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Research paper

Influence of *Eimeria* spp. infection and dietary inclusion of arginine on intestine histological parameters, serum amino acid profile and ileal amino acids digestibility in broiler chicks

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ABSTRACT

Coccidiosis is considered to be one of the most important challenge in the poultry industry causes economic losses due to the destruction in the digestive tract of chicken. It disturbs amino acids profile and their digestibility, leading to weight lost and economic burden. Using dietary arginine may decrease the adverse effects of coccidiosis on chicken digestive tract. This study aimed to evaluate the effects of dietary inclusion of arginine on intestine histological parameters, serum amino acid concentration and ileal amino acid digestibility of broiler chicks infected with coccidiosis. A total number of 384 one-ᴅ-old broiler chicks (Ross 308) of mixed sex with initial weight of 42 ± 2 g was allocated into 8 groups with 8 birds/pen from grower period. At 21 days of age, broiler chicks were infected with a mixture of *Eimeria* spp. Broiler chicks were divided into infected and uninfected groups and received arginine at recommended levels of 85, 100, 125 and 150 %. Intestinal morphology and lesions, serum amino acid concentration and ileal amino acid digestibility were evaluated. Broiler chicks infected with *Eimeria* spp. showed lower villus height and villus height: crypt depth ratio and also higher intestinal lesions ($P < 0.05$). Coccidia infection decreased the ileal amino acid digestibility for all studied amino acids and also reduced serum concentrations of amino acids, except lysine and isoleucine ($P < 0.05$). Dietary supplementation of arginine especially in higher levels significantly increased villus height and villus height:crypt depth ratio and decreased lesions ($P < 0.05$). Moreover, dietary supplementing of arginine increased the serum concentration of arginine ($P < 0.05$), but it did not have any significant effect on its digestibility ($P > 0.05$). In sum, coccidiosis decreases amino acid digestibility and serum amino acid concentration, but dietary inclusion of higher levels of arginine significantly improved histological parameters of broiler chicks infected with coccidiosis.

1. Introduction

Poultry breeders usually try to obtain maximum profit and best efficiency without the risk of challenge with infectious pathogens. Infectious challenges like parasite infections considerably decrease productivity and economic efficiency in herd (El-katcha et al., 2018). Coccidiosis is an important infectious challenge in the poultry industry that causes much economic loss. The protozoan parasite, *Eimeria* genus causes avian coccidiosis which is associated with decrease productivity and economic loss and anticoccidial drugs are used to control this parasite (Chapman, 2014). Coccidiosis vaccines are usually used for controlling *Eimeria* infections and decreasing dependence on anticoccidial drugs (Lee et al., 2009). Coccidial infections not only increase oxidative and induce gut damages (Tan et al., 2014), but also increase histopathological changes in avian intestine and parenchymal organs (Koinarski et al., 2005). Coccidial infections cause injuries in epithelial cells and induce diarrhea, osmotic stress in the intestine (Perez-Carbajal et al., 2010) and decrease nutrients absorption (Metzler-Zebeli et al., 2009). Coccidiosis causes ileal amino acid digestibility malfunction of diets in broiler chicks (Amerah and Ravindran, 2015; Rochell et al.,

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2016). Disproportionate alterations in digestibility of amino acids increase the difference between the target and actual profile (Rochell, 2015). The blood amino acid concentration of an animal at a given time-point shows the net effect of amino acid appearance from dietary absorption and tissue release and its disappearance (Cynober, 2002). Coccidial infections negatively affect the morphology of the intestine, such as villi length and width (Nabian et al., 2018).

Coccidiosis decreases the plasma concentrations of arginine (Rochell et al., 2016) due to increased need for production of nitric oxide (Allen and Fetterer, 2000). Arginine is an essential amino acid in birds, because birds cannot synthesize arginine due to lack of urea cycle (Sathyapriya et al., 2018). Arginine promotes cell proliferation (Murakami et al., 2004) and cellular migration (Rhoads et al., 2006). Dietary inclusion of arginine (500 mg/kg) significantly decreased lesion scores of broiler chicks (Rochell, 2015). Supplementation with some specific dietary additives decreases the negative effects of infection induced by *Eimeria* (Rochell, 2015). Presence of essential amino acids, protein digestibility, and bioavailable amino acids are the key factors for evaluating the protein's quality (Gilani et al., 2005), especially under challenge conditions. Seemingly, arginine can decrease negative effects of coccidiosis on intestinal injuries, but the effects of arginine on serum amino acid profile and ileal digestibility under challenge conditions have not been investigated so far. This study was conducted to evaluate the effects of different levels of arginine on histological parameters, serum amino acid concentration and ileal amino acid digestibility of broiler chicks infected with coccidiosis.

2. Materials and methods

2.1. Broiler chicks and experimental treatments

The animal experimental procedures described in this study were approved by the Animal Care Committee of Tabriz University (East Azerbaijan-Iran) (No. 1621). A total number of 384 one-d-old broiler chicks (Ross 308) of mixed sex with initial weight of 42 ± 2 g was purchased from Kimia joojeh Amol Company (Mazandran, Iran). A lighting program (23 h light: 1 h darkness) was applied within experimental period. Broiler chicks were reared in pens covered with fresh wood shavings. Water and feed were *ad libitum* provided during experiment.

