogorov–Smirnov test. For the zero assumption, the normality was examined.

The analyses of variance were performed using the GLM procedure of SAS (2005) as a completely randomized design. The results are presented as mean ± standard error of the means (SEM). The significantly different treatment means were investigated using Duncan's new multiple rang test. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The IOF of Gln reduced the hatchability and increased ($P<0.05$) the body weights of newly-hatched chicks compared to the C group (Table 2). Furthermore, All Gln treatments improved the body weight gain and food conversion ratio through the whole experimental period (Table 3).

In this study, the IOF of Gln did not affect the amylase and lipase activities in newly-hatched chickens and on 10 d old (Table 4).

As seen in Tables 5, the IOF of Gln caused an increase in blood glucose of newly-hatched chicks compared to the S and the C group ($P<0.01$). Also, the number of lymphocytes and hetrophils and heterophil to lymphocyte ratios (H/L) of the newly-hatched broiler chickens as well as the heterophil to lymphocyte ratios (H/L) of broiler chickens of 10 days old were increased ($P<0.0$, Table 6).

All Gln levels increased linear the relative weights of the spleen and the bursa of newly-hatched chicks and the weight of the spleen of broilers when they were 10 days old (Table 7). On the 26th day, increasing of Gln dose increased concentration immunoglobulines G and M which this increasing was significant compared to the S and C groups ($P<0.05$, Table 8). The results of the present study indicated that the injection of different levels of Gln into the albumen at the 14th day of incubation caused a decrease in hatchability. By contrast, in the Uni *et al.* (2005) study, the *in ovo* administration at late-term chicken embryos has a positive effect on hatchability. On the other hand, Ipek *et al.* (2004) reported that IOF had no significant effect on the hatchability.

Table 2 Effects of *in ovo* feeding of glutamine (Gln) on hatchability, body weight and body length of newly-hatched chickens

Treatments	Hatchability (%)	Body weight (g)	Body length (Cm)
C	88.9[a]	43.5[b]	19.7
S	73.1[b]	43.3[b]	20.0
10Gln	76.0[b]	43.9[a]	20.1
20Gln	75.7[b]	43.8[ab]	20.3
30Gln	75.7[b]	44.1[a]	20.3
40Gln	76.8[b]	44.0[a]	20.7
50Gln	77.5[b]	44.2[a]	20.5
SEM	2.00	0.18	0.38
P-value	0.0005	0.0091	0.3405

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of *in ovo* feeding of glutamine (Gln) on body weight gain, feed intake and feed conversion ratio in the broilers chickens in different period

Treatments	Body weight gain, g/bird			
	0 to 10 days	11 to 26 days	27 to 42 days	1 to 42 days
C	196.2[b]	643.2[b]	1405.3[c]	2244.7[c]
S	198.5[b]	649.7[b]	1410.3[c]	2258.4[c]
10Gln	206.9[a]	665.7[ab]	1443.2[bc]	2315.8[b]
20Gln	208.8[a]	671.3[ab]	1458.8[ab]	2338.9[ab]
30Gln	211.2[a]	689.8[a]	1463.6[ab]	2364.6[ab]
40Gln	209.6[a]	983.9[a]	1484.7[a]	2378.3[a]
50Gln	214.2[a]	691.1[a]	1479.1[ab]	2384.5[a]
SEM	2.81	9.08	12.50	19.37
P-value	0.0013	0.0050	0.0006	0.0001
	Feed intake, g/bird			
C	250.0	917.7	3148.2	4315.9
S	250.5	919.1	3144.8	4314.4
10Gln	248.0	906.1	3131.9	4286.1
20Gln	245.6	908.3	3128.5	4282.4
30Gln	247.8	903.7	3139.1	4290.6
40Gln	246.7	891.4	3116.5	4254.6
50Gln	244.3	894.7	3112.9	4251.8
SEM	6.56	8.10	11.02	22.84
P-value	0.9934	0.1755	0.2256	0.3262
	Feed conversion ratio, g feed:g weight gain			
C	1.27[a]	1.42[a]	2.24[a]	1.92[a]
S	1.26[a]	1.41[a]	2.23[a]	1.91[a]
10Gln	1.19[ab]	1.36[b]	2.17[b]	1.85[b]
20Gln	1.17[ab]	1.35[bc]	2.14[bc]	1.83[b]
30Gln	1.17[ab]	1.31[cd]	2.14[bc]	1.81[bc]
40Gln	1.17[ab]	1.30[d]	2.09[c]	1.78[c]
50Gln	1.14[b]	1.29[d]	2.10[c]	1.78[c]
SEM	0.03	0.01	0.01	0.01
P-value	0.0901	0.0001	0.0001	0.0001

