PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 11 (3): 258-264, 2012
ISSN 1680-5194

The Influence of Dietary Arginine Supplementation on Blood Traits of Broiler Chickens

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Abstract: A total of 300 one day old Ross 308 broiler chicks were used in this study to determine the effect of supplementing ration with different levels of arginine on productive performance of broiler chickens. The chicks were allocated for 4 treatment groups (75 chicks for each group) and each treatment was consisted of five replicates with 15 chicks each. Treatment groups were: C: control group (without any addition of arginine); T1, T2 and T3: adding arginine to the diet of broiler chickens at levels of 0.02, 0.04 and 0.06%, respectively. Two types of diets were used over the period of experiment, starter diet was used from one to 20 days of chicks' age and then grower diet was used till the end of the experiment (46 days of age). Blood traits included in this study were total erythrocytes count (RBC), Packed Cell Volume (PCV), Hemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), total leucocytes count (WBC), hetrophil to lymphocyte ratio (H/L) and thrombocytes count (Thr.). Results indicated that adding arginine to the diet of broiler chickens (T1, T2 and T3) resulted in significant increase in RBC, PCV, Hb, MCV, MCH, MCHC, WBC and Thr. and significant decrease in H/L ratio during all periods of experiment and regarding the total means of these traits as compared with control group. In conclusion, supplementation of the broiler ration with L-arginine resulted in significant improvement with respect to blood traits included in this study. Therefore, arginine can be used as effective feed additive for enhancing physiological status of broiler chickens.

Key words: Arginine, blood traits, broiler chickens

INTRODUCTION

The amino acid arginine is essential for optimal growth and nitrogen balance in growing animals. Whereas most mature mammals can synthesize arginine to meet their requirements, chickens cannot synthesize arginine therefore, chicks are completely dependent on dietary arginine to meet their needs for protein synthesis and other functions (Tamir and Ratner, 1963). Moreover arginine has more benefits and has vast effects when it is added as supplementary diet, for instance arginine increases the release of growth hormone and arginine facilitates muscle growth (by inhibiting muscle loss) and it is required for the transport of the nitrogen used in muscle metabolism and arginine improves muscle performance, also improves glucose uptake into muscle cells (Stevens *et al*., 2000). Moreover arginine improves blood circulation, by stimulating the production of nitric oxide, an endogenous neurotransmitter that helps to prevent vasoconstriction which initiates vasodilation by relaxing the smooth muscle cells of the blood vessels, arginine also helps to prevent abnormal blood clotting by stimulating the production of plasmin and by increasing vasodilation and also inhibits the adhesion of monocytes to the endothelium, besides arginine reduces pulmonary blood pressure and improves blood circulation in pulmonary hypertension syndrome that known as ascites (Nakaki, 1990; Nagaya, 2001). Arginine lowers total serum cholesterol levels and serum Low-Density Lipoprotein (LDL) levels, it also reduces insulin resistance and improves blood sugar disposal in diabetes, then arginine improves diabetes and reverses damage caused by diabetes and may prevent diabetes (Mohan and Cas, 1998).

Furthermore arginine helps to prevent bacterial and viral diseases and enhance immune system functions and increases the size of the thymus. Arginine also stimulates the production of helper T-cells by the thymus and restores the production of thymic hormones to youthful levels (Dean, 1999). Increasing dietary arginine improved blood plasma parameters (Emadi *et al*., 2010). Deficiency of arginine impaired insulin production, glucose production and liver lipid metabolism (Balch *et al*., 1997), because it is involved in the production of variety of enzymes and hormones. Arginine facilitated the release of Growth Hormone (GH) and stimulated the pancreas for insulin production (Balch *et al*., 1997). It also increased the levels of glucose and GH (Braverman, 1997). As far as we know relevant information about the arginine status in chickens as regards their physiological performance is rare. The purpose of this study was therefore to investigate the effects of dietary supplementation of arginine on physiological performance of broiler chickens.