Temperature was maintained at 35 °C during the first week and then gradually reduced on the basis of common management practices. Sanitation procedures were considered before and during the rearing periods. All the broiler chicks received a corn-soybean meal basal diet (lack of coccidiostats) that met all the Ross catalogue requirements (Aviagen, 2014) for broiler chicks (Table 1). The rearing periods included starter (1–10 days), grower (11–24 days) and finisher (25–42 days).

Broiler chicks were randomly allocated into 4 treatments with 6 replications and 16 birds per pen. Arginine was mixed with fed diet which was supplemented with 85, 100, 125 and 150 % of recommended digestible arginine in the first three weeks. 21 days after the study was started, half of broiler chicks was infected with *Eimeria* and then divided into 8 groups with 6 replications and 8 birds/replicate. Experimental treatments included: The challenged broiler chicks treated with 85 % (E-85), 100 % (100-E), 125 % (125-E) and 150 % (150-E) of recommended arginine. Other treatments included: The non-challenged broiler chicks treated with 85 % (N-85), 100 % (N-100), 125 % (N-125) and 150 % (N-150) of recommended arginine. To induce infection, an oral inoculum (1.5 mL) containing 2×10^5 sporulated oocysts of *E.necatrix* (7.5 %), *E. maxima* (10 %), *E.acervulina* (7.5 %) and *E.tenella* (75 %) was administrated per broiler chicks, at day 21. The Eimeria inoculum preparation was obtained from the Faculty of Veterinary Medicine, University of Tehran (Tehran-Iran). The inoculation of oocysts of Eimeria was made by a suspension of mixed Eimeria oocysts and their percentages were calculated by morphological characteristic of oocysts. The species of oocysts was selected based on the more pathogenic species present in our study area.

Table 1
Feed ingredients and nutrient composition of experimental diets during different periods.

Ingredients (g/kg)	Starter	Grower	Finisher
Corn	506.2	541.79	587.9
Soybean Meal	369.54	329.81	289.26
Corn Gluten Meal	50	50	40
Di-Calcium Phosphate	19.42	17.88	15.09
Calcium carbonate	12.07	11.27	10.53
Mineral Mixture[1]	2.5	2.5	2.5
vitamin Mixture[2]	2.5	2.5	2.5
DL-methionine	1.349	1.1	1.02
L-lysine	2.719	2.218	1.94
L-threonine	1.506	1.05	0.846
L-Arginine	0.676	0.242	0.074
Vegetable oil	27.55	35.640	44.32
Salt	2.77	2.8	2.82
Sodium bicarbonate	1.2	1.2	1.2
Nutrients			
ME (Mcal/Kg)	3000	3100	3200
Crude protein (%)	23	21.5	19.5
Ca (%)	0.96	0.87	0.79
Available phosphorous (%)	0.48	0.435	0.395
K (%)	0.91	0.84	0.77
Na (%)	0.17	0.17	0.17
Cl (%)	0.19	0.2	0.2
Met (%)	0.51	0.47	0.43
Met + Cys (%)	0.85	0.79	0.72
Lys (%)	1.28	1.15	1.03
Arg (%)	1.37	1.23	1.10
Thr (%)	0.86	0.77	0.69
Try (%)	0.2	0.2	0.18
Crude Fiber (%)	3.67	3.46	3.24
Ether Extract (%)	4.78	5.69	6.6
Choline	1.7	1.6	1.5
Linoleic Acid (%)	2.23	2.61	3.01

[1] Mineral premix provided per kilogram of diet: Mn (MN_3O_4), 120 mg, Zn ($ZnSO_4{\cdot}H_2O$), 102 mg, Fe ($FeSO_4{\cdot}5H_2O$), 40 mg, Cu ($CuSO_4{\cdot}5H_2O$), 10 mg, I (ca $(Io_3)_2$, X H_2O), 1.5 mg, Se (Na_2seo_3), 0.35 mg.

[2] Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU, cholecalciferol, 4500 IU, vitamin E (DL-α-tocopheryl acetate), 62.5 IU, vitamin K (menadione sodium bisulfite), 3 mg, thiamine, 3 mg, riboflavin, 6.6 mg, nicotin amide, 55 mg, calcium pantothenate, 20 mg, pyridoxine, 5 mg, folic acid, 1.92 mg, biotin, 0.20 mg, vitamin B12, 0.016 mg, choline (choline chloride, 60 %), 500 mg, and Antioxidant, 150 g.

Arginine was purchased from CJ Corporation (South Korea) and in the form of L-arginine HCl. Arginine contents of feed samples were determined by an ion exchange high performance liquid chromatography (HPLC) (Biochrom 20 Amino Acid Analyzer; Biotronik GmbH, Maintal, Germany), by post-column ninhydrin derivatization and fluorescence detection. Arginine amount in the different periods is shown in Table 2.

