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Effects of different level of arginine on antioxidant status, serum carotenoid levels and carcass traits in broilers challenged with *Eimeria* spp

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Abstract

Introduction: Coccidiosis is one of the most common diseases in poultry industry in all over the world that is characterized by enteritis. Coccidiosis causes economic losses in chicks, because it induces diarrhea and deaths. This disease decreases the plasma concentration of arginine and suppresses antioxidant system. This study aimed to evaluate the effects of different levels of arginine on antioxidant status, carcass traits, and serum carotenoid levels in broiler chicks challenged with *Eimeria* spp. **Materials and Methods:** A total number of 384 one-day-old broiler chicks (Ross 308) of mixed sex was allocated into 8 groups with 8 birds/pen from grower period. At 21 days, broiler chickens were challenged with a mixture of *Eimeria* species. Birds were divided into infected and uninfected groups and received arginine at 85, 100, 125, and 150 % of recommended levels. The levels of antioxidant enzymes, malondialdehyde (MDA), nitric oxide (NO), and serum carotenoid levels were assessed in blood sera and also carcass traits were evaluated. **Results:** Coccidiosis decreased total antioxidant capacity, and serum carotenoid levels, but increased MDA and NO in comparison with uninfected Birds (p<0.05). However, 125 and 150% diets, increased total antioxidant capacity and serum carotenoid levels, but decreased MDA (P<0.05). **Conclusion:** In conclusion, coccidiosis decreased antioxidant status and serum carotenoid levels in broiler, but dietary inclusion of higher levels of arginine improved antioxidant status and serum carotenoid levels. In summary, higher levels of arginine could be recommended to improve antioxidant capacity and serum carotenoid levels in broiler challenged with coccidiosis.

Keywords: Antioxidant capacity, Broiler chickens, Coccidiosis, Malondialdehyde, Serum carotenoid

Introduction

Coccidiosis is known as one of the most common diseases in poultry industry in all over the world that is characterized by enteritis (Habibi et al., 2016). It annually causes economic losses in broiler chicks production industry (Dalloul and Lillehoj, 2006). *Eimeria* spp causes coccidiosis in broiler chickens. Insufficient ventilation and humidity, inappropriate stocking density, deficient immune responses, bacterial enteritis, and lack of efficient anticoccidial drugs facilitate development of coccidiosis (Shivaramaiah et al., 2014). The use of feed drugs or

coccidiostats and vaccination cannot immunize poultry industry against coccidiosis (Peek and Landman, 2011), because they do not efficiency act. Avian coccidiosis increases oxidative stress in broiler chicks (Bun *et al.*, 2011) and decreases the levels of carotenoids (Zhao et al., 2006).

Nutrition is known to have significant role in improving immune response in broiler chickens. Studies have shown that amino acid profile can have significant effect on *Eimeria* pathogenicity and also improve bird ability for response to infection (Lehman et al., 2009; Lee et al., 2011). Coccidiosis impairs nutrient digestion and absorption. Amerah and Ravindran (2015) reported that coccidial infection adversely influences ileal amino acid digestibility of diets in broiler broilers (Amerah and Ravindran, 2015). It is well accepted that coccidiosis changes ideal amino acid profile in broiler chicks, because it creates biochemical and physiological changes in animals (Rochell et al., 2016). These changes likely influence free amino acid pool in broiler chicks (Rochell et al., 2016). Among amino acids, arginine and tryptophan efficiently affect immune response in birds (Allen and Fetterer, 2000). Broiler chicks fed with 2.5 times of recommended levels of National Research Council (NRC) showed better growth performance and health status (Emadi et al., 2011). Coccidiosis decreases the plasma concentration of arginine (Allen and Fetterer, 2000). Decreased plasma concentration of arginine in infected birds could be attributed to increase demand for the production of nitric oxide (Allen and Fetterer, 2000). Nitric oxide (NO) production depends on extracellular availability of arginine (Chang et al., 1998). Arginine is an essential amino acid for broilers that is normally founded in food (Ebrahimi et al., 2015). Arginine is required as a precursor for synthesis of proteins, nitric oxide, creatine, ornithine, glutamate, proline, glutamine, and poly-amines (Azimi Youvalari et al., 2017). Studies have reported that dietary inclusion of arginine significantly improved immune responses in healthy and challenged broiler chicks (Deng et al. 2005; D'Amato and Humphrey 2010). Studies have reported the impact of using arginine on improving of metabolism (Atakisi et al., 2009) and alleviating adverse effects of abnormal condition in poultry (Attia et al., 2011). Studies have also reported that total body fat deposition decreases with increasing in the levels of arginine (Al-Daraji et al., 2011, Wu et al., 2011). Arginine supplementing of broiler chickens'diet significantly improved antioxidant status and nitric oxide production in poultry (Atakisi et al., 2009; Bun et al., 2011). Seemingly, excess dietary arginine may improve levels of carotenoids and thigh and breast weights.by modulating in antioxidant status of broiler chickens challenged with *Eimeria*. Thus, the present study aimed to evaluate the effects of different levels of arginine on antioxidant status, carcass traits, and serum carotenoid levels in broilers challenged with *Eimeria.*

