Research Article



Effect of Extra Arginine Supplementation on Growth Performance, Carcass Characteristics and Immune Response in Broilers

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Abstract | The trial was conducted to examine the effect of varying levels of supplementation of arginine on different parameters of growth, carcass and immune system in broilers birds. A total of 240 day old cobb-500 male chicks were purchased and were divided into 4 treatments in completely randomized design. Each treatment further subdivided into six replicates and each replicate contained 10 chicks. Four iso-nitrogenous and iso-caloric starter (CP and ME as 22% and 2850 kcal/kg, respectively) and finisher (CP and ME as 20% and 2950 kcal/kg, respectively) diets (A, B, C and D) were formulated. Diet A contained arginine level according to the NRC (1994) recommendations both in starter and finisher phase. Remaining diets (B, C and D) were supplemented with arginine as 0.2, 0.4 and 0.6% of diet, respectively. The duration of experiment was 35 days. Weekly feed consumption, weekly weight gain and mortality were recorded. Data on carcass characteristics like percentage of dressed meat, thigh meat yield, breast meat yield, relative organs weight (heart, liver and gizzard) and immune organs such as bursa, spleen and thymus weight were also recorded. Weekly feed intake (3506g, 3531g) and body weight (2080g, 2139g) were better (P<0.05) in group fed arginine supplementation as 0.4 and 0.6%, respectively as compared to control group. Better feed conversion ratio (FCR, P<0.05) was shown by the all arginine treated groups with best FCR result in D group. Birds fed diet containing higher level of arginine (0.6%) had higher (P<0.05) dressing percentage, thigh yield and breast yield. However, relative organ (liver, gizzard and heart) weights and immune organ (thymus, bursa and spleen) weights were not affected (P>0.05) by arginine supplementation. Higher (P<0.05) titer against ND virus and IBD virus were recorded in birds fed diet containing arginine at 0.6%. It can be concluded that inclusion of arginine at 0.6% had improved growth performance and carcass yield.

Keywords | Arginine, Growth parameters, Carcass attributes, Immune functions

Received | June 15, 2021; Accepted | July 25, 2021; Published | January 10, 2022

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INTRODUCTION

Essential amino acids are not synthesized by the bird's body so their synthetic forms are used in diet to optimize the performance. Arginine is included in essential amino acids and important for optimal growth performance (Jankowski et al., 2020; Ruan et al., 2020) and creatine supply in the body (Khajali and Wideman, 2010). It is also critical for digestive functions in birds (Ghamari et al., 2020) and mammals as it decreases permeability of intestines because it has role in nitric oxide synthesis. It also plays a vital role in ulcers healing present in G. I. tract (Wideman et al., 2007; Neyens et al., 2017) wound healing and infection (Alexander and Supp, 2014). Arginine supplementation in hens diet improve the immunity of offspring (Li et al., 2021) broiler (Khatun et al., 2020) and turkey (Jankowski et al., 2020).

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Birds lacking the key enzyme carbamoyl phosphate synthase-I that is necessary for the synthesis of arginine inside the body therefore, birds also have less activity of hepatic arginase and ornithine transcarbamylase. However, carbamoyl phosphate synthase-I is present in many mammals thus they have an ability to synthesize arginine to fulfill their body requirements. Therefore, chicken diet must contain an adequate amount of arginine according to body requirements in order to maintain immune functions, protein accretion and physiological functions. (Khajali and Wideman, 2010).

According to the recommendations of NRC, birds require arginine as 12.5 and 11.0 g / kg of the diet in starter (first 3 weeks) and finisher phase (from 3-6 weeks), respectively (NRC, 1994).

Extra supplementation of Arginine for broilers than the recommendations of NRC increased the weight gain and FCR (Fernandes et al., 2009; Khajali et al., 2011). Arginine in their diet is necessary as it improves growth performance (Castro et al., 2019). Birds fed diet supplemented with arginine improved the breast muscle weight (Yu et al., 2018). In ovo administration of arginine also reported to improve post-hatch performance and bone morphometry in quails (Luqman et al., 2021). Bulbul et al. (2014) observed that antibody titer against Newcastle disease (ND) was not improved by super dosing of arginine.