2.2. Ileal digesta collection, serum and ileal amino acid profile

On day 42, two birds per replication were euthanized by intravenous administration of Ketamine hydrochloride. The contents of the ileum were collected in plastic bags by gently flushing with distilled water and air pressure. Ileal digesta was kept at -20 °C, and subsequently freeze dried. It was then ground by a coffee grinder for passing by a 0.5 mm sieve and kept in plastic tubes at-4 °C for chemical analyses (Bandegan

Table 2
Digestible arginine (%) in the different periods.

Periods	85 % Arg	100 % Arg	125 % Arg	150 % Arg
Starter	1.164	1.37	1.712	2.055
Grower	1.045	1.23	1.537	1.845
Finisher	0.935	1.1	1.375	1.65

et al., 2009). The amino acid digestibility of the ileal digesta were assessed as reported by Palliyeguru et al. (2010) using an AA analyzer (Biochrom 30 plus, Biochrom Ltd. Cambridge, UK). In summary, hydrogen peroxide-formic acid-phenol solution was used for oxidizing the samples. To degrade the excess oxidation reagent, sodium disulphite was used. Following oxidation, samples were hydrolyzed by 6 M HCl for 24 h (pH = 2.20). Amino acid analyzer was used for separation of amino acids (AOAC, 2000). To analyze the serum samples, the blood samples were collected from 2 birds per replication, centrifuged at 2500 *g* for 15 min and sera samples were analyzed by HPLC using a lithium cation-exchange column (Model 0,354,100 T, Pickering Laboratories, Inc., Mountain View, CA) and then by post-column ninhydrin derivatization and UV light detection of individual amino acids.

2.3. Lesion scoring and histopathological assessment

At day 42, the small intestine (duodenum and jejunum) and ceca were excised for lesion scores. Tissues samples were randomly taken from the three cross sections for each intestinal segment (duodenum, jejunum, and ceca) as reported by Ozdemir et al. (2009). Scoring was conducted from light (+) to severe (+++). For histopathological evaluation, 2 cm sections of duodenum and jejunum were collected and placed in 10 % buffered formalin. The duodenal and jejunal sections were processed, examined and scored in the manner previously described by Ozdemir et al. (2009). Furthermore, the hematoxylin and eosin staining were performed for assessment of histopathological changes.

2.4. Jejunal morphology

At the end of trial, jejunal segments were separated from midpoint between the bile duct entry and Meckel's diverticulum, washed in saline 85 % and fixed in 10 % buffered formaldehyde, dehydrated by ethanol and xylene, embedded in paraffin wax, cut into 5 µm thickness, and stained with hematoxylin and eosin. The prepared samples were evaluated by light microscope. Villus width (VW) was evaluated for each villus; villus height (VH) was evaluated from the top of the villus to the villus-crypt junction, and crypt depth (CD) was evaluated from the base of the villus to the submucosa.

2.5. Statistical analysis

This study was conducted based on a completely randomized design in a 4 × 2 factorial arrangement with infection (challenged and non-challenged) and dietary supplement of arginine (85, 100, 125 and 150 % of the recommended levels). The data for lesion scores were analyzed as a completely randomized design by ANOVA procedure. Other parameters were analyzed for the main effects of infection and arginine and the interactions between infection and arginine. The parameters were analyzed as follows:

$$Yijk=\mu+(Ii)+(Aj)+(IAij)+(eijk)$$

Where Yijk is the assessed variable, µ is the overall average, (Ii) is the main effect of infection, (Aj) is the main effect of arginine, (IAij) is interaction between infection and arginine and (eijk) is the residual error. If interaction was significant, main effects were not considered. The data were analyzed by General Linear Model procedure of SAS (SAS software, 2008). The differences among group was calculated by Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. Serum amino acid concentration

The results for serum concentrations of amino acids are shown in Table 3. The results showed that amino acid concentrations of histidine, threonine, tryptophan, valine and leucine were not influenced by challenge with coccidiosis ($P > 0.05$). Moreover, our results showed that challenge significantly increased the serum concentrations of lysine and isoleucine ($P < 0.05$) and decreased the serum concentration of methionine, arginine, glycine, glutamic acid, aspartic acid and alanine ($P < 0.05$) compared to non-challenged broiler chicks. Broiler chicks fed with higher levels of arginine (125 and 150 %) showed higher serum concentrations for arginine, glutamic acid, aspartic acid and alanine compared to broiler chicks fed with 85 % arginine ($P < 0.05$). Interactive effects for arginine and challenge were not observed ($P > 0.05$).

3.2. Ileal amino acids digestibility

The results for ileal amino acids digestibility are presented in Table 4. The results showed that ileal amino acids digestibility for all the amino acids was significantly lower in the infected broiler chicks compared to non-infected broiler chicks ($P < 0.05$). Dietary supplementing of arginine did not have any significant effect on ileal amino acids digestibility ($P > 0.05$) and only isoleucine digestibility was significantly lower in broiler chicks fed with 150 % arginine compared to those received other levels ($P < 0.05$). Interactive effects between arginine and *Eimeria* infection was only observed for isoleucine digestibility ($P < 0.05$) and non-infected broiler chicks receiving arginine in highest level showed lower digestibility compared to non-infected broiler chicks that received lower levels ($P < 0.05$).

