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The Effects of Dietary Inclusion of Arginine and Rice Hull on Growth Performance, Immune Responses, Intestinal Morphology, Tibia Bone Mineral Content and Intestinal Microbial Population of Broiler Chickens

Research Article

M. Abbasabadi[1*], S.M. Hosseini[1], H. Naeimipour[1] and F. Izadi Yazdanabadi[2]

[1] Department of Animal Science, Faculty of Agriculture, University of Birjand, Birjand, Iran
[2] Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

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*Correspondence E-mail: abbasabadi_mehdi@birjand.ac.ir

ABSTRACT

Arginine and fibers may have positive effects on gastrointestinal tract and indirectly affect immune responses, growth performance, intestinal morphology and microbial population. This study was conducted to evaluate the effects of dietary inclusion of arginine and rice hull on growth performance, immune responses, intestinal morphology and intestinal microbial population of broiler chickens. Three hundred and sixty male broiler chickens were randomly allocated into nine dietary treatments with four replications of 10 broiler chickens based on a factorial arrangement (3×3) in a completely randomized design. Experimental treatments were including basal diets supplemented with arginine (90, 100, and 110% recommended levels), and fiber (0.00, 2.50, and 5.00%). Growth performance, immune responses, intestinal morphology, tibia bone mineral content and intestinal microbial population of broiler chickens were measured. The effects of dietary inclusion of rice hull, arginine, and their interactions were significant on average daily gain ($P<0.05$) and feed conversion ratio (FCR) ($P<0.05$), so that highest gain and the lowest feed conversion ratio were observed in broiler chickens fed with highest levels of arginine (110.00%) and fiber (5.00%). Dietary supplementation of arginine progressively increased immune responses ($P<0.05$), while inclusion of rice hull increased villus height and crypt depth ($P<0.05$) and decreased the concentrations of calcium ($P<0.05$), phosphorous ($P<0.05$) and magnesium ($P<0.05$). The inclusion of arginine ($P<0.05$), and fiber ($P<0.05$) in higher levels increased *Lactobacilli* population ($P<0.05$). In total, higher levels of fiber (5.00%) and arginine (110.00%) improved the growth performance and are suggested in diet of broiler chickens for improving growth performance.

KEY WORDS arginine, body weight, broiler chickens, fiber, gastrointestinal tract.

INTRODUCTION

The requirement of the poultry industry for more production can be responded by adding safe growth promoters into diet (Sabour *et al.* 2019). Dietary supplementation of growth promotors may have the positive effects on the microbial profile of gastrointestinal tract (GIT), and influence growth performance in broiler chickens (Abazari *et al.* 2016). The intestinal microbiota effectively affect host for regulating homeostasis, organ development, metabolic processes and immune responses (Tremaroli and Backhed, 2012). It was reported a relationship between gut microflora and immune responses in broiler chickens (Sabour *et al.* 2019). Diet changes can rapidly influence the composition of the gut microbial community (David *et al.* 2014). Protein has a significant importance in nutrition of monogastric animals and they need a range from 15 to 22% protein (Ravindran, 2016). Essential amino acids such as arginine must be supplied in poultry nutrition from external sources (Aguzey *et al.* 2020).

L-arginine is an amino acid that must be supplied in broiler chicken diet and it can be metabolized for producing important molecules, such as nitric oxide, polyamines and creatine (Zhang *et al.* 2018). Ghamari *et al.* (2020) showed that arginine supplementation into diets alleviated intestinal villus damage, crypt dilation and goblet cell depletion induced by coccidia. It was also reported that dietary supplementation of L-arginine protects the gut mucosa by improving innate immune responses, intestinal absorption and barrier function via reducing the colonization of harmful bacteria in broiler chickens (Zhang *et al.* 2017). Studies have reported the positive effects of arginine supplementation on amino acid digestibility, growth performance and immunity in broiler chickens (Yazdanabadi *et al.* 2020a; Yazdanabadi *et al.* 2020b). Dietary supplementation of arginine into mice diet changed the intestinal microbiota, activated intestinal innate immunity by nuclear factor-kB, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase Akt signaling pathways (Ren *et al.* 2014). Hu *et al.* (2019) reported that dietary supplementation of arginine in combination with leucine reduced body fat weight and increased colonic butyrate and propionate concentrations that are associated with the changes of gut microbiota composition in finishing pigs.