There is lack of information regarding arginine supplementation on broiler performance under indigenous environment. Thus, this study has been planned to evaluate the influence of different levels of super dosing of arginine on growth, carcass characteristics and antibody titers in broilers birds.

MATERIALS AND METHODS

University of Agriculture Faisalabad, Pakistan has its experiment station named as "Raja Muhammad Akram Animal Nutrition Research center" where we examined the effect of adding different levels of arginine to diet on growth performance, carcass characteristics and immune response of broilers. This research station is under the Institute named as "Institute of Animal and Dairy Sciences". House was completely sealed and air tight after dry cleaning and white wash. Saw dust was spread in all pens. Then poultry shed was preheated with the help of brooder to achieve desired house temperature twenty four hours prior to the arrival of chicks. Feed was prepared before the flock arrivals. All equipment's were cleaned, disinfected and in good working order. Availability of clean and fresh drinkable water was present throughout the whole experiment.

Total number of chicks was 240 that were procured from local hatchery. Four dietary treatments named as "A, B, C and D" were randomly allocated to the day-old chicks in completely randomized design. Each group had 6 replicates and each replicate contained 10 chicks. In diet formulation we fulfilled all the nutritional requirement of broilers. All diets were iso-nitrogenous and iso-caloric. Starter and finisher diets contained CP as 22 and 20% and ME as 2850 and 2950 kcal/kg, respectively (Tables 1-4). Diet A was without supplementation and contained arginine according to the NRC (1994). Birds require 12.5 and 11.0 g / kg arginine in their starter and finisher phases, respectively (NRC, 1994). Remaining three diets (B, C and D) were extra supplemented with arginine as 2, 4 and 6 g/ kg, respectively. Chicks were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) as also mentioned in Ali et al. (2012).

Weekly feed consumption and body weight was recorded for each replicate separately. Feed conversion ratios were calculated for each treatment. Blood samples (two birds / replicate) were collected at 28th day and 35th day of age. First collected samples (28th day) were used to determine antibody titer of IBD (Infectious Bursal Disease). While last samples were used to determine antibody titer against ND (Newcastle disease).

On 35th day, 2 birds per replicate were randomly selected for slaughtering to determine dressing percentage, relative weight of breast meat yield, thigh, heart, liver, gizzard, spleen, thymus and bursa.

STATISTICAL ANALYSIS

From SAS 9.2, we analyzed our research data by using procedures of GLM (SAS, 2007). Treatments means were compared using Tukey's Test (Steel et al., 1997).

RESULTS

Better feed intake (P<0.05) was found in group of birds provided supplemented diet with arginine as 0.4 and 0.6% whereas, lowest value of the amount of feed intake (3364g) was shown in birds fed diet A. Overall, weight gain was better in birds fed diet D in which arginine was supplemented as 0.6% than other groups. Results about feed conversion ratios was significant (P<0.05) within all supplemented groups. Diet D showed better (P<0.05) FCR as compared to other diets. Mortality of birds was remained similar across the treatments (Table 5).

Similarly, diet D showed more dressing percentage than other groups, whereas, lowest value for dressing percentage was observed in control diet (Table 6). Relative breast meat yield were higher in birds fed diet containing arginine OPENOACCESSJournal of Animal Health and ProductionTable 1: Ingredients composition of starter diets (0-21 days)*