Materials and methods

Broiler chickens and treatments

This experiment was conducted according to protocols approved by the Animal Care Committee of Tabriz University. A total number of 384 one-d-old broiler chicks (Ross 308; Kimia joojeh Amol Co., Mazandran, Iran) of mixed sex (each sex 50%) with initial weight of 42±2 g purchased. A lighting program (23h light: 1 h darkness) was considered. Broiler chicks were reared in pens covered with fresh wood shavings. Water and feed were provided *ad libitum* during experiment. Temperature was set in 35 ˚C during several first days and it was gradually decreased 23.9 ˚C in the end of 21 days. Sanitation procedures were certainly conducted in the house before and during the trial. All the broiler chicks received a corn-soybean meal basal diet (lack of coccidiostats) which met all the Ross catalogue requirements (Aviagen, 2014) for broiler chicks (Tables 1 and 2). Experimental periods consisted of: starter (1-10 days), grower (11-24 days) and finisher (25-42 days). Chicks were randomly allocated into 4 treatments with 6 replicates and 16 birds each up to 21 days. The animals

fed diets supplemented with 85, 100, 125 and 150% of recommended digestible arginine. On 21 days, broiler were divided into 8 groups and half of birds were infected by *Eimeria*. On other words, broiler chickens were divided into 8 groups with 6 replications and 8 birds/ replicate. A suspension containing 200000 sporulated oocysts of *E. negatrix* (7.5%), *E. maxima* (10%), *E. acervulina* (7.5%) and *E. tenella* (75%) was used for induction of *Eimeria* infection. Experimental groups included:

-Broiler chickens challenged with *Eimeria spp.* and treated with 85% of recommended arginine (E-85 group)

- Broiler chickens challenged with *Eimeria spp.* and treated with 100% of recommended arginine (E-100, positive control)

- Broiler chickens challenged with *Eimeria* spp.and treated with 125% of recommended arginine (E-125)

- Broiler chickens challenged with *Eimeria* spp and treated with 150% of recommended arginine (E-150)

- Broiler chickens unchallenged with *Eimeria* spp. and treated with 85% of recommended arginine (N-85)

- Broiler chickens unchallenged with *Eimeria* spp. and treated with 100% of recommended arginine (N-100, Negative control)

- Broiler chickens unchallenged with *Eimeria* spp. and treated with 125% of recommended arginine (N-125)

- Broiler chickens unchallenged with *Eimeria* spp. and treated with 150% of recommended arginine (N-150).

Table 1- Diet ingredients fed to broiler

Age periods (days) Ingredients (g/kg)	1-10	11-24	25-42
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
Total	1000	1000	1000

[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, 4,500 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.

Table 2- Calculated concentrations of nutrient in broiler's diets during different rearing periods

	Age period(days)		
	1-10	**11-24**	**25-42**
ME (Kcal/kg)	3000	3100	3200
Crude Protein (%)	23	21.5	19.5
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
Amino acids (%)			
Methionine	0.51	0.47	0.43
Met + Cys	0.85	0.79	0.72
Lysine	1.28	1.15	1.03
Arginine	1.37	1.23	1.10
Threonine	0.86	0.77	0.69
Tryptophan	0.2	0.2	0.18
Ions (%)			
Calcium	0.96	0.87	0.79
Available phosphorus	0.48	0.435	0.395
Sodium	0.17	0.17	0.17
Potassium	0.91	0.84	0.77
Chloride	0.19	0.2	0.2

Table 3- Digestible arginine (g/kg) in the different periods

Periods	85% Arg	100 % Arg	125 % Arg	150 % Arg
Starter	11.64	13.7	17.12	20.55
Grower	10.45	12.3	15.37	18.45
Finisher	9.35	11	13.75	16.5

Arginine was purchased from CJ Corporation (South Korea) and in from of L-arginine HCl (98.5% purity). Arginine contents of feed samples were determined using an ion exchange 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 3. At d 21, broiler chickens were challenged with a mixture of *Eimeria* spp. A 1.5 ml solution contained sporulated oocysts (2×10^5 oocysts; *E.necatrix* (7.5%), *E.maxima* (10%), *E.acervulina* (7.5%) and *E.tenella* (75%) was orally inoculated to broiler chickens by automatic drencher.