On the other hand, dietary inclusion of fibers may change the intestinal system. Rice hull is a fiber source that can be used in poultry diet. It is containing 2.4% crude protein, 0.5% ether extract, 12.9% ash, 54.2% crude fiber, 74% total dietary fiber, 72% insoluble fiber, and 2.00% soluble fiber (Jiménez-Moreno *et al.* 2016). Soluble fiber may influence GIT microflora by increasing fermentation in the hindgut, producing short chain fatty acids, and their effects on the pathogenic bacteria (Van der Wielen *et al.* 2001). Abazari *et al.* (2016) reported that dietary inclusion of rice hull into diets of broiler chickens could increase the population of *Lactobaccili*, and decrease pathogenic bacteria in intestinal system. Dietary inclusion of insoluble fiber improved utilization of other nutrients, healthiness in intestinal system and growth performance of broiler chickens (Hartini *et al.* 2019). There are reports for the positive effects of rice hull on growth performance (Hartini and Purwaningsih, 2018) and improving bone-breaking in broiler chicks (Nakhon *et al.* 2019).

Seemingly, arginine and fiber can have positive effects on GIT system and indirectly influence growth, immunity and intestinal morphology. So far, any study has not been conducted to evaluate the combined effects of dietary supplementation of arginine and rice hull on growth performance, immune responses, intestinal morphology, and intestinal microbial population of broiler chickens. This study aimed to evaluate the combined effects of dietary supplementation of arginine and rice hull on growth performance, immune responses, intestinal morphology, and intestinal microbial population of broiler chickens.

MATERIALS AND METHODS

Materials

Arginine amino acid (purity of 98.50%) was prepared from CJ Company (South Korea). Rice hull was also provided by Animal and Poultry Feed Company of Shargh Oloofeh (Birjand-Iran).

Chemical composition

Chemical composition of fibers is shown in Table 1. Rice hull was measured for crude fiber, dry matter, crude protein, ether extract, gross energy, calcium, phosphorous, ether extract and ash (Debon and Tester, 2001).

Table 1 Chemical composition of rice hull

Composition	%
Dry matter	93-95
Gross energy, kcal/kg	834
Crude protein, %	3-4
Calcium, %	0.7-0.8
Phosphorous, %	0.25-0.30
Crude fiber, %	36-38
Ether extract, %	3-4
Ash, %	16-17

Ethical standard

All the used procedures were approved by Ethical Committee of University of Birjand (BU, No. 12014).

Animals

This study was conducted in the Research poultry house of University of Birjand. In this study, 360 male broiler chickens of Ross 308 strain were reared in cages with dimensions of 45 cm height × 100 cm width × 100 cm length. Broiler chickens were reared in a three-phases period of starter (1-10 days), grower (11-24 days), and finisher (25-42 days).

The birds were randomly allocated into nine dietary treatments with four replicates and 10 broiler chickens in each cage based on a factorial arrangement of treatments (3×3) in a completely randomized design. Experimental treatments included basal diets supplemented with arginine (90, 100, and 110% recommended levels), and fiber (0, 2.5, and 5%). Based diet composition is presented in Table 2. Experimental diets were prepared based on the nutritional requirements of broiler chickens as provided by Ross 308 broiler management manual (Aviagen, 2014). The diets were prepared in mash form and formulated in iso-proteinous and iso-energetic forms. Broiler chickens were raised in a controlled windowless environment. A lighting program of 23 h light and 1 h darkness was considered. Environmental temperature was set at 32-34 ˚C for the first week, 30-32 ˚C for the second week, 28-30 ˚C for the third week, 26-28 ˚C for the fourth week, and 25-26 ˚C until end of the study. The experimental treatments included: 1) Diets supplemented with 90% arginine and without fiber, 2) Diets supplemented with 90% arginine and 2.50% rice hull, 3) Diets supplemented with 90% arginine and 5.00% rice hull, 4) Diets supplemented with 100% arginine and without fiber (control), 5) Diets supplemented with 100% arginine and 2.50% rice hull, 6) Diets supplemented with 100% arginine and 5.00% rice hull, 7) Diets supplemented with 110% arginine and without fiber, 8) Diets supplemented with 110% arginine and 2.50% rice hull, and 9) Diets supplemented with 110% arginine and 5.00% rice hull.

Data collection and sampling

Average daily feed intake (ADFI) and average daily gain (ADG) were recorded in different periods, and the feed conversion ratio (feed intake/weight gain) was also calculated. Mortality was considered for feed conversion ratio.