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In parallel, Pedroso *et al.* (2006) stated that the *in ovo* administration of Gln into the amniotic fluid of embryos on day 18 of incubation did not have a positive effect on hatchability. Dos Santos *et al.* (2010) demonstrate the IOF of 0.5 mL of a 10% Gln solution did not affect the hatching of chickens. Salmanzadeh *et al.* (2016) reported that hatchability reduced when injecting Gln at day 7 of incubation. Chen *et al.* (2009) stated that the hatchability of control and treated groups with sucrose and maltose (DS), L-alanyl-L-glutamine (Ala-Gln), sucrose, maltose and L-alanyl-L-glutamine (Ds+Ala-Gln), groups were 85%, 65%, 70% and 82% respectively.

Table 4 Effects of *in ovo* feeding of glutamine (Gln) on amylase and lipase in newly hatched chickens and 10 days of age

Treatments	Newly hatched chickens		10 days of age	
	Amylase (IU/g protein)	Lipase (IU/g protein)	Amylase (IU/g protein)	Lipase (IU/g protein)
C	3761.4	1621.2	4074.1	1941.9
S	3826.9	1842.3	3756.7	2094.9
10Gln	3561.2	1567.8	3988.6	1874.2
20Gln	3382.4	1742.4	4224.7	2212.3
30Gln	3641.8	1924.5	4396.3	1735.5
40Gln	3906.4	1701.8	3980.5	2331.7
50Gln	4016.4	2024.8	4338.5	2154.7
SEM	167.63	159.26	228.32	190.54
P-value	0.0654	0.5784	0.1719	0.3293

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
SEM: standard error of the means.

Table 5 Effects of *in ovo* feeding of glutamine (Gln) on concentration of blood of glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP) and alkaline phosphatase (ALP) of newly hatched chickens and broilers on 10 d old

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TP (g/dL)	ALP (U/L)
				Newly hatched chickens			
C	182.8^{e}	347.4	75.1	97.4	248.4	1.79^{c}	2598.2
S	179.3^{e}	383.2	78.3	92.5	272.7	1.80^{c}	2731.7
10Gln	212.4^{c}	395.7	62.8	103.4	302.5	1.89^{bc}	2629.5
20Gln	198.2^{d}	406.3	84.4	95.9	278.9	1.90^{bc}	2501.3
30Gln	205.6^{cd}	402.9	73.2	75.2	295.8	2.12^{a}	2484.3
40Gln	225.4^{b}	434.5	69.3	88.1	264.3	2.09^{ab}	2667.1
50Gln	241.3^{a}	448.5	64.8	77.2	232.0	2.16^{a}	2572.0
SEM	3.10	26.62	7.99	10.36	22.23	0.07	118.92
P-value	0.0001	0.0727	0.5363	0.6041	0.2587	0.0018	0.2771
				Broilers on 10 d old			
C	244.7	109.3	95.4	48.6	70.0	2.83	2945.2
S	260.2	101.2	90.3	50.7	83.2	2.56	3165.7
10Gln	228.2	95.1	79.2	45.5	74.4	3.05	3238.0
20Gln	238.8	109.4	107.1	39.6	59.7	2.79	2798.8
30Gln	254.3	119.7	103.1	36.8	78.2	2.94	2855.2
40Gln	249.4	111.6	109.0	32.3	64.7	2.87	2659.3
50Gln	234.5	98.3	98.8	43.4	69.4	2.73	3064.8
SEM	9.80	922	7.38	5.19	6.82	0.11	171.93
P-value	0.4458	0.6349	0.0904	0.1365	0.1164	0.0898	0.0595

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of *in ovo* feeding of glutamine (Gln) on number of heterophil and lymphocytes and heterophil to lymphocyte ratios (H/L) of newly-hatched chickens and broilers on 10 d old