3.3. Histopathological assessment

Lesions in different parts of intestine were not observed in the un-infected broiler chicks. The results for the intestinal gross investigation are shown in Table 5. Dietary inclusion of arginine significantly decreased lesion scores in duodenum, jejunum and ceca ($P < 0.05$). The lowest values for lesion scores were observed in broiler chicks fed diets containing 125 and 150 % arginine compared to those received lower levels ($P < 0.05$). Histological parameters are shown in Fig. 1. The results showed that extra arginine decreases various coccidial stages in the cecal mucosal tissues (Fig. 1C and D) compared to those received lowest level (85) and recommended level (100) of arginine (Fig. 1 A and B).

3.4. The intestinal morphometric parameters

The data for jejunal morphology at 21 d post-infection are summarized in Table 6. Interactive effects between coccidial infection and arginine supplementation were observed for jejunal morphometric parameters, including villus height, villus width, crypt depth, villus height-to-crypt depth ratio). Infection with *Eimeria* spp significantly decreased the jejunal villus height and villus height: crypt depth ratio and increased villus width and crypt depth ($P < 0.05$). Supplementation of higher level of arginine (125 and 150 %) in the diet significantly increased villus height and crypt depth compared to lowest level (85 %).

4. Discussion

This study was conducted to evaluate the effect of *Eimeria* spp infection and dietary inclusion of arginine on intestine histological parameters, serum amino acid profile and ileal amino acid digestibility of broiler chicks. The results showed that challenge with coccidiosis infection increased the serum concentrations of lysine and isoleucine ($P < 0.05$), but decreased the serum concentration of methionine, arginine, glycine, glutamic acid, aspartic acid and alanine ($P < 0.05$) compared to un-infected broiler chicks. Inclusion of arginine (125 and 150 %) into feed increased the serum concentrations for arginine, glutamic acid, aspartic acid and alanine compared to broiler chicks fed with 85 % arginine ($P < 0.05$). It means that coccidial infection can have a substantial effect on the serum concentrations of amino acids. Previous

Table 3
Effects of dietary arginine supplementation and coccidiosis on serum concentration of amino acids in healthy and challenged broiler chicks.

Groups	Met	Lys	Arg	His	Thr	Trp	Val	Leu	Ile	Gly	Glu	Asp	Ala
N-85	198.66^{a}	257.00^{d}	509.33^{b}	117.16	1224.33	58.33	240.83	277.50	158.00^{ab}	709.66^{a}	215.33^{bc}	30.50^{b}	1324.00^{b}
N-100	201.33^{a}	260.00^{cd}	528.66^{a}	121.16	1209.66	59.16	241.16	276.66	157.50^{abc}	712.16^{a}	218.00^{ab}	33.16^{a}	1357.66^{a}
N-125	201.00^{a}	261.83^{cd}	521.66^{a}	122.00	1226.66	59.83	240.94	276.66	153.00^{c}	712.83^{a}	219.66^{a}	33.70^{a}	1359.33^{a}
N-150	200.30^{a}	261.33^{cd}	525.33^{a}	120.66	1203.33	60.41	241.00	276.06	156.33^{bc}	718.00^{a}	220.41^{a}	33.00^{a}	1358.66^{a}
E-85	180.66^{c}	278.33^{abc}	498.00^{c}	116.75	1210.00	59.50	243.83	276.66	161.83^{a}	652.00^{c}	208.33^{d}	23.66^{c}	1235.66^{d}
E-100	188.00^{b}	265.00^{bcd}	500.66^{bc}	121.00	1168.33	59.00	242.66	275.00	161.50^{a}	659.33^{bc}	209.00^{d}	24.16^{c}	1262.33^{c}
E-125	187.66^{b}	280.33^{ab}	499.66^{bc}	121.66	1224.33	59.50	242.33	274.00	161.83^{a}	658.33^{bc}	211.00^{d}	25.16^{c}	1264.66^{c}
E-150	185.66^{b}	284.00^{a}	500.83^{bc}	122.00	1216.33	58.88	240.81	272.83	161.16^{a}	664.16^{b}	211.66^{cd}	23.33^{c}	1257.33^{cd}
P-value	0.000	0.019	0.000	0.558	0.303	0.633	0.751	0.381	0.005	0.000	0.000	0.000	0.000
SEM	1.72	2.66	2.65	0.79	6.09	0.211	0.47	0.53	0.76	5.82	1.01	0.93	10.49
Infection													
Non-challenged	200.30^{a}	260.00^{b}	521.30^{a}	120.30	1216.00	59.56	241.00	276.70	156.20^{b}	713.20^{a}	218.40^{a}	32.59^{a}	1350.00^{a}
Challenged	185.50^{b}	276.90^{a}	499.80^{b}	120.40	1205.00	59.22	242.40	274.60	161.60^{a}	658.50^{b}	210.00^{b}	24.08^{b}	1255.00^{b}
Arginine													
85	189.66	267.66	503.66^{b}	116.95	1217.66	59.16	242.33	277.08	159.91	680.83	211.83^{b}	27.08^{b}	1279.83^{b}
100	194.66	262.50	514.66^{a}	121.08	1189.00	59.08	241.91	275.83	159.50	685.75	213.50^{ab}	28.66^{a}	1310.00^{a}
125	194.33	271.08	510.66^{a}	121.83	1225.50	59.66	241.64	275.33	157.41	685.58	215.33^{a}	29.43^{a}	1312.00^{a}
150	193.00	272.66	513.08^{a}	121.33	1209.83	59.65	240.90	274.45	158.75	691.08	216.04^{a}	28.16^{ab}	1308.00^{a}
Infection	0.000	0.001	0.000	0.949	0.348	0.449	0.183	0.063	0.000	0.000	0.000	0.000	0.000
Arginine	0.101	0.327	0.015	0.164	0.188	0.691	0.795	0.379	0.386	0.372	0.027	0.027	0.001
Interaction	0.642	0.414	0.087	0.978	0.430	0.394	0.753	0.855	0.330	0.878	0.872	0.254	0.847