Table 1: Ingredients composition of starter diets (0-21 days)*							
Ingredients %	Diet A	Diet B	Diet C	Diet D			
Maize	39.33	39.01	39.07	39.14			
Rice broken	15.00	15.00	15.00	15.00			
Soybean Meal	21.38	21.59	22.37	23.15			
Canola Meal	15.00	14.79	13.73	12.66			
Corn Gluten 60%	2.50	2.50	2.50	2.50			
Guar Meal	2.00	2.00	2.00	2.00			
Vegetable Oil	0.89	1.00	1.00	1.00			
Lime stone	1.06	1.06	1.07	1.08			
DCP	1.77	1.77	1.77	1.78			
Salt NaCl	0.25	0.25	0.25	0.25			
Sodium Bicarbonate	0.00	0.00	0.01	0.01			
Premix**	0.10	0.10	0.10	0.10			
DL Methionine	0.24	0.24	0.24	0.25			
L Threonine	0.06	0.06	0.06	0.06			
L. Lysine HCl	0.31	0.31	0.30	0.30			
L-Arginine	0.00	0.20	0.40	0.60			
Betafin [®] (natural betaine)	0.04	0.04	0.04	0.04			
Axtra XAP (enzyme)	0.01	0.01	0.01	0.01			
AGP***	0.01	0.01	0.01	0.01			
Phytase	0.01	0.01	0.01	0.01			

Total 1 *Diets A, B, C and D contained 0, 0.2, 0.4 and 0.6% of L-Arginine.

 ** Vitamins and minerals premix provides per kg of diet: 10000 IU Vitamin A, 11.0 IU Vitamin E, 1.1 mg Vitamin K, 1100 IU Vitamin D3, 5 mg Riboflavin, 12 mg Ca Pantothenate, 12.1 μg Vitamin B12, 2.2 mg Vitamin B6, 2.2 mg Thiamin, 44 mg Nicotinic acid, 250 mg Choline chloride, 1.55 mg Folic acid, 0.11 mg d-biotin, 60 mg Mn, 50 mg Zn, 0.3 mg I, 0.1 mg Co, 30 mg Fe, 5 mg Cu and 1 mg Se.

0.05

100.0

0.05

100.0

0.05

100.0

0.05

100.0

*** Antibiotic growth promoter (Maxus®, Germany).

M-tox+ (mycotoxin preventer)

Table 2: Nutrients composition of starter (0-21 days) diets*

Nutrients (%)	Diet A	Diet B	Diet C	Diet D
Metabolizable Energy KCal/Kg	2850.00	2850.00	2850.00	2850.00
Crude Protein %	22.00	22.00	22.00	22.00
Crude Fat / E.E	2.76	2.85	2.84	2.82
Linoleic Acid	1.03	1.04	1.04	1.05
Crude Fiber	4.66	4.64	4.56	4.48
Ash	6.30	6.30	6.30	6.30
Dig. Lysine	1.20	1.20	1.20	1.20
Dig. Metheonine	0.58	0.58	0.58	0.59
Met+Cyst	0.96	0.96	0.96	0.96
Dig. Threonine	0.78	0.78	0.78	0.78
Dig. Arginine	1.26	1.45	1.65	1.85
Glycine + Serine	1.87	1.87	1.87	1.86
Dig. Histidine	0.53	0.53	0.53	0.53
Dig. Leucine	1.68	1.68	1.68	1.69
Dig. Isoleucine	0.84	0.84	0.85	0.85

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Dig. Tryptophan	0.25	0.25	0.25	0.25	
Dig. Phenylalanine	0.94	0.94	0.95	0.95	
Dig. Valine	0.95	0.95	0.95	0.96	
Calcium	1.00	1.00	1.00	1.00	
Phosphorous	0.42	0.42	0.42	0.42	
Na	0.15	0.15	0.15	0.15	
Κ	0.85	0.85	0.85	0.85	
C1	0.20	0.20	0.20	0.20	

*Diets A, B, C and D contain 0, 0.2, 0.4 and 0.6% L-Arginine in broiler diet.