Treatments	Newly hatched chickens			10 days of age		
	Heterophil (%)	Lymphocytes (%)	H/L	Heterophil (%)	Lymphocytes (%)	H/L
C	22.3^{bc}	71.3^{b}	0.3b^{cd}	21.1	76.2	0.27^{bc}
S	21.4^{c}	72.1^{b}	0.29^{d}	20.2	78.2	0.25^{c}
10Gln	25.1^{a}	74.0^{a}	0.33^{a}	24.1	73.2	0.33^{a}
20Gln	24.4^{a}	75.9^{a}	0.32^{abc}	22.1	74.9	0.29^{abc}
30Gln	24.7^{a}	74.8^{a}	0.33^{ab}	23.2	75.4	0.30^{ab}
40Gln	24.0^{a}	74.5^{a}	0.32^{abc}	20.7	77.0	0.26^{c}
50Gln	24.4^{a}	74.3^{a}	0.32^{abc}	19.9	76.2	0.26^{c}
SEM	0.52	0.63	0.006	1.05	1.14	0.01
P-value	0.0001	0.0002	0.0048	0.0667	0.1033	0.0007

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Moreover, Ds and Ala-Gln decreased hatchability by 24% and 18% (P<0.01), respectively.

The difference in among hatchability results of the IOF in the present and previous studies indicated that nutrient specificity might result in the differing response of embryos. The reduction in hatchability may probably result in the IOF into albumin (Salmanzadeh *et al.* 2016) or the sensitivity of the cavity under the air sac causing the respiration of developing an embryo to stop (Salmanzadeh *et al.* 2012). In the studies (Ohta *et al.* 2001; Kop-Bozbay and Ocak, 2015; Bozbay *et al.* 2016) showed that the effect of IOF of a substance on chicken hatchability might be related to the *in ovo* injection site. Thus, it was seen that any IOF could be harmful to the internal environment susceptibility and have negative effects on the hatching rate (Kop Bozbay and Ocak, 2019). This effect may largely depend on the effect of Gln injection. Moreover, one of the important factors affecting the embryo mortality is the osmolality of the solution, the maximum osmolality of the solution was 400-600 miliosmol (mOsm) suggested by Uni and Ferket (2003).

The acceptable hatchability of chicks was observed when the eggs were injected with solutions having an osmolarity ranging below 800 mOsm. Also, Kop Bozbay and Ocak (2019) noted that higher mortality in the IOF groups may be related to the high osmotic pressure of the injected solution, allergic cavities or the pure toxicity of the high amino acid levels concerned. Unacceptable hatching rates were observed when the *in ovo* feeding solution exceeded 800 mOsm. In the present study, the osmolality of all the injected solutions was adjusted to about 472 mOsm. Thus, osmolarity could not be the reason for the decrease of hatchability. This is consistent with the results obtained in the present study, in which the injection of different levels of Gln and deionized water caused a significant decrease in the hatchability. Also, in the experiment of Salmanzadeh *et al.* (2016) the osmolality of all the injections of the solution was adjusted to about 450 mOsm and the injection of different levels of Gln and deionized water in the albumin at the 7th day of incubation caused a significant decrease in the hatchability.

More reviewed studies related to the *in ovo* feeding showed that the IOF can be seen as an effective tool to improve the mean body weights of newly-hatched chickens. Moreover, a novel method of supplementing the *in the* (IO) nutrition of oviparous species, described as the *in ovo* feeding (IOF) within the US Patent (6592878) of Uni and Ferket (2003), was demonstrated to be an effective way to administer exogenous nutrient to support the development of the embryos and neonates in broiler hens (Uni and Ferket, 2004).

Also, the *in ovo* feeding of supplemental nutrients may help to overcome the constraint of limited egg nutrients (Foy *et al.* 2006). Previous studies demonstrated that the improved embryo development and nutritional status afforded by the *in ovo* feeding improved the hatching weight and growth rate (Al-Murrani, 1982; Ohta *et al.* 1999; Bhanja *et al.* 2004). Salmanzadeh *et al.* (2016) showed that the injection of different levels of Gln into albumin on the 7th day of incubation increased the weight of newly-hatched chickens. Chen *et al.* (2009) stated that the *in ovo* injection of carbohydrates improved duck weight gain in the early days of post-hatch. In the present study, the weight of newly-hatched chickens was significantly higher when Gln was injected *in ovo* compared with that of the control and sham groups. According to the past studies and our present observations, the late-term embryo and neonatal chicken depends on gluconeogenesis from amino acids, resulting in the depletion of muscle protein reserves and the reduction of hatching weight. To reduce the depletion of muscle protein, we carried out IOF Gln into albumen before hatching to support the energy status of the hatching by moderating the use of muscle that consequently increases body weight at hatch. In addition, food deprivation causes a 50% reduction in plasma IGF-I levels in chicks (Kita *et al.* 2002). Therefore, providing nutrients before hatching seems to support the growth of the neonate and help to overcome nutrient limitations of egg (Foye *et al.* 2006).