Antibody titers against SRBC

At 18 and 35 days of the study, two broiler chickens per treatment were randomly selected and 1 mL of 10% SRBC was administrated into the breast muscle of the broiler chickens. To investigate the antibody titer against SRBC, 2 mL blood sample was taken from the wing vein of the broiler chickens on next week. The sera samples were investigated as reported by He *et al.* (2020). Antibody values were reported as log2.

Morphology of small intestine

At 42 d of age, two broiler chickens from each cage were slaughtered and small intestinal segments were sampled from all the sections. The samples were assessed for the villus height, villus width, and crypt depth. To remove intestinal contents, intestinal parts were flushed by a physiological saline solution. The samples were investigated in a tissue processor with ethanol as dehydrated and were embedded in paraffin. A microtome (Rotary Microtome, Model MK1120, Pooyan medical Co., Mashhad, Iran) was used to prepare sections (5 mm) and stained with hematoxylin-eosin. An optical microscope (Olympus CX31, Tokyo, Japan) was used to investigate the morphological properties. Villus width (VW), villus height (VH), and crypt depth (CD) were investigated. Villus height was investigated from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between 2 villi.

Microbial populations

To assess the intestinal microbial populations, intestinal samples (from Meckel's diverticulum to the ileocecal colon junction) were directly prepared into 80-mL sampling cups under CO2, sealed, and put on ice until they were transported to the laboratory for enumeration of bacterial populations. The samples were investigated for *Lactobacilli* bacteria and coliforms. The samples were serially diluted in 0.85% sterile saline solution for enumeration of L*actobacilli* bacteria and coliforms by conventional microbiological procedures with the help of a selective agar media. All microbiological analyses were conducted in duplicate and the average values were used for statistical analysis. MRS agar and MacConkey agar were used to enumerate the population of *Lactobacilli* and *Coliform* bacteria, respectively. Colonies per agar media were counted, log transformed and expressed per gram of digesta.

Tibia bone mineral content

Tibia bone mineral contents were measured as reported by others (Moyo *et al.* 2021). In total, bone samples were ashed in a muffle furnace at 550 ˚C for 6 h. An amount of 0.2 g of ash was weighted and digested using hydrochloric and nitric acid in a digestion tube. The minerals were then assessed with the help of an ICP Mass Spectrometer (Perkin-Elmer, Model 3110, 1994).

Data analysis

The data were analyzed as a 3 × 3 factorial arrangement on the basis of a completely randomized design by the GLM procedure of SAS 9.2 (SAS ???). The statistical model included the main effects of arginine (90, 100, and 110% of recommended level) and rice hull source (0.00, 2.50 and 5.00% of diet) and their interactions. The data were analyzed for all birds in a cage as an experimental unit. The data were compared by Duncan test. For all the analyses, significant was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 showed chemical composition of hull rice. It was containing 93-95% dry matter, 3.00-4.00% crude protein, 0.7-0.8% calcium, 0.25-0.30% phosphorous, 36.00-38.00% crude fiber, 3.00-4.00% ether extract and 16.00-17.00% ash.

The results for the effects of the supplementation of rice hull and arginine on growth performance of broiler chickens are shown in Table 3.

Table 2 Diet composition of based diet in the different periods

Ingredients	**Starter (1-10 d)**	**Grower (11-24 d)**	**Finisher (25-42 d)**
Corn	59.20	64.64	68.77
Soybean meal	29.26	24.73	20.77
Corn gluten	5.62	4.87	4.82
Calcium carbonate	1.66	1.53	1.41
Dicalcium phosphate	1.64	1.45	1.28
Bentonite	1.21	1.43	1.57
L-lysine	0.44	0.41	0.38
Salt	0.38	0.39	0.36
Mineral and vitamin supplement[1]	0.25	0.25	0.25
L-threonine	0.20	0.17	0.14
DL-methionine	0.14	0.13	0.11
Arginine	-	-	-
Oil	-	0.0004	0.14
Rice hull	-	-	-
Chemical composition			
Metabolizable energy (kcal/kg)	2900	2950	3000
Calcium (%)	0.96	0.87	0.79
Potassium (%)	0.78	0.71	0.64
Phosphorous (%)	0.48	0.43	0.39
Sodium (%)	0.18	0.18	0.17
Chloride (%)	0.26	0.27	0.25
Arginine (%)	1.23	1.10	0.99
Methionine (%)	0.51	0.47	0.43
Methionine + Cysteine (%)	0.81	0.75	0.69
Lysin (%)	1.28	1.15	1.03
Threonine (%)	0.86	0.77	0.69
Tryptophan (%)	0.20	0.18	0.16
Linoleic acid (%)	1.28	1.35	1.42
Ether extract (%)	2.33	2.48	2.61
Crude fiber (%)	3.30	3.08	2.88
Protein (%)	21.50	19.50	18.00
Calcium/phosphorous	2.00	2.00	2.00
Energy/protein ratio	134.8	151.1	166.6