Table 3: Ingredients	composition	of finisher	(21-35	days) diets	
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Ingredients	Diet A	Diet B	Diet C	Diet D
Maize	36.81	38.11	39.41	40.71
Rice Polish	1.00	1.00	1.00	1.00
Rice broken	23.87	22.67	21.46	20.26
Soybean Meal	22.44	23.23	24.01	24.80
Canola Meal	7.82	6.71	5.60	4.50
Corn Gluten 60%	2.50	2.50	2.50	2.50
Guar Meal	0.50	0.50	0.50	0.50
Vegetable Oil	1.00	1.00	1.00	1.00
Lime stone	1.04	1.07	1.09	1.11
DCP	1.88	1.88	1.88	1.88
Salt NaCl	0.25	0.25	0.25	0.25
Sodium Bicarbonate	0.02	0.02	0.02	0.02
Premix**	0.10	0.10	0.10	0.10
DL Methionine	0.26	0.26	0.27	0.28
L Threonine	0.07	0.07	0.07	0.07
L Lysine HCl	0.32	0.31	0.31	0.31
L Arginine	0.00	0.20	0.40	0.60
Betafin® (natural betaine)	0.04	0.04	0.04	0.04
Axtra XAP (enzyme)	0.01	0.01	0.01	0.01
AGP***	0.01	0.01	0.01	0.01
Phytase	0.01	0.01	0.01	0.01
M-tox+ (mycotoxin preventer)	0.05	0.05	0.05	0.05
Total	100.0	100.0	100.0	100.0

*Diets A, B, C and D contain 0, 0.2, 0.4 and 0.6% L-Arginine in broiler diet.

** Vitamins and minerals premix provides per kg of diet: 10000 IU Vitamin A, 11.0 IU Vitamin E, 1.1 mg Vitamin K, 1100 IU Vitamin D3, 5 mg Riboflavin, 12 mg Ca Pantothenate, 12.1 µg Vitamin B12, 2.2 mg Vitamin B6, 2.2 mg Thiamin, 44 mg Nicotinic acid, 250 mg Choline chloride, 1.55 mg Folic acid, 0.11 mg d-biotin, 60 mg Mn, 50 mg Zn, 0.3 mg I, 0.1 mg Co, 30 mg Fe, 5 mg Cu and 1 mg Se.

*** Antibiotic growth promoter (Maxus®, Germany).

Table 4: Nutrients composition of finish	her (21-35 days) diets*
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Nutrients (%)	Diet A	Diet B	Diet C	Diet D
Metabolizable Energy KCal/Kg	2950.00	2950.00	2950.00	2950.00
Crude Protein %	20.00	20.00	20.00	20.00
Crude Fat / E.E	2.86	2.89	2.91	2.94
Linoleic Acid	1.05	1.08	1.10	1.13
Crude Fiber	3.86	3.80	3.74	3.68

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Ash	6.02	6.04	6.05	6.07	
Dig. Lysine	1.12	1.12	1.12	1.12	
Dig. Methionine	0.56	0.57	0.57	0.58	
Met+Cyst	0.89	0.89	0.89	0.89	
Dig. Threonine	0.72	0.72	0.72	0.72	
Dig. Arginine	1.16	1.35	1.55	1.74	
Glycine + Serine	1.65	1.65	1.66	1.66	
Dig. Histidine	0.48	0.48	0.48	0.48	
Dig. Leucine	1.54	1.55	1.57	1.58	
Dig. Isoleucine	0.79	0.80	0.80	0.81	
Dig. Tryptophan	0.23	0.23	0.23	0.23	
Dig. Phenylalanine	0.89	0.89	0.90	0.90	
Dig. Valine	0.90	0.90	0.90	0.90	
Calcium	1.00	1.00	1.00	1.00	
Phosphorous	0.42	0.42	0.42	0.42	
Na	0.15	0.15	0.15	0.15	
K	0.79	0.79	0.79	0.79	
Cl	0.20	0.20	0.20	0.20	

*Diets A, B, C and D contain 0, 0.2, 0.4 and 0.6% L-Arginine in broiler diet.