Also, because myofibers of chicks are already formed at hatching, higher availability of nutrients may cause hyperplasia in myofibers and then, higher muscle production (Ebrahimi *et al.* 2017). Foye *et al.* (2006) showed that the supply of the nutrient by IOF improved avian energy status, which spared energy used for metabolism and consequently, increased postnatal performance. Today, the whole embryonic life is almost 35% of the productive life of broilers. Thus, the stimulation of the development of the chick embryo is an important factor to increase the weight of newly-hatched chicks. Gln is a glucogenic amino acid. It is an amino acid that can be converted into glucose through gluconeogenesis. The production of glucose from glucogenic amino acids involves these amino acids being converted to alpha-keto acids and then to glucose, with both processes occurring in the liver and as a result increases blood glucose concentrations. Also, Gln is the precursor of some amino acids and contributes to protein synthesis. Consequently, Gln increases the blood protein concentration, muscle building and improves growth performance (Bartell, 2006).

Previous studies showed that the weight of newly-hatched chickens was a major predictor for marketing weight in modern broilers.

Table 7 Effects of *in ovo* feeding of glutamine (Gln) on weight of spleen, thymus and bursa of newly-hatched chickens (based on percentage of live body weight)

Treatments	Spleen (%)	Thymus (%)	Bursa (%)
		At hatch	
C	0.029[b]	0.502	0.096[c]
S	0.031[b]	0.489	0.108[c]
10Gln	0.041[a]	0.557	0.142[b]
20Gln	0.046[a]	0.498	0.145[ab]
30Gln	0.048[a]	0.564	0.152[ab]
40Gln	0.044[a]	0.595	0.149[ab]
50Gln	0.046[a]	0.585	0.161[a]
SEM	0.002	0.033	0.005
P-value	0.0001	0.0558	0.0001
		On 10 d old	
C	0.053	0.454	0.145[d]
S	0.056	0.447	0.138[d]
10Gln	0.068	0.464	0.169[bc]
20Gln	0.063	0.439	0.174[b]
30Gln	0.069	0.492	0.172[b]
40Gln	0.065	0.485	0.181[ab]
50Gln	0.076	0.458	0.195[a]
SEM	0.007	0.024	0.006
P-value	0.3557	0.7140	0.0001

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effects of *in ovo* feeding of glutamine (Gln) on Immunoglobulins G (IgG) and M (IgM) of broilers in 26

Treatments	Anti-SRBC (log_2)	IgG (mg/dL)	IgM (mg/dL)
C	5.23	1.79[cd]	2.41[c]
S	5.04	1.58[d]	2.36[c]
10Gln	5.73	1.86[c]	2.68[b]
20Gln	5.85	2.12[b]	2.74[b]
30Gln	5.34	2.42[a]	2.80[b]
40Gln	6.18	2.51[a]	2.62[b]
50Gln	5.56	2.34[a]	3.05[a]
SEM	0.34	0.07	0.07
P-value	0.1502	0.0001	0.0001

C: without injection of any solution (control); S: *in ovo* injection of 0.5 mL of deionized water (sham group) and 10Gln, 20Gln, 30Gln, 40Gln and 50Gln: *in ovo* injection of 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of Gln dissolved in 0.5 mL of deionized water, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Uni *et al.* (2005) observed that the inoculation of β-hydroxy β-methyl butyrate and carbohydrates improved body weight at hatch, improved breast yield at hatch and at 10 and 25 d of age, and increased liver glycogen content in chicks. According to these authors, the supplied energy-reduced muscle protein degradation serves as the energy source at hatch, resulting in higher body weight and breast percentage at hatch and this can improve feed conversion ratio (FCR).

Based on the results of this study, the growth performance of broiler chickens improved when injecting Gln at day 14 of incubation. These results are in agreement with the results of Salmanzadeh *et al.* (2016) who demonstrated that the injection of Gln on the 7th day of incubation stimulated the development of the gastrointestinal tract and improved the growth performance.