[1] Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate): 12000 IU; cholecalciferol: 4500 IU; vitamin E (DL-α-tocopheryl acetate): 62.5 IU; Menadione (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.

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 and Cu ($CuSO_4 \cdot 5H_2O$).

The results showed that the effects of dietary inclusion of rice hull, arginine, and their interaction on ADG ($P<0.05$) and FCR ($P<0.05$) were significant. The ADG was significantly higher, while FCR was significantly lower in broiler chickens fed with 5.00% rice hull ($P<0.05$). The broiler chickens fed with arginine at level of 110.00% showed higher ADG, and lower FCR compared to other broiler chickens fed with levels of 100.00%, and 110.00% ($P<0.05$). Feed conversion ratio was significantly higher in broiler chickens fed with 90.00% arginine compared to other groups ($P<0.05$). In total, broiler chickens fed with 110.00% arginine and 5.00% rice hull showed higher ADG, and lower FCR.

The results for the effects of dietary additives showed that adding arginine into diet significantly increased immune responses ($P<0.05$) (Table 4). Dietary supplementation of arginine progressively increased antibody titer on days 25 ($P<0.05$) and 42 ($P<0.05$), while addition of fiber into diet did not have significant effects on immune responses ($P>0.05$). It was not observed a significant interaction between fiber and arginine ($P>0.05$) for immune responses.

The results for intestinal morphology are shown in Table 5. The results showed that dietary inclusion of rice hull could significantly increase VH and crypt depth in jejunal section ($P<0.05$) compared to control group. Main effects of arginine and its interaction with fiber did not have significant effects on intestinal morphology ($P>0.05$).

The results for microbial population in Table 6 showed that dietary inclusion of arginine and fiber in higher levels increased *Lactobacilli* population ($P<0.05$) but their interactions were not significant. The results showed that coliform population was not influenced by the experimental treatments ($P>0.05$).

Table 3 The effects of experimental on growth performance (1-42 days)

Groups	ADG (g)	ADFI (g)	FCR
Rice hull, % of diet			
0.00	53.71^{b}	112.97	2.12^{a}
2.50	53.21^{b}	109.66	1.86^{c}
5.00	56.06^{a}	109.93	1.97^{b}
Arginine, % of recommendation			
90.00	55.94^{b}	110.66	2.02^{ab}
100.00	53.61^{b}	110.87	2.08^{a}
110.00	58.20^{a}	111.03	1.97^{b}
SEM	0.495	0.478	0.010
P-value			
Rice hull	0.045	0.453	0.031
Arginine	0.031	0.852	0.042
Interaction	0.001	0.999	0.025

ADG: average daily gain; ADFI: average daily feed intake and FCR: feed conversion ratio.
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 4 The effects of experimental on immune responses (log2)

Groups	25	42
Rice hull		
0.00	4.46	7.13
2.50	4.45	7.15
5.00	4.48	7.17
Arginine		
90.00	4.18^{c}	6.80^{c}
100.00	4.48^{b}	7.24^{b}
110.00	4.73^{a}	7.42^{a}
SEM	0.029	0.034
P-value		
Rice hull	0.661	0.686
Arginine	0.000	0.000
Interaction	0.997	0.902

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.

The results for tibia bone mineral content are presented in Table 7. The results showed that feeding fiber in levels of 2.50% and 5.00% decreased the concentrations of calcium, phosphorous and magnesium, but other elements were not influenced (P>0.05). Arginine and its interaction with fiber did not have significant on tibia bone mineral content (P>0.05).

The results for chemical composition showed that hull rice was containing 93-95% dry matter, 3.00-4.00% crude protein, 0.7-0.8% calcium, 0.25-0.30% phosphorous, 36.00-38.00% crude fiber, 3.00-4.00% ether extract and 16.00-17.00% ash. The results agree with those reported by Jiménez-Moreno *et al.* (2016) who reported 2.4% crude protein, 0.5% ether extract, 12.9% ash, 54.2% crude fiber, 74% total dietary fiber, 72% insoluble fiber, and 2% soluble fiber in hull rice.