Carcass traits

At the end of trial, 2 birds per replicate were weighted and killed by decapitation (Deheading). Weight percentage of carcass, spleen, heart, liver, and pancreas were calculated as percentage of live body weight. The different parts were weighted by a scale (Sartorius AG, Weender land strasse, 94-108, 370075 Goettingen, Germany with sensitivity of 0.1 g).

Antioxidant status

At 42 days of trial, blood samples were collected from 2 birds/ replicate in tubes which did not have any anticoagulant. Part of blood was used as a whole blood for measurement of glutathione peroxidase (GPx) and superoxide dismutase (SOD), and total antioxidant capacity (TAC), while the other part of blood samples was centrifuged at 2500 rpm for 12 minutes and blood serum was stored at -80°C. In order to assay nitric oxide, samples were firstly thawed at 4°C. Specified commercial kit {NO: Zellbio (Germany), Cat No.: ZB-NO.96 A} was used to evaluate the levels of NO_2^- + NO_3^- as previously recommended by producer company. Colorimetric detection of $NO2^-$ was

considered as a colored azo dye product of the Griess reaction and was assayed by absorbance at 585 nm. Malondialdehyde (No: 15023, ZellBio, Germany), GPx (No: 430430, Randox Laboratories, Ardmore, Crumlin, UK), SOD (No: 439108, Randox Laboratories, Ardmore, Crumlin, UK), TAC (NX 2332, Zellbio Germany), were assessed based on recommendations of producer company

Serum carotenoid levels

In 3, 5, 7 and 9 days after induction of infection, blood samples were collected from 2 broiler chickens per replicate and were centrifuged. The samples were investigated as reported by previous studies (Mougeot et al. 2010) by spectrophotometer apparatus (shimadzu, Japan).

Statistical analysis

The study was conducted based on a completely randomized design in a 4×2 factorial arrange with infection (challenged and unchallenged) and dietary arginine supplemental (85, 100, 125, and 150% of the recommended levels of arginine). However, parameters were analyzed for the main effects of infection and arginine and the interaction 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 the infection, (Aj) is the main effect of the arginine, (IAij) is the 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 2001). The differences among group were calculated by Duncan's multiple range test (P<0.05).

Results

The effects of different levels of arginine and challenged with *Eimeria* on antioxidant status are shown in Table 4. The results showed that challenging with *Eimeria* significantly decreased TAC, but increased NO and MDA (P<0.05). However, challenging with *Eimeria* did not have any significant effect on SOD and GPx (P>0.05). Dietary arginine treatments did not show any significant effect on SOD and GPx (P>0.05). Dietary treatments of 125 and 150% arginine could significantly increase TAC, and NO, but decrease MDA (P<0.05). arginine treatments based on 100% of the recommended level could significantly improve antioxidant status in comparison with those received lower level of 85% arginine in challenged broiler chickens. The effect of dietary arginine supplementation and challenge with *Eimeria spp.* on carcass characteristics and internal organs' weight are shown in Table 5. The results showed that percentage of carcass, heart and liver were significantly smaller in broiler chickens challenged with *Eimeria spp.* (P<0.05). Dietary arginine treatments and interaction between arginine and infection on carcass traits were significant (P<0.05). Broilers fed with higher levels of arginine (125 and 150%) showed better carcass percentage in comparison with those received lower levels (85 and 100%), (P<0.05). Coccidiosis had not a significant effect on pancreas and spleen percentage weight (P>0.05), however, arginine supplementation did not have a significant effect on pancreas, spleen, heart, liver, and carcass (P>0.05).

The effects of different levels of arginine on the serum concentration of carotenoids are shown in Table 6. The results showed that infection significantly decreased the serum concentration of carotenoids in all the days (P<0.05), but higher levels of arginine (125 and 150%) increased the serum concentration of carotenoids in challenged broiler chickens.