Superscripts show significant differences in per column ($P \leq 0.05$). SEM: Standard error of means.

Table 4
Effects of dietary arginine supplementation on ileal amino acids digestibility of healthy and challenged broiler chicks.

Groups	Met	Lys	Arg	His	Thr	Trp	Val	Leu	Ile	Gly	Glu	Asp	Ala
N-85	91.50^{a}	85.13^{a}	87.96^{a}	82.50^{a}	74.06^{a}	76.13^{a}	76.96^{a}	82.83^{a}	79.50^{a}	75.00^{b}	84.83^{a}	77.83^{a}	78.00^{a}
N-100	91.00^{a}	85.83^{a}	87.50^{a}	82.50^{a}	74.00^{a}	76.16^{a}	77.50^{a}	83.31^{a}	80.50^{a}	75.83^{ab}	85.20^{a}	78.50^{a}	78.30^{a}
N-125	91.16^{a}	85.66^{a}	87.46^{a}	82.00^{a}	75.00^{a}	76.01^{a}	77.83^{a}	83.50^{a}	80.50^{a}	76.16^{ab}	85.50^{a}	78.44^{a}	73.50^{a}
N-150	91.33^{a}	85.50^{a}	87.98^{a}	82.16^{a}	75.00^{a}	76.45^{a}	77.66^{a}	83.10^{a}	75.53^{b}	76.66^{a}	85.75^{a}	77.65^{a}	79.33^{a}
E-85	86.17^{b}	81.48^{b}	82.50^{b}	76.50^{b}	69.16^{b}	70.83^{b}	70.00^{b}	75.78^{b}	75.63^{b}	71.81^{c}	81.50^{b}	72.00^{b}	73.16^{b}
E-100	86.83^{b}	80.83^{b}	82.00^{b}	77.96^{b}	69.83^{b}	70.50^{b}	71.00^{b}	75.86^{b}	76.16^{b}	71.31^{c}	82.14^{b}	72.06^{b}	72.50^{b}
E-125	86.66^{b}	80.33^{b}	82.00^{b}	77.67^{b}	69.66^{b}	71.00^{b}	71.16^{b}	76.50^{b}	75.83^{b}	71.33^{c}	81.66^{b}	71.83^{b}	72.83^{b}
E-150	86.50^{b}	81.16^{b}	83.00^{b}	78.33^{b}	69.16^{b}	70.83^{b}	71.16^{b}	76.33^{b}	76.16^{b}	72.00^{c}	82.15^{b}	72.08^{b}	72.83^{b}
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0000	0.000	0.000	0.000	0.000	0000
SEM	0.53	0.52	0.60	0.55	0.56	0.58	0.71	0.74	0.45	0.47	0.38	0.65	0.65
Infection													
Non-challenged	91.25^{a}	85.53^{a}	87.73^{a}	82.29^{a}	74.52^{a}	76.19^{a}	77.49^{a}	83.19^{a}	79.01^{a}	76.04^{a}	85.32^{a}	78.11^{a}	78.53^{a}
Challenged	86.54^{b}	80.95^{b}	82.20^{b}	77.62^{b}	69.46^{b}	70.79^{b}	70.83^{b}	76.12^{b}	75.95^{b}	71.62^{b}	81.86^{b}	72.00^{b}	72.83^{b}
Arginine													
85	88.83	83.33	85.23	79.50	71.61	73.48	73.48	79.30	77.56^{a}	73.65	83.16	74.91	75.58
100	88.91	83.33	84.75	80.23	71.91	73.33	74.25	79.59	78.33^{a}	73.57	83.67	75.28	75.50
125	88.91	83.30	84.73	79.83	72.33	73.50	74.50	80.00	78.16^{a}	73.75	83.58	75.13	75.56
150	88.91	83.33	85.14	80.25	72.08	73.64	74.41	79.72	75.85^{b}	74.33	83.95	74.86	76.08
Infection	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Arginine	0.999	0.949	0.785	0.776	0.695	0.958	0.243	0.326	0.000	0.096	0.338	0.861	0.901
Interaction	0.861	0.615	0.998	0.610	0.581	0.931	0.967	0.825	0.000	0.283	0.810	0.757	0.798

Superscripts show significant differences in per column ($P \leq 0.05$). SEM: Standard error of means.