Table 5: Effect of different levels of arginine on growth performance and mortality in broiler at 35 day

	Dietary treatments *					
Parameters	Α	В	С	D	SEM	P value
Feed intake (g)	3364.35 ^b	3435.64 ^{ab}	3506.33ª	3531.34ª	30.2	0.00
Weight gain (g)	1903.68°	2002.43 ^b	2080.18^{a}	2139.17ª	19.3	0.00
Feed conversion ratio	1.77ª	1.72 ^b	1.69 ^c	1.65 ^d	0.007	0.00
Mortality (%)	6.67	5.00	5.00	3.33	2.53	0.83

* Dietary treatments (A, B, C and D) include arginine at 0, 0.2, 0.4 and 0.6% in broiler diet.

Values with different super-scripts (a, b, c and d) are significantly different (P < 0.05)

Table 6: Effect of different levels of arginine on carcass characteristics and immune organs weight in broiler at 35 day

	Dietary tr	eatments *			0	
Parameters (%)	Α	В	С	D	SEM	P value
Dressing percentage	55.74°	56.51°	57.98 ^b	59.81ª	0.380	0.00
Breast meat weight	36.07 ^b	37.17 ^{ab}	37.71ª	38.05 ^a	0.324	0.00
Thigh meat weight	19.67 ^b	19.34 ^b	20.27 ^{ab}	21.76ª	0.425	0.00
Heart weight	0.46	0.48	0.44	0.47	0.023	0.57
Gizzard weight	1.50	1.50	1.51	1.52	0.030	0.93
Liver weight	2.45	2.41	2.43	2.44	0.019	0.62
Immune organs						
Spleen weight	0.17	0.18	0.18	0.18	0.009	0.71
Thymus weight	0.22	0.21	0.21	0.21	0.011	0.85
Bursa weight	0.07	0.08	0.08	0.08	0.008	0.87

* Dietary treatments (A, B, C and D) include arginine at 0, 0.2, 0.4 and 0.6% in broilers diet.

Values with different super-scripts (a, b, and c) are significantly different (P < 0.05)

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Table 7: ND and IBD titer of broilers fed diets containing different levels of arginine.									
Dietary treatments *									
Parameters	Α	В	С	D	SEM	P value			

Newcastle Disease Titer82.54b83.95ab95.33ab106.34aInfectious Bursal Disease titer37.52b46.44ab55.15a58.28a* Dietary treatments (A, B, C and D) include arginine at 0, 0.2, 0.4 and 0.6% in broiler diet.

Values with different super-scripts (a and b) are significantly different (P < 0.05)

at 0.4 and 0.6% than those fed NRC recommended diet. Birds fed diet containing arginine at 0.6% showed higher value of relative thigh meat yield than other dietary treatments. However, treatments were remained similar (P>0.05) regarding weights of liver, heart, gizzard and spleen in birds among treatments. The bursa and thymus weights also showed non-significant differences (P>0.05) among the experimental diets (6).

Titers against ND and IBD were different (P<0.05) in birds fed diets containing supplemental levels of arginine. Higher titers of ND and IBD were recorded in birds offered diet supplemented with 0.6 % arginine whereas, birds fed control diet showed lower titer of ND (Table 7).

DISCUSSION

GROWTH PERFORMANCE

Increase in feed intake and weight gain in birds supplemented with arginine in current study might be arginine function of protein synthesis, secretion glucagon, insulin and growth hormone. It has also been stated that arginine supplementation increases the villus height of intestine which increases absorption of nutrients results in increase feed intake and growth (Al-Daraji and Salih, 2012). The other possible reason of improved the amount of feed intake and growth in arginine supplemented birds could be due to increase in villus height of intestine. These results imply the critical role of arginine to maintain growth performance in terms of protein synthesis and accretion. However, our findings are not agreed with the that of Fernandes et al. (2015) and Murakami et al. (2012). They reported no effect of the supplementation of L-arginine on the amount of feed intake in broilers. Whereas, Khajali et al. (2011) and Emadi et al. (2011) observed lower feed intake by the birds fed diet containing supplemented arginine.