Table 4- The effects different levels of diet arginine on antioxidant status (mean ± SE) of chickens at 21 d after infection (42 d of age) with mixed *Eimeria* spp

Groups	TAC (mmol/L)	NO (nmol/mg Hb)	MDA (nmol/mL)	SOD (U/g Hb)	GPx (U/g Hb)
N-85	1.43±0.04^{e}	3.64±0.11^{g}	2.13±0.07^{c}	920.00±224.49	58.00±17.90
N-100	1.64±0.05^{c}	4.86±0.07^{f}	2.14±0.11^{c}	980.00±231.86	60.33±9.01
N-125	1.87±0.04^{b}	6.43±0.05^{d}	2.13±0.05^{c}	960.00±274.80	61.66±11.58
N-150	1.92±0.03^{a}	6.30±0.06^{d}	2.10±0.07^{c}	947.50±220.62	64.00±13.59
E-85	1.30±0.04^{f}	5.21±0.11^{e}	2.74±0.05^{a}	860.00±198.25	54.91±8.61
E-100	1.57±0.04^{d}	7.14±0.14^{c}	2.31±0.03^{b}	873.33±159.49	55.58±13.08
E-125	1.68±0.02^{c}	7.81±0.11^{b}	2.10±0.05^{c}	866.66±178.28	54.41±11.08
E-150	1.85±0.06^{b}	8.58±0.14^{a}	2.09±0.08^{c}	902.66±159.34	55.91±12.92
Infection					
Unchallenged	1.71±0.04^{a}	5.31±0.07^{b}	2.12±0.07^{b}	951.88±220.12	61.00±10.43
Challenged	1.60±0.03^{b}	7.18±0.12^{a}	2.31±0.05^{a}	875.67±175.31	55.20±10.07
Arginine (%)					
85	1.36±0.04^{d}	5.42±0.11^{d}	2.43±0.06^{a}	890.00±202.10	56.45±12.21
100	1.60±0.04^{c}	6.50±0.11^{c}	2.22±0.07^{b}	926.67±185.89	57.95±11.07
125	1.77±0.03^{b}	7.62±0.08^{a}	2.12±0.06^{c}	913.33±220.41	58.04±11.23
150	1.88±0.04^{a}	7.14±0.10^{b}	2.10±0.07^{c}	925.08±190.41	59.95±12.71
SE	0.02	0.452	0.01	85.42	
			P-Values		
Infection	0.007	0.002	0.005	0.214	0.117
Arginine	0.021	0.003	0.001	0.971	0.924
Infection × Arginine	0.003	0.001	0.001	0.982	0.959

TAC – total antioxidant capacity; NO – nitric oxide; MDA – malone dialdehyde; SOD – superoxide dismutase; GPx– glutathione peroxidase. Superscripts (a-g) show significant differences per column based on P<0.05.

Table 5- Effect of dietary arginine supplementation on carcass characteristics and internal organs weight (mean ± SE) (as the percentages of live body weight) of challenged and unchallenged broiler chickens.

Groups	**Pancreas**	**Spleen**	**Heart**	**Liver**	**Carcass**
N-85	0.23±0.01	0.125±0.03	0.49±0.02	2.17±0.14	62.14 ±1.01^{b}
N-100	0.23±0.01	0.128±0.02	0.48±0.04	2.16±0.16	63.06±1.18^{b}
N-125	0.22±0.03	0.135±0.01	0.47±0.02	2.18±0.18	65.97±1.16^{a}
N-150	0.20±0.02	0.130±0.02	0.47±0.02	2.19±0.26	65.12±0.56^{a}
E-85	0.20 ±0.01	0.105±0.02	0.45±0.05	2.01±0.09	60.81±0.97^{c}
E-100	0.21 ±0.03	0.130±0.02	0.45±0.03	1.97±0.08	61.03±1.01^{c}
E-125	0.20 ±0.01	0.106±0.01	0.43±0.03	2.01±0.21	63.21±1.52^{b}
E-150	0.20 ±0.02	0.140±0.02	0.46±0.03	2.04±0.22	63.11±0.79^{b}
Infection					
Unchallenged	0.129±0.10	0.129±0.02	0.48±0.02^{a}	2.17±0.16^{a}	64.07±0.97^{a}
Challenged	0.120±0.10	0.120±0.02	0.44±0.02^{b}	2.01±0.11^{b}	62.04±1.03^{b}
Arginine (%)					
85	0.115±0.09	0.129±0.02	0.47±0.03	2.07±0.12	61.47±1.00^{b}
100	0.129±0.11	0.115±0.02	0.47±0.03	2.09±0.12	62.04±1.04^{b}
125	0.120±0.12	0.120±0.01	0.45±0.02	2.09±0.21	64.59±1.23^{a}
150	0.135±0.10	0.135±0.02	0.46±0.03	2.12±0.22	64.11±1.01^{a}
SE	0.009	0.003	0.005	0.027	