Table 5
Effect of dietary arginine supplementation on intestinal lesions.

Groups	Lesion score duodenum	Lesion score jejunum	Lesion score ceca
85	0.77^{a}	0.70^{a}	1.22^{a}
100	0.62^{b}	0.59^{b}	0.70^{b}
125	0.59^{c}	0.51^{c}	0.66^{c}
150	0.55^{d}	0.51^{c}	0.64^{c}
SEM	0.021	0.020	0.051
P-values	0.0001	0.0005	0.0001

Superscripts show significant differences in per column ($P \leq 0.05$). SEM: Standard error of means.

studies have reported that environmental factors such as heat stress, microbial environment, and disease condition can influence the serum concentrations of amino acids (Corzo et al., 2007; Star et al., 2012). The blood amino acid profile in an animal shows net effect of amino acid appearance from dietary absorption and tissue release and amino acid disappearance that can be due to participation in proteins, oxidation, metabolism and excretion (Cynobar, 2002). It can be stated that decreased level of some amino acids under infection condition is due to demand for participation in protein structure, oxidation and metabolism in a time point manner which lead to malabsorption and decreased bioavailability. Blood amino acid profile is a criteria for identifying limiting amino acids that is known with low concentration, and also excess amino acids (Fernandez-Fıgares et al., 2005). Our findings showed that lysine and isoleucine are in excess when compared with uninfected birds, but other amino acids are indispensable. Lysine is the reference amino acid, since it is the second limiting amino acid commonly used in broiler diets. It is one of the main amino acid used for protein production and it is not used as a precursor for other amino acids

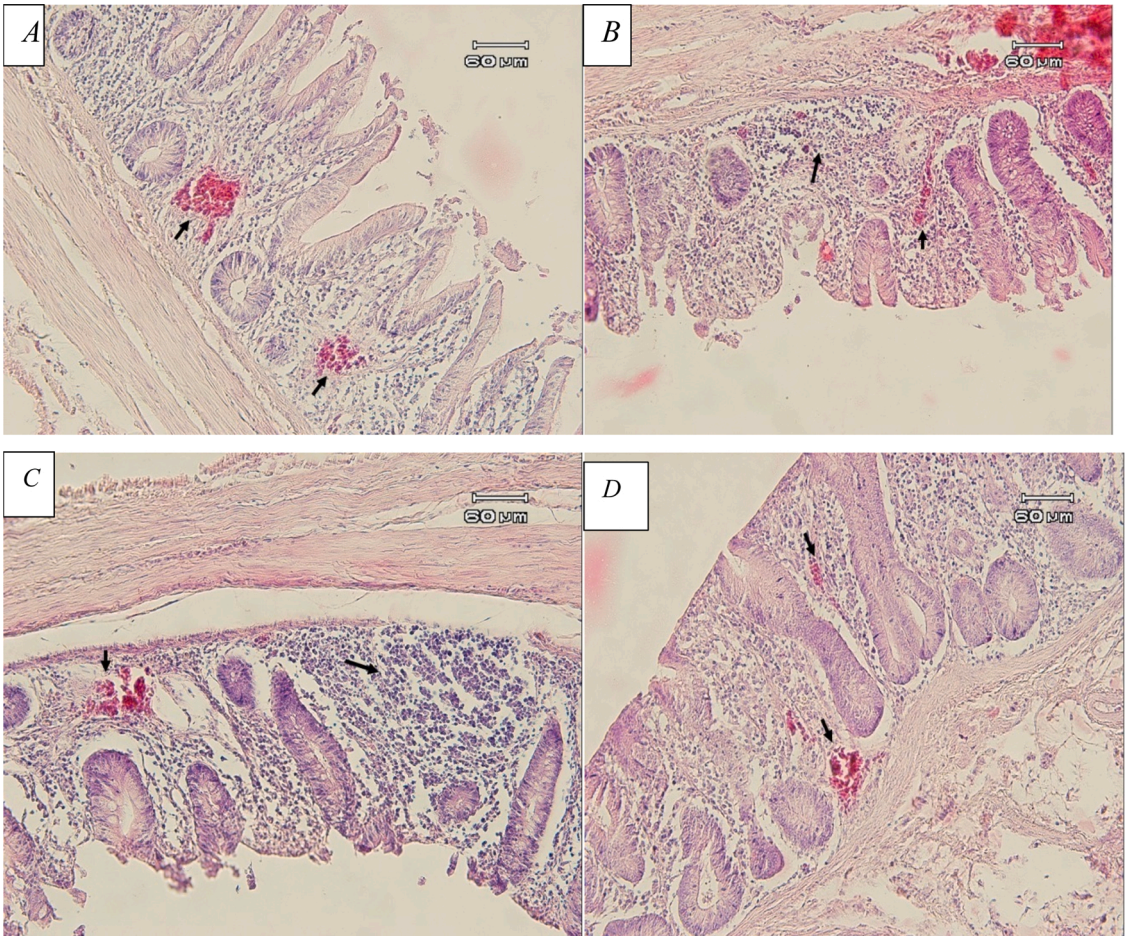

Fig. 1. The effect of dietary arginine supplementation on coccidial associated intestinal lesions. 85 % (A), 100 % (B), 125 % (C) and 150 % (D) on cecal mucosa layer in coccidial infected broiler chicks. Figure A shows medium bleeding in sub-mucosa vessels. Figures B & C show mild bleeding and lymphatic discharge in follicles. Figure D shows mild inflammation and bleeding.