Also, these researchers stated that1g a difference in the weight of newly-hatched chickens due to IOF resulted in 109 to 130 g of increase in the bodyweight on the 42nd day of age. Bartell and Batal (2007) demonstrated that the addition of Gln to the diet of broiler chickens improved growth performance.

Previous studies showed that the dietary supplementation with Gln stimulated the intestine development and improved the growth performance of turkey poults one week after hatching (Yi *et al.* 2001), weanling pigs (Kitt *et al.* 2002), broilers (Bartell and Batal, 2007) and Japanese quails (Salmanzadeh and Shahryar, 2013b). On the other hand, in this study, the *in ovo* injection of Gln did not have any statistically significant effect on the activities of the

digestive enzyme (amylase and lipase) of newly-hatched chickens at 10 days of age.

Thus, it is seen that the IOF of Gln can be beneficial for the development of the gastrointestinal tract and have positive effects on growth performance. This effect could largely independent of the activities of the digestive enzyme. It is well demonstrated that Gln is the principal metabolic fuel for lymphocytes, macrophages and fibroblasts (Andrews and Griffths, 2002). Likewise, Calder and Yaqoob (1999) showed that in the absence of Gln immune system cells do not proliferate, but as the Gln concentration in the culture, medium increases lymphocyte proliferation. Gln is also the precursor for the net synthesis of Arginine, which has been shown to increase the size of thymus and spleen in mice (Adjei *et al.* 1998), increase the production of cytokine, and enhance the proliferation of the lymphocyte (Reynolds *et al.* 1988).

On the whole, the immune tissue development is the basis of immune functionality. Bartell and Batal (2007) showed that the relative weights of thymus and spleen of the broilers were significantly more when 1% Gln was supplemented in the food and water compared by the birds fed the control Corn-SBM diet.

Despite the Gln supplementation in the food, water or both did not affect the chicks' bursa weight. Also, supplementing the diet with 1% Gln significantly increased IgA, IgG, IgM and IFN-γ concentrations, and anti-SRBC titers in broiler chicks. Thus, the increase in the immune tissue weight resulting from Gln supplementation correlated with the functionality of thymus and spleen in terms of IgG and IgM production. Furthermore, It was not surprising to note an increase in the antibody concentrations and hemagglutination titers since the antibodies in the serum are mainly produced by antibody-forming cells located in the spleen and the development of the immune tissue is the basis of immune functionality (Russell and Ezeifeka, 1995; Al-Garib *et al.* 2000), which may explain the increase in spleen size observed in the broilers from injected eggs with Gln. The present study indicates that the modulation of antibody responses of chickens to SRBC was possible via the IOF of Gln.

As a conclusion, broiler breeder eggs treated *in ovo* with 10, 20, 30, 40 and 50 mg of Gln linearly increased weight of newly hatched chickens and stimulated immune response of broilers, and consequently, improved the growth performance whereas hatchability significantly depressed in all injected eggs compared to the not injected ones. Also, 50 mg is the best dose for Gln.

Data of this study showed that the IOF of Gln into the albumin can be seen as an effective tool to improve the mean body weights of newly hatched chickens. Previous studies showed that stimulating the development of the chick embryo is an important factor in increasing the weight of newly hatched chickens (Tako *et al.* 2004a; Uni and Ferket, 2004; Uni *et al.* 2005; Smirnov *et al.* 2006).

Also, previous studies showed that the weight of newly hatched chickens is a major predictor for marketing weight in modern broilers. Wilson (1991) stated that each 1 g of improvement in weight of newly hatched chickens leads to 8-13 g of improvement in body weight at marketing. In this study, we demonstrate that a 0.83 g difference in weight of newly hatched chickens due to IOF resulted in 139 g increase in body weight on day 42.

CONCLUSION

As a conclusion, *in ovo* administration of Gln into broiler breeder eggs significantly improve hatch weight, performance and immune response whereas hatchability significantly depressed in all injected eggs compared to the not injected ones. These results showed that reduction in hatchability may be related to the injection site. Consequently, further studies are needed to explore the effects of different sites of injection to determine the proper injection in broiler breeder eggs. On the other hand, with the advancement of science and technology of *in ovo*, hatchability is increasing.

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REFERENCES

Adjei A.A., Matsumoto Y., Oku T., Hiroi Y. and Yamamoto S. (1998). Dietary arginine and glutamine combination improves survival in septic mice. *Nutr. Res.* **14,** 1591-1599.