			P-Values		
Infection	0.198	0.198	0.003	0.003	0.004
Arginine	0.206	0.206	0.597	0.934	0.001
Infection × Arginine	0.195	0.195	0.600	0.990	0.001

Table 6- The effect of dietary arginine supplementation on serum carotenoid levels (mean ± SE), (µg/mL) of challenged and unchallenged broiler chickens in 3, 5, 7, and 9 days after induction of infection

Groups	Day 3	Day 5	Day 7	Day 9
N-85	62.54±2.56[a]	62.75±3.43[b]	64.08±2.49[a]	64.83±1.72[a]
N-100	60.50±2.96[a]	61.75±1.99[b]	61.58±1.90[a]	62.17±1.80[a]
N-125	64.50±1.84[a]	65.00±1.22[a]	63.33±1.53[a]	63.75±2.27[a]
N-150	62.00±2.00[a]	62.58±2.20[a]	63.33±1.96[a]	65.17±2.75[a]
E-85	48.58±2.20[c]	38.50±1.94[c]	28.97±1.76[c]	21.58±1.14[c]
E-100	47.67±1.86[c]	37.33±1.32[c]	29.33±1.40[c]	21.92±1.28[c]
E-125	55.00±1.41[b]	48.42±1.74[b]	33.33±4.08[b]	32.67±1.03[b]
E-150	54.67±1.21[b]	48.08±2.06[b]	39.25±1.72[b]	37.83±3.54[b]
SE	0.923	1.579	2.277	2.701
Infection				
Unchallenged	62.39±2.66[a]	63.02±2.51[a]	63.08±2.09[a]	63.98±2.00[a]
Challenged	51.48±3.79[b]	43.08±5.55[b]	32.72±4.82[b]	28.50±4.39[b]
Arginine (%)				
85	55.56±7.63[b]	50.63±6.94[b]	46.53±7.45[b]	43.21±6.53[b]
100	54.08±7.10[b]	49.54±5.85[b]	45.46±6.92[b]	42.04±10.26[b]
125	59.75±5.20[a]	56.71±8.77[a]	48.33±5.94[a]	48.21±6.32[a]
150	58.33±4.14[a]	55.33±7.84[a]	51.29±2.70[a]	51.50±4.18[a]
		P-Values		
Infection	0.000	0.000	0.000	0.000
Arginine	0.000	0.000	0.000	0.000
Infection × Arginine	0.001	0.000	0.000	0.000

Discussion

In the present study, challenge with *Eimeria* spp significantly reduced TAC, but increased NO and MDA serum levels, however, blood SOD and GPx activities were not affected by infection. Avian coccidiosis increases oxidative stress in broiler chicks (Bun et al., 2011). The oxidative stress as a consequence of coccidial infection is associated with an imbalance between free radical production and endogenous antioxidants (Estevez, 2015). It is well known that invasion of Eimeria sporozoites to intestinal epithelium, can destroy intestinal epithelial barrier through impairing tight junctions and induce inflammation and produce reactive nitrogen species (RNS) and other powerful pro-oxidants belonging to reactive oxygen species (ROS) including superoxide, hydroxyl radical, and hydrogen peroxide. Both ROS and RNS at physiological levels are signaling molecules that are involved in homeostasis. However, overproduction of ROS and RNS are known to have adverse effects on the host (Halliwell and Gutterige, 1989). RNS is by-product of nitric oxide synthases (NOS). The NOS converts arginine into citrulline and products NO radical (NO•). NO acts as a potent vasodilator and neurotransmitter and modulates in some physiological, pharmacological, and pathological activities (Moncada et al., 1997). NO is known as one mediator of innate immunity (Allen 1997), however, excessive production of NO radical destroys intestinal