Table 6
The effect of graded supplementation of arginine concentrations on jejunal morphology (μm) of chickens at 21 d after infection (42 d of age).

Groups	Villus height	Villus width	Crypt depth	VH:CD
N-85	1002.00 ± 37.10^{b}	137.30 ± 9.07^{c}	160.00 ± 5.50^{c}	6.27 ± 0.42^{b}
N-100	1083.00 ± 11.31	121.20 ± 9.04^{e}	145.00 ± 4.09^{d}	7.47 ± 0.26^{a}
N-125	1073.00 ± 21.19^{a}	127.30 ± 5.92^{d}	164.30 ± 5.04^{c}	6.53 ± 0.27^{b}
N-150	1028.00 ± 9.43^{b}	123.80 ± 3.97^{e}	162.80 ± 8.32^{c}	6.32 ± 0.37^{b}
E-85	771.70 ± 23.88^{e}	158.20 ± 4.75^{a}	180.20 ± 3.65^{a}	4.28 ± 0.13^{d}
E-100	852.50 ± 59.22^{d}	143.30 ± 4.25^{b}	169.20 ± 5.84^{b}	5.05 ± 0.50^{c}
E-125	922.50 ± 25.20^{c}	152.80 ± 6.14^{a}	182.50 ± 3.67^{a}	5.05 ± 0.15^{c}
E-150	905.80 ± 55.72^{c}	159.80 ± 3.12^{a}	180.30 ± 6.02^{a}	5.03 ± 0.39^{c}
Infection				
Non-challenged	1046.37 ± 39.66^{a}	127.42 ± 9.26^{b}	158.04 ± 9.62^{b}	6.65 ± 0.59^{a}
Challenged	863.12 ± 72.84^{b}	153.54 ± 7.90^{a}	178.04 ± 7.03^{a}	4.85 ± 0.45^{b}
Arginine (%)				
85	967.75 ± 127.05^{a}	132.25 ± 13.39^{b}	157.08 ± 13.50^{b}	6.26 ± 1.32^{a}
100	887.00 ± 124.08^{b}	147.75 ± 12.88^{a}	170.08 ± 11.44^{a}	5.28 ± 1.08^{b}
125	997.58 ± 81.50^{a}	140.08 ± 14.50^{ab}	173.42 ± 10.37^{a}	5.79 ± 0.80^{b}
150	966.67 ± 74.08^{a}	141.83 ± 19.10^{ab}	171.58 ± 10.46^{a}	5.67 ± 0.76^{b}
SEM	15.77	2.26	1.89	0.15
Infection	0.001	0.001	0.001	0.001
Arginine	0.001	0.001	0.001	0.001
Infection × Arginine	0.001	0.019	0.001	0.001

Superscripts show significant differences in per column ($P \leq 0.05$). SEM: Standard error of means.

or derivatives. Increased serum concentration of lysine may be attributed to cellular response for increasing lysine. Lysine may be released from tissues and transferred into blood for responding to infection. Similarly, it was reported that coccidiosis decreases the plasma concentrations of arginine (Rochell et al., 2016). Decreased arginine is due to demand for arginine as a substrate for the production of nitric oxide (Allen and Fetterer, 2000). Dietary inclusion of arginine increased the serum concentration of arginine. It means that external arginine increases serum arginine. Decreased level of other amino acids under infection condition may be attributed due to their participation as fuel sources for immune cells and enterocytes and also malabsorption and decreased bioavailability (Rochell et al., 2016). In the present study, it was shown that ileal amino acids digestibility for all the amino acids was significantly lower in the *Eimeria* infected broiler compared to non-infected broiler chicks ($P < 0.05$). Moreover, dietary supplementing of arginine did not have any significant effect on ileal amino acids digestibility ($P > 0.05$). It means that coccidiosis infection decreases amino acid digestibility in broiler chicks. Decreased digestibility is due to decreased expression of amino acid transport systems in the duodenum of birds during an *E. acervulina* infection (Paris and Wong, 2013; Su et al., 2014, 2015) and/or might be attributed to decreased digestibility. Coccidiosis not only decreases the expression of amino acid transport systems in the duodenum, but it also reduces the expression of aminopeptidase N, a brush border aminopeptidase (Su et al., 2014, 2015).