Al-Garib S.O., Gielkens A.L.J., Kok G. and Koch G. (2000). Double immunoenzymatic technique for *in situ* Ig-isotype-specific detection of cells producing antibody directed against Newcastle disease virus. Pp. 44-46 in Proc. 21st World's Poult. Congr., Montreal, Canada.

Al-Murrani W.K. (1982). Effect of injecting amino acids into the egg on embryonic and subsequent growth in the domestic fowl. *British Poult. Sci.* **23,** 171-174.

Andrews F.J. and Griffiths R.D. (2002). Glutamine: essential for immune nutrition in the critically ill. *British J. Nutr.* **1,** 3-8.

Ardawi M.S.M. and Newsholme E.A. (1983). Glutamine metabol-

ism in lymphocytes of the rat. *Biochem. J.* **212,** 835-842.

Bartell S.M. (2006). The effect of supplemental glutamine on growth performance, development of gastrointestinal tract, and immune response of broiler. MS Thesis. The University of Georgia, Athens, Georgia.

Bartell S.M. and Batal A.B. (2007). The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poult. Sci.* **86,** 1940-1947.

Bhanja S.K., Mandal A.B. and Goswami T.K. (2004). Effect of *in ovo* injection of amino acids on growth, immune response, development of digestive organs and carcass yield of broiler. *Indian J. Poult. Sci.* **39,** 212-218.

Bozbay C.K., Konanç K., Ocak N. and Öztürk E. (2016). The effects of *in ovo* injection of propolis and injection site on hatchability, hatching weight and survival of chicks. *Turkish J. Agric. Res.* **3,** 48-54.

Calder P.C. and Yaqoob P. (1999). Glutamine and the immune system. Pp. 227-241 in Proc. Int. Congr. Amino Acids., Mainz, Germany.

Chen W., Wang R., Wan H.F., Xiong X.L., Peng P. and Peng J. (2009). Influence of *in ovo* injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. *British Poult. Sci.* **50,** 436-442.

Cruzat V., Rogero M.M., Keane K.N., Curi R. and Newsholme P. (2018). Glutamine: Metabolism and immune function, supplementation and clinical translation. *Nutrients.* **10,** 1564.

Curi T.C., De Melo M.P., De Azevedo R.B., Zorn T.M.T. and Curi R. (1997). Glutamine utilization by rat neutrophils: Presence of phosphate-dependent glutaminase. *Am. J. Physiol.* **273,** 1124-1129.

Dong X.Y., Jiang Y.J., Wang M.Q., Wang Y.M. and Zou X.T. (2013). Effects of *in ovo* feeding of carbohydrates on hatchability, body weight, and energy status in domestic pigeons. *Poult. Sci.* **92,** 2118-2123.

Dos Santos T.T., Corzo A., Kidd M.T., McDaniel C.D., Torres Filho R.A. and Araújo L.F. (2010). Influence of *in ovo* inoculation with various nutrients and egg size on broiler performance. *J. Appl. Poult. Res.* **19,** 1-12.

Ebrahimi M., Janmohammadi H., Daghigh kia H., Moghaddam G., Rajabi Z., Abbas raft S. and Javanmard A. (2017). The effect of L-lysine *in ovo* feeding on body weight characteristics and small intestine morphology in a day-old Ross broiler chicks. *Rev. Med. Vet*. **168,** 116-124.

Foye O.T., Uni Z. and Ferket P.R. (2006). Effect of *in ovo* feeding egg white protein, β-hydroxy-β-methylbutyrate, and carbohydrateson glycogen status and neonatal growth of turkeys. *Poult. Sci.* **85,** 1185-1192.

Gross W.B. (1978). Comparison of dose effect of sheep red blood cells on antibody response in two lines of chickens. *Avian. Dis.* **23,** 526-528.

Ipek A., Sahan U. and Yilmaz B. (2004). The effect of *in ovo* ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. *Arch. Geflugelkd.* **63,** 132-135.

Jazideh F., Farhoomand P., Daneshyar M. and Najafi G. (2014). The effects of dietary glutamine supplementation on growth performance and intestinal morphology of broiler chickens reared under hot conditions. *Turkish J. Vet. Anim. Sci.* **38,** 264-270.

Kew S., Wells S.M., Yaqoob P., Wallace F.A. and Miles E.A. (1999). Dietary glutamine enhances murine T-lymphocyte Responsiveness. *J. Nutr.* **129,** 1521-1531.