mucous membrane and spoils nutrient utilization (Sklyarov et al., 2011). In the luminal part, NO reacts with superoxide anion and produces peroxynitrite ($ONOCO_2^-$), which is one of the most originator of oxidative damage (Pacher et al., 2007). In normal physiological conditions, over production of intracellular oxidative radicals are removed by a series of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase (Kurutas, 2016). Superoxide dismutase converts superoxide anion (O^{-2}) to H_2O_2 and oxygen. In turn, H_2O_2 broke down by catalase and GPx to H_2O and oxygen (Fukai and Ushio-Fukai, 2011). The antioxidant properties of dietary arginine supplementation and nitric oxide production at physiological levels have been reported (Atakisi et al., 2009). Wu et al. (2004) demonstrated that dietary arginine increased serum and skeletal muscle TAC levels. It was displayed that supplementation with L-arginine reduced superoxide release in rats (Wascher et al.,1997). In contrast to our findings, Georgieva et al. (2006) have reported a decrease in SOD activities in birds infected with *E. tenella* in comparison with control birds. Ma et al. (2010) reported that dietary supplementation with 0.5% and/or 1% arginine increased serum activity of GPx, though it decreased the hydroxyl radical level in the serum of pigs. In infected chicks, oxidative detriment to lipids happens due to an imbalance between the production of free radical and the animal's antioxidant defense system. Malondialdehyde is a soluble degradation product of lipids, so serum level of MDA is used as a biomarker for lipid peroxidation and oxidative stress (Wang *et al.*, 2006, Ayala *et al*. 2014). Therefore, the increase of serum MDA concentration in infected birds is attributed to increase in ROS, as a consequence of lipid peroxidation. Wang et al. (2008) reported that challenging with *E. tenella*, increased serum level of MDA in broiler chicks. Fouad et al. (2012) have reported an increase in GPx as result of MDA increase in the infected broiler chicks; implicating antioxidant activity for decreasing MDA. In summary, our findings showed that inclusion of arginine into diet increased serum nitric oxide and TAC levels; suggesting better antioxidant activity in broiler chicks fed arginine supplement.

Percentages of carcass, heart, liver, and pancreas were significantly smaller in broiler chickens challenged with *Eimeria* and arginine supplementation did not have a significant effect on carcass traits except for carcass percentage. Previous study indicated that dietary supplementation of L-arginine at the level of 0.04% significantly increased carcass traits (Al-Daraji and Salih, 2012). Improving carcass percentage in the present study could be attributed to participation of arginine in biosynthesis of several molecular structures that improves growth performance (Bartell and Batal, 2007; Khajali and Wideman, 2010; Chen et al., 2011). In addition, arginine acts as an important regulator in nutrient metabolism and immune responses and influences breast and thigh weights (Wu et al., 2011). In contrast, Bozkurt *et al.* (2016) showed that challenging with coccidiosis increased relative weights of liver (by 24%) and pancreas (by 11%) in comparison with uninfected broiler chickens. They showed that an increase in liver and pancreas weights is due to increased production of digestive enzymes and bile salts response to parasitic infection (Bozkurt et al., 2016). Since coccidiosis adversely influences growth performance, thus, it can have significant effects on carcass traits.

The results showed that induction of infection significantly decreased serum concentrations of carotenoids, but higher levels of arginine could maintain concentrations of carotenoids. The results showed that with increasing time (from day 3 to day 9), the level of carotenoids was decreased in infected groups (51.48 (µg/ml) in day 3 as compared with .28.5 (µg/ml) in day 9). It is believed that serum carotenoid tends to be maintained until storage pools are depleted, so, carotenoids are not considered reliable indicators of nutritional status (Swayne, 2013). Zhao et al., (2006) showed that serum carotenoids were decreased during *E. tenella* infections due to

haemorrhage. Carotenes are known to have ability for scavenging radicals in the lipid phase, because, these are mostly found deep in the apolar core of lipid membranes (El-Agamey and McGarvey, 2008). Carotenoids are micronutrients that play essential physiological roles during early life, due to their immunostimulant and antioxidant properties (Bai et al., 2011). Then, carotenoids can act an antioxidant and its concentration decreased during infection. Decreased concentration of carotenoids was parallel with increasing in MDA and TAC. It means that it can have synergism activity with TAC for decreasing ROS. The reason of increasing carotenoids in arginine groups is unknown. It seems that improving in TAC, but deceasing in MDA spare carotenoids in arginine groups. On the other hand, it has been shown that increasing dietary carotenoids delayed the *E. tenella* reproductive cycle, supporting earlier studies demonstrating the protective effect of carotenoids (Figuerola et al., 2014) It was reported that plants and herbal products rich in carotenoids prevent against coccidiosis (Dragan et al., 2014). The mechanism is unknown. Seemingly, carotenoids act by modulating antioxidant system and the present findings for antioxidant parameters confirm this claim. A study showed high levels of β-carotene and zeaxanthin oxidation products present in blood, breast, thigh and shank skin, and fat of broiler chicks (Swayne, 2013). It means that the carotenoids were involved in antioxidant activity in these tissues in infected birds.