In the present study, higher levels of arginine decreased intestinal lesions. The data for necropsy of the small intestine showed a higher survival and health in the broiler chicks treated with higher levels of arginine. The *Eimeria* challenge is an experimental model for induction of intestinal infection (Amat et al., 1996). Under coccidiosis challenge, sporozoites infect the cells of the intestinal lining and cause tissue damage and trauma in the intestinal mucosa and submucosa (Pirali Kheirabadi et al., 2011). Coccidia challenge negatively influence the morphology of the intestine, such as shortening villi length and width (Amat et al., 1996). Mucosal layers of intestine are barriers between luminal contents and enterocytes lining the intestine. Mucin type glycoproteins make up the mucosal layer, aggregate different bacterial species, and prevent adjoining pathogens to the intestinal epithelium (Koinarski et al., 2005). The intestinal epithelium of the chicken is continuously produced and differentiated in the mucosal crypts tissues (Mondal et al., 2011). Intestinal challenges increase turnover rate, but its rate differs among intestinal regions and regional of infection (Fernando and McCraw, 1973). Enteric infection such as coccidiosis increase the immune response, and some nutrients such as amino acids may be limiting factors for producing the key proteins required for appropriate immune function. Higher levels of amino acids increase the development and immunity of the gastrointestinal tract in broiler chickens under normal and challenged conditions (Tan et al., 2014; Rochell et al., 2016). Higher levels of digestible amino acids may improve malabsorption under intestinal challenge (Tan et al., 2014). Our results showed that dietary inclusion of arginine increased the serum concentrations of arginine that may have beneficial effects under challenge conditions. Beneficial effects of arginine on morphological indices of jejunal epithelial cells are due to production of nitric oxide (Moncada and Higgs, 1993). It causes toxic condition for coccidia due to its oxidant properties and also its ability for reacting with intracellular iron-containing compounds (Ovington and Smith, 1992). Increased serum concentration of arginine results in increased production of nitric oxide and these leads to increase plasma $NO2^{-} + NO3^{-}$ levels and reduce lesion scores in coccidian infected broiler chickens. Dietary supplementation of arginine stimulates cell proliferation (Murakami et al., 2004) and migration (Rhoads et al., 2006) that decrease lesions.

Broiler chicks infected with *Eimeria* spp in our study, showed lower VH and VH:CD ratio and higher VW and CD while broiler chicks fed with arginine (125 and 150 %) showed higher VH and CD compared to broiler chicks received lowest level (85 %). Coccidiosis increases intestinal inflammation and expression of pro-inflammatory genes (iNOS, IL-1β, IL-8, and MyD88) and decreases villus: crypt ratio, crypt dilatation and goblet cell number (Tan et al., 2014). Epithelial cells that cover the margin create a process that is so called restitution in response to injuries. The crypt allows to increase proliferation of the progenitor cell population. Infectious diseases such as coccidiosis have noxious effects on the intestinal microarchitecture and decrease absorptive surface, and nutrient absorption (Ruff and Edgar, 1982). Using coccidiosis prophylaxis such as probiotic and prebiotic supplements, alleviated negative effect of coccidiosis on gut health and intestinal integrity and increase the digestibility of the diet (Giannenas et al., 2012 & 2014). Diet supplementing of arginine increased crypt depth and villi height diameter. Xiao et al. (2016) showed that dietary inclusion of 1% arginine increased VH: CD ratio and also improved morphological structure in rats challenged with oxidative stress. Arginine participates in synthesis of some polyamines such as putrescine, spermine and spermidine that the polyamines play major role in development of small intestine, colonic mucosa, cell division and tissue growth (Loser et al., 1999). Khajali et al. (2014) showed that dietary inclusion of arginine (10 g/kg) increased blood nitric oxide, duodenum and jejunum VH, VW and surface area in broiler challenged with hypoxia condition. Increased VH and CD in broiler chicks supplemented with arginine is due to the role of arginine in synthesis of polyamines, because these molecules participate in gut development and nutrient absorption in small intestine (Abdulkarimi et al., 2017).

In conclusion, coccidia infection decreased the ileal amino acid digestibility for all amino acids studied in this research and also reduced serum concentrations of amino acids except for lysine and isoleucine. Infection with *Eimeria* spp decreased VH and VH:CD ratio which was accompanied with increased lesions. Dietary inclusion of arginine especially in higher levels, increased VH and VH:CD ratio with decreased lesions. Additionally, dietary supplementing of arginine increased the serum concentration of arginine, but it did not have any significant effect on its digestibility. Therefore, under infection condition, higher levels of arginine are recommended for decreasing digestive tract injuries.

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CRediT authorship contribution statement

Fatemeh Izadi Yazdanabadi: Conceptualization, methodology, Data curation, Writing- Original draft preparation. Hadi Mohebalian: Advise throughout the project. Gholamali Moghaddam: Conceptualization, Supervision. Mehdi Abbasabadi: Advise throughout the project. Hadi Sarir: Conceptualization, Supervision. Advise. Seyyed Javad Hosseini Vashan: Advise throughout the project. Alireza Haghparast: Conceptualization, Supervision, Writing- Reviewing, Revising and Editing.

Declaration of Competing Interest

The authors declare that they did not have any conflict of interest.

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