Dietary inclusion of rice hull, arginine, and their interactions had significant effects on ADG (P<0.05) and FCR (P<0.05).

In contrast to our findings, Sadeghi *et al.* (2015) showed that dietary inclusion of 3.00% rice hulls (2 mm in size) into diet did not have any significant effect on growth performance. Partly similar findings, Hartini and Purwaningsih (2018) found that addition of 40 g kg^{-1} rice hull in a commercial starter diet did not improve ADG and ADFI. In the current study, higher levels of fiber had better effects on growth performance.

Differences between our findings and others could be attributed to several factors. It was reported that the effects of inclusion of rice hulls into diet on growth performance depends on the type and composition of diet, and the condition in that the rice hulls are added into the diet (Hartini *et al.* 2019). An improvement in growth performance in this study could be attributed to several factors.

The results showed that adding fiber into diet improved VH and crypt depth, and also increased population of benefit bacteria. The improved of intestinal morphology and increased beneficial bacteria help to increase in feed utilization and improve growth performance.

The results showed that adding arginine in higher levels into diet could improve ADG and FCR in broiler chickens. Ghamari *et al.* (2020) showed that diet supplementation of arginine improved growth performance of broiler chickens challenged with *Eimeria* spp. In another study, Li *et al.* (2007) showed that dietary inclusion of arginine in higher levels recommended by NRC improved growth performance in broiler chickens. In this study, the results showed that levels of 90, and 100% are not enough to improve growth performance in broiler chickens. Improved growth performance in broiler chickens fed with higher levels of arginine could be attributed to several factors. Arginine is essential for improving growth performance of broiler chickens, because poultry cannot synthesize urea cycle (Perez-Carbajal *et al.* 2010).

Arginine also participates in structure of body protein and improves weight (Emadi *et al.* 2011). It also promotes secretion of metabolic hormones, increases protein production and feed intake (Davila *et al.* 1987) that improve growth performance. Other reason is attributed to protective effects of arginine on intestinal system by decreasing the colonization of harmful bacteria in broiler chickens (Zhang *et al.* 2017). In total, the results showed that higher levels of arginine and fiber improved body weight and FCR that might be attributed to an increase in *Lactobacilli* bacteria and fiber.

Table 5 The effects of experimental on intestinal morphology

Groups	Duodenum			Jejunum			Ileum		
	VH (mm)	VW (mm)	CD	VH (mm)	VW (mm)	CD	VH (mm)	VW (mm)	CD
Rice hull									
0.00	1.01^{c}	0.151	0.094^{c}	0.644^{c}	0.175	0.079^{b}	0.536^{b}	0.115	0.068^{c}
2.50	1.04^{b}	0.152	0.150^{a}	0.724^{a}	0.172	0.125^{a}	0.590^{a}	0.119	0.078^{b}
5.00	1.06^{a}	0.163	0.23^{a}	0.692^{b}	0.178	0.126^{a}	0.590^{a}	0.122	0.087^{a}
Arginine									
90.00	1.03^{b}	0.159	0.158	0.692	0.178	0.108	0.583	0.123	0.077
100.00	1.03^{b}	0.154	0.159	0.685	0.175	0.107	0.575	0.120	0.071
110.00	1.05^{a}	0.153	0.167	0.682	0.175	0.111	0.558	0.113	0.085
SEM	0.003	0.002	0.007	0.005	0.002	0.004	0.540	0.002	0.003
P-value									
Rice hull	0.000	0.115	0.000	0.000	0.507	0.000	0.000	0.495	0.020
Arginine	0.005	0.609	0.466	0.666	0.847	0.584	0.128	0.203	0.250
Interaction	0.999	0.622	0.803	0.620	0.439	0.611	0.620	0.059	0.084

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 The effects of experimental on microbial population (CFU/g)

Groups	*Lactobacilli*	*Coliforms*
Rice hull		
0.00	5.23^{b}	4.16
2.50	5.49^{b}	4.14
5.00	5.65^{a}	4.14
Arginine		
90.00	5.45^{ab}	4.15
100.00	5.39^{b}	4.15
110.00	5.53^{a}	4.15
SEM	0.028	0.009
P-value		
Rice hull	0.000	0.643
Arginine	0.009	1.000
Interaction	0.091	0.992