Kita K., Nagao K., Taneda N., Inagaki Y., Hirano K., Shibata T., Yaman M.A., Conlon M.A. and Okumura J. (2002). Insulin-like growth factor binding protein-2 gene expression can be regulated by diet manipulation in several tissues of young chickens. *J. Nutr.* **132,** 145-151.

Kitt S.J., Miller P.S., Lewis A.J. and Fischer R.L. (2002). Effects of glutamine on growth performance and small intestine villus height in weanling pigs. *Nebraska Swine Report*. **82,** 29-32.

Kop-Bozbay C. and Ocak N. (2015). Growth, digestive tract and muscle weights in slow-growing broiler is not affected by a blend of branched-chain amino acids injected into different sites of egg. *J. Agric. Environ. Sci.* **4,** 261-269.

Kop Bozbay C. and Ocak N. (2019). *In ovo* injection of branched chain amino acids: Embryonic development, hatchability and hatching quality of turkey poults. *J. Anim. Physiol. Anim. Nutr.* **103,** 1135-1142.

Moghaddam A., Karimi I., Borji M., Bahadori S. and Abdolmohammadi A. (2013). Effect of royal jelly *in ovo* injection on embryonic growth, hatchability, and gonadotropin levels of pullet breeder chicks. *Theriogenology.* **80,** 193-198.

Newsholme P. (2001). Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? *J. Nutr.* **131,** 2515-2522.

Newsholme P., Curi R., Gordon S. and Newsholme E.A. (1986). Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem. J.* **239,** 121-125.

Nowaczewski S., Kontecka H. and Krystianiak S. (2012). Effect of *in ovo* injection of vitamin C during incubation on hatchability of chickens and ducks. *Folia. Biol. (Kraków).* **60,** 93-97.

Ohta Y., Kidd M.T. and Ishibashi T. (2001). Embryo growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after *in ovo* administration of amino acids. *Poult. Sci.* **80,** 1430-1436.

Ohta Y., Tsushima N., Koide K., Kidd M.T. and Ishibashi T. (1999). Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. *Poult. Sci.* **78,** 1493-1498.

Ohta Y., Yoshida T. and Tsushima T. (2004). Comparison between broilers and layers for growth and protein use by embryos. *Poult. Sci.* **83,** 783-787.

Pedroso A.A., Chaves L.S., Cafe M.B., Leandro N.S.M., Stringhini J.H. and Menten J.F.M. (2006). Glutamine as broilers embryos nutrient. *Brazilian J. Poult. Sci.* **8,** 43-49.

Piquer F.J., Sell J.L., Al-Batshan H.A., Mallarino E.G., Soto-Salanova M.F. and Angel C.R. (1991). Post-hatching changes in the immunoglobulin a concentration in the jejunum and bile of turkeys. *Poult. Sci.* **70,** 2476-2483.

Reynolds J.V., Daly J.M. and Zhang S. (1988). Immunomodulatory mechanism of arginine. *Surgery.* **104,** 141-151.

Rifai N., Bachorik P.S. and Albers J.J. (1999). Lipids, lipoproeins and apolipoproteins. Pp. 809-861 in Tietz Textbook of Clinical

Cgemistry. C.A. Burtis and E.P. Ashwood, Eds. W.B Saunders Company, Philadelphia, Pennsylvania.

Roberts I., Kwan I., Evans P. and Haig S. (2002). Does animal experimentation inform human healthcare? Observations from a systematic review of international animal experiments on fluid resuscitation. *British Med. J.* **324,** 474-476.

Russell P.H. and Ezeifeka G.O. (1995). The hitchner B1 strain Newcastle disease virus induces high levels of IgA, IgG, and IgM in newly hatched chicks. *Vaccine.* **13,** 61-66.

Salahi A., Mousavi S.N., Foroudi F., Moosanezhad Khabisi M. and Norozi M. (2011). Effects of *in ovo* injection of butyric acid in broiler breeder eggs. *Global Vet.* **7,** 468-477.

Salmanzadeh M. (2011). The effects of *in ovo* injection of glucose on hatchability, hatching weight and subsequent performance of newly-hatched chicks. *Brazilian J. Poult. Sci.* **14,** 137-140.