Conclusion

Carcass traits, serum carotenoid levels, and total antioxidant status were negatively influenced by coccidiosis. Adding arginine into the diet could alleviate adverse effects of challenging on serum carotenoid levels and TAC. With regards to achieved results of this experiment, arginine in higher levels (125 and 150%) could be recommended in chickens challenged with coccidiosis.

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Conflict of Interest Declaration

The authors have not any conflict of interest.

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اثرات سطوح مختلف آرژینین بر وضعیت آنتی اکسیدانی، صفات لاشه و سطح سرمی کاروتنوئیدها در جوجه‌های گوشتی چالش یافته با *Eimeria* spp

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چکیده

زمینه مطالعاتی: کوکسیدیوز یکی از رایج‌ترین بیماری‌ها در صنعت طیور در سرتاسر جهان است که با انتریت شناخته می‌شود. کوکسیدیوز باعث ایجاد زیان‌های اقتصادی در طیور جوان می‌شود، زیرا سبب مرگ‌ومیر و اسهال می‌شود. این بیماری غلظت پلاسمایی آرژنین را کاهش می‌دهد و سیستم آنتی اکسیدانی را سرکوب می‌کند. **هدف**: این مطالعه با هدف ارزیابی اثرات آرژینین بر وضعیت آنتی اکسیدانی، صفات لاشه و سطوح سرمی کاروتونوئیدها در جوجه‌های چالش یافته با گونه‌های آیمریا انجام شد. **روش کار**: تعداد ۳۸٤ جوجه‌ی گوشتی نژاد راس از هردو جنس به ۸ گروه، با ٦ تکرار و ۸ پرنده در هر تکرار تقسیم بندی شدند و تا ۲۱ روزگی در شرایط مشابهی پرورش یافتند. در روز ۲۱، جوجه‌های گوشتی با گونه‌های آیمریا چالش یافتند. جوجه‌های گوشتی به دو بخش عفونی و غیر عفونی تقسیم بندی شدند و سطوح (۸۵، ۱۰۰، ۱۲۵ و ۱۵۰٪) آرژنین را دریافت کردند. سطوح آنزیم‌های آنتی اکسیدان، مالون دی‌آلدهید، نیتریک اکسید، صفات لاشه و سطوح کاروتنوئیدها ارزیابی شدند. **نتایج**: نتایج نشان داد که چالش با کوکسیدیوز ظرفیت تام آنتی اکسیدانی و سطح کاروتنوئیدها را کاهش داد ولی سطح مالون دی‌آلدهید و سطح نیتریک اکسید را در مقایسه با گروه‌های غیر عفونی افزایش داد ($P<۰,۰۵$). افزودن آرژینین به جیره در سطوح ۱۲۵٪ و ۱۵۰٪ ظرفیت تام آنتی اکسیدانی و سطح کاروتنوئیدها را افزایش داد ولی سطح مالون دی‌آلدهید و سطح نیتریک اکسید را در مقایسه با گروه‌های غیر عفونی کاهش داد ($P<۰,۰۵$). **نتیجه‌گیری**: در مجموع، کوکسیدیوز ظرفیت تام آنتی اکسیدانی و سطح کاروتنوئیدها را کاهش داد و سطح مالون دی‌آلدهید و سطح نیتریک اکسید را در مقایسه با گروه‌های غیر عفونی افزایش داد، ولی سطوح بزرگتر آرژینین اثرات منفی عفونت را کاهش داد. در مجموع، می‌توان بیان نمود که سطوح بزرگتر آرژینین وضعیت آنتی اکسیدانی را در شرایط عفونی بهبود بخشید.

واژگان کلیدی: ظرفیت آنتی اکسیدانی، جوجه‌های گوشتی، کوکسیدیوز، مالون دی‌آلدهید، کاروتنوئید