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 7 The effects of experimental treatments on tibia bone mineral content

Groups	Calcium (g/kg)	Phosphorous (g/kg)	Magnesium (g/kg)	Manganese (mg/kg)	Zinc (mg/kg)	Copper (mg/kg)
Rice hull						
0.00	173.25^{a}	92.29^{a}	4.26^{a}	4.00	172.41	0.80
2.50	171.50^{b}	90.87^{b}	3.70^{b}	3.99	172.66	0.79
5.00	164.62^{c}	85.95^{c}	3.51^{c}	3.84	170.87	0.80
Arginine						
90.00	165.45	90.25	3.96	4.02	172.62	0.80
100.00	165.95	90.20	3.85	3.91	172.25	0.80
110.00	165.95	88.66	3.67	3.90	171.08	0.79
SEM	0.471	0.39	0.059	0.043	0.305	0.007
P-value						
Rice hull	0.000	0.000	0.000	0.251	0.052	0.669
Arginine	0.439	0.054	0.051	0.471	0.091	0.911
Interaction	0.575	0.319	0.672	0.727	0.873	0.837

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.

Arginine may have synergistic interaction effects for improving growth performance via influencing on *Lactobacilli* bacteria.

Dietary supplementation of arginine, but no fiber increased immune responses in broiler chickens. SRBC is commonly employed to stimulate humoral immune responses in poultry (Maghsoudi *et al.* 2020). The administration of non-pathogenic antigens such as SRBC stimulates the immune response but does not cause toxicity to cells. The results show that arginine increases humoral responses to stimulant of SRBC. Similarly, dietary supplementation of arginine significantly improved immune responses in healthy and challenged broiler chickens (D'Amato and Humphrey, 2010). It was also reported that arginine supplementation increased immune responses by reducing concentration of inflammatory cytokines (Guo *et al.* 2015). Arginine participates in nitric oxide structure, because nitric oxide is toxic for bacteria and some parasites, and has significant roles in resistance and immunity to infectious diseases (Allen and Fetterer, 2002). In total, the results show that arginine influences immune system by influencing on gastrointestinal tract and inflammatory responses. It was expected that fiber influences benefit bacteria and subsequently immune system, but such result was not observed that its mechanism is unknown.

The results showed that dietary supplementation of rice hull could significantly increase VH and crypt depth. Villi height shows the absorptive capacity of the intestine (Teirlynck *et al.* 2009). Similar to our results, Rezaei *et al.* (2011) showed an increase in ileal villus height in broiler chickens fed with fiber. Wils-Plotz and Dilger (2013) showed an increase in duodenal crypt depth in broiler chickens fed with diets containing cellulose. In diets containing fiber, more substrate is available for microbial fermentation and increases production of fatty acids. An increase in production of fatty acids promotes proliferation and cell growth in the gut (Montagne *et al.* 2003) that influence intestinal morphology. It was expected that arginine improves intestinal morphology due to its role in intestine structure, but such result was not observed that its reason is unknown.

The results showed that *Lactobacilli* population was significantly higher in broiler chickens fed with arginine and fiber. Similar to our findings, Abazari *et al.* (2016) reported that dietary inclusion of rice hull in diets of broiler chickens improved the population of *Lactobacilli*. Soluble fiber can influence GIT microflora by increasing the fermentation in the hindgut, producing short chain fatty acids, and their bacteriostatic effects on the pathogenic bacteria (Van der Wielen *et al.* 2001). Seemingly, inclusion of fibers decreases the pathogenic bacteria and increases benefit bacteria. With regards to arginine, it was reported that dietary supplementation of L-arginine decreases colonization of harmful bacteria in broiler chickens (Zhang *et al.* 2017) and changes the intestinal microbiota in mice (Ren *et al.* 2014). Thus, fiber and arginine have synergistic effects on *Lactobacilli* bacteria.

The results showed that feeding fiber in levels of 2.50% and 5.00% decreased the concentrations of calcium, phosphorous and magnesium that is due to interaction between fiber compounds and elements in gut system. Seemingly, fiber compounds prevent absorption of minerals in gut and decrease their concentration in the bone.

CONCLUSION

In total, a combination of fiber and arginine improved growth performance by increasing beneficial bacteria. Rice hull and arginine can have synergism interaction effects in broiler chicken diets. We suggest to include arginine (110% recommended levels) and fiber (5.00%) for improving growth performance in broiler chickens.

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