Salmanzadeh M., Ebrahimnezhad Y., Shahryar H.A. and Beheshti R. (2012). The effects of *in ovo* injection of glucose and magnesium in broiler breeder eggs on hatching traits, performance, carcass characteristics and blood parameters of broiler chickens. *Arch. Geflügelkd.* **76,** 277-284.

Salmanzadeh M., Ebrahimnezhad Y., Shahryar H.A. and Ghiasi Ghaleh-Kandi J. (2016). The effects of *in ovo* feeding of glutamine in broiler breeder eggs on hatchability, development of the gastrointestinal tract, growth performance and carcass characteristics of broiler chickens. *Arch. Anim. Breed.* **59,** 235-242.

Salmanzadeh M., Ebrahimnezhad Y., Shahryar H.A. and Lotfi A. (2011). The effects of *in ovo* injection of L-threonine in broiler breeder eggs on characters of hatching and growth performance broiler chickens. *European J. Exp. Biol.* **1,** 164-168.

Salmanzadeh M., Ghanouni Bostanabad J. and Arva S. (2014). The effects of *in ovo* injection of butyric acid into quails breedereggs on hatchability, growth performance, development of the gastrointestinal tract, and carcass traits of Japanese quails. *Bull. Environ. Pharmacol. Life Sci.* **3,** 126-130.

Salmanzadeh M. and Shahryar H.A. (2013a). Effects of dietary supplementation with glutamine on growth performance, small intestinal morphology and carcass traits in turkey poults under heat stress. *Rev. Méd. Vét.* **164,** 476-480.

Salmanzadeh M. and Shahryar H.A. (2013b). Effects of dietary glutamine addition on growth performance, carcass characteristics and development of the gastrointestinal tract in Japanese quails. *Rev. Méd. Vét.* **164,** 471-475.

Samli H.E., Senkoylu N., Koc F., Kanter M. and Agma A. (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, guthistomorphology and microbiota. *Arch. Anim. Nutr.* **61,** 42-49.

SAS Institute. (2005). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Smirnov A., Tako E., Ferket P.R. and Uni Z. (2006). Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by *in ovo* feeding of carbohydrates. *Poult. Sci.* **85,** 669-673.

Tako E., Ferket P.R. and Uni Z. (2004a). Effects of *in ovo* feeding of carbohydrates and β- hydroxy-β-methylbutyrate on the development of chicken intestine. *Poult. Sci.* **83,** 2023-2028.

Tako E., Ferket P.R. and Uni Z. (2004b). Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra-amniotic zinc-methionine administration. *J. Nutr. Biochem.* **16,** 339-346.

Teshfam M. (1984). Comparison of the effects of the high-acid milk replacer with conventional skim milk replacer. Ph D. Thesis. University of Bristol, Bristol, United Kingdom.

Uni Z. and Ferket P.R. (2003). Enhancement of development of oviparous species by *in ovo* feeding. *US Patent*. **6,** 878.

Uni Z. and Ferket R.P. (2004). Methods for early nutrition and their potential. *World's Poult. Sci.* **60,** 101-111.

Uni Z., Ferket P.R., Tako E. and Kedar O. (2005). *In ovo* feeding improves energy status of lateterm chicken embryos. *Poult. Sci.* **84,** 764-770.

Wells S.M., Kew S., Yaqoob P., Wallace F. and Calder P. (1999). Dietary glutamine enhances cytokine production by murine macrophages. *Nutrition.* **15,** 881-884.

Wilson J.H. (1991). Bone strength of caged layers as affected by dietary calcium and phosphorus concentrations, reconditioning, and ash content. *British Poult. Sci.* **32,** 501-508.

Yeh S.L., Yeh C.L., Lin M.T., Lo P.N. and Chen W.J. (2001). Effects of glutamine-supplemented total parenteral nutrition on cytokine prodution and T cell population in septic rats. *J. Parent. Enteral. Nutr.* **25,** 269-274.

Yi G.F., Allee G.L., Knight C.D. and Dibner J.J. (2005). Impact of glutamine and oasis hatchling supplement on growth performance, small intestinal morphology, and immune response of broilers vaccinated and challenged with *Eimeria maxima*. *Poult. Sci.* **84,** 283-293.

Yi G.F., Allee G.L., Spencer J.D., Frank J.W. and Gaines A.M. (2001). Impact of glutamine, menhaden fish meal and spray-dried plasma on the growth performance and intestinal morphology of turkey poults. *Poult. Sci.* **80,** 201-226.