Highest amount of weight gain was recorded in group of birds fed diet containing arginine as 0.4 and 0.6% of diet. Similar results are observed by Basoo et al. (2012) who reported improved weight gain in birds fed arginine supplemented diets. This might be due to that arginine role in supporting chickens for maximum growth. Arginine acts as important amino acid in maintaining the growth in terms of protein synthesis and accretion and biosynthesis of nitric oxide. It has been reported that it has role in the synthesis of numerous factors that are related to growth including spermine, putrescine and spermidine. It also increases the hydroxyproline and proline through the synthesis of glutamate, these amino acids are required for the synthesis of connective tissue. Results are not agreed with the results of Fernandes et al. (2015) who reported no effect on weight gain of birds fed L-arginine supplemented diets. Mejia et al. (2012) showed that bird fed diet containing additional arginine, had no effect on weight gain.

5.94

3.05

0.03

0.00

Better (P<0.05) FCR was seen in experimental group offered diet containing 0.6 % arginine. Results are in line with the outcomes of Basoo et al. (2012) who showed that birds fed arginine supplemented diet had better FCR. Al-Daraji and Salih (2012) recorded that arginine supplementation in broiler diet had improved FCR. Arginine is present in the structure of ornithine that is used for the formation of polyamines, which in turn act on the maturation and regeneration of the intestinal mucosa, resulted in increased absorption and improved feed efficiency. Further, the super dosing of arginine than recommended by NRC may be needed for the stability of the intestinal epithelium which ultimately improves FCR.

IMMUNE RESPONSE

Higher titer of ND and IBD were recorded in birds fed diet containing arginine at 0.6%. It modifies or boosts cellular and humoral immune responses to the challenges of experimental infection. Further, it increases the lymphocytes proliferation in the blood, increase phagocytic activity of alveolar macrophages and suppressor T-cell counts. The increase in ND and IBD titer in current study might be factor of arginine influence on immune defense mechanism of the birds. Our findings are in agreement with the results of Tan et al. (2014) who showed that arginine supplementation in broiler diet had higher ND titer than control group. Kidd et al. (2001) who reported that arginine supplementation in broiler grower had higher IBD titer. Findings are not in favor of the results of Bulbul et al. (2014) who found that birds provided diet varying levels of arginine supplementation had no effect of ND and IBD titer.

SLAUGHTERING DATA

Arginine supplementation in the diet increases the carcass yield. Mendes et al. (1997) also found better carcass

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yield in broilers when they supplemented the arginine. Fernandes et al. (2009) found that arginine super dosing during starter phase may be useful for increased muscle development in broilers birds. It may be due to effect of arginine on blood vessels dilation, basically through enhanced nitric oxide production (Huk et al., 1997). It is important phenomenon because it increased the blood flow to the body muscles and due to this improved blood flow permits more distribution of portions, hormones, carbohydrates and many more nutrients to the muscular system and thus aids in the nourishment of body muscles (Chamruspollert et al., 2002). More nitric oxide has a positive influence on muscle mass by encouraging the rate of protein formation within cells of different muscle (Stevens et al., 2000).

Relative organ (gizzard, heart and liver) weights and immune organ (bursa, thymus and spleen) weights were remained same by supplementation of arginine. Our findings are closed to the results of Yang et al. (2016). They also reported no effect of arginine supplementation on organ weights of spleen, liver, gizzard and heart. Similarly, Guo et al. (2015) also revealed that birds provided diet supplemented with arginine had no effect on spleen weight. Cengiz and Kucukersan (2010) reported that immune organ (bursa and spleen) weights were not affected by arginine supplementation.

CONCLUSION

On the basis of the study, it can be concluded that inclusion of arginine at 0.6% in the diet of broilers had improved growth performance and carcass yield.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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