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Table 1: Ingredient of the starter diet used in the experiment

	Treatments			
Ingredients	Control	Trt. 1	Trt. 2	Trt. 3
Yellow corn	58.00	58.00	58.00	58.00
Soya bean meal	27.00	27.00	27.00	27.00
Protein conc.*	9.00	9.00	9.00	9.00
Wheat	4.00	3.98	3.96	3.94
Sunflower oil	1.50	1.50	1.50	1.50
DCP**	0.30	0.30	0.30	0.30
Salt	0.20	0.20	0.20	0.20
Arginine	0.00	0.02	0.04	0.06
Total	100.00	100.00	100.00	100.00
Calculated composition* **				
Protein	22.01	22.00	22.00	22.00
ME kcal/kg	3045.00	3040.00	3040.00	3040.00
Calcium	0.74	0.74	0.74	0.74
Phosphorus	0.41	0.41	0.41	0.41
Lys.	1.30	1.30	1.30	1.30
Meth.	0.62	0.62	0.62	0.62
Meth. to Cyst.	0.96	0.96	0.96	0.96
Arginine	1.55	1.57	1.59	1.61
Arg:Lys	1.19:1	1.20:1	1.22:1	1.23:1

*Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40% crude protein, 2100 kcal ME/kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, 3.85% lysine, 3.70% methionine and 4% cystine.
**DCP: Dicalcium Phosphate.
***The calculated composition of the diets was determined according to NRC (1994). Trt. = Treatment

Table 2: Ingredient of the grower diet used in the experiment

	Treatments			
Ingredients	Control	Trt. 1	Trt. 2	Trt. 3
Yellow corn	61.00	61.00	61.00	61.00
Soya bean meal	24.00	24.00	24.00	24.00
Protein conc.*	7.00	7.00	7.00	7.00
Wheat	5.00	4.98	4.96	4.94
Sunflower oil	2.50	2.50	2.50	2.50
DCP**	0.30	0.30	0.30	0.30
Salt	0.20	0.20	0.20	0.20
Arginine	0.00	0.02	0.04	0.06
Total	100.00	100.00	100.00	100.00
Calculated composition* **				
Protein	20.15	20.13	20.13	20.13
ME kcal/kg	3150.00	3145.00	3145.00	3145.00
Calcium	0.60	0.60	0.60	0.60
Phosphorus	0.35	0.35	0.35	0.35
Lys.	1.16	1.16	1.16	1.16
Me.	0.54	0.54	0.54	0.54
Meth. to Cyst.	0.85	0.85	0.85	0.85
Arginine	1.387	1.407	1.427	1.447
Arg:Lys	1.19:1	1.21:1	1.23:1	1.24:1

*Protein concentrate used in the diets was produced in Holland (WAFI) which contains: 40% crude protein, 2100 kcal ME/kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, 3.85% lysine, 3.70% methionine and 4% cystine.
**DCP: Dicalcium Phosphate.
***The calculated composition of the diets was determined according to NRC (1994). Trt. = Treatment

MATERIALS AND METHODS

A total of 300 one day old Ross 308 broiler chicks were housed at clean well - ventilate room previously disinfected and the management of the four treatment groups were identically carried out, where the new hatched chicks have been raised at poultry experimental fields, which consisted of several separate rooms from each other and the area of each room were 5.75 x 3.75 m and the chicks have been randomly distributed into 3 rooms and chicks were raised on floor cages (140 x 120 x 90 cm). Feed and water were provided *ad libitum* during the whole period of experiment which lasted 6 weeks. Two types of diets were used over the period of experiment, starter diet was used from one to 20 days of chicks age and then grower diet was used till the end of the experiment (Table 1 and 2). The chicks were allocated for 4 treatment groups (75 chicks for each group) and each treatment was consisted of five replicates with 15 chicks each. Treatment groups were: C: control group (without any addition of arginine); T1, T2 and T3: adding arginine to the diet of broiler chicken at levels of 0.02, 0.04 and 0.06%, respectively. Blood traits included in this study were total erythrocytes count (RBC), Packed Cell Volume (PCV), Hemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), total leucocytes count (WBC), Hetrophil to Lymphocyte ratio (H/L) and Thrombocytes count (Thr.). These traits were determined according to Al-Daraji *et al*. (2008). Data of experiment were analyzed using XLStat, version 7.5, 2004. The significant differences between means of traits included in this study were determined using Duncan's multiple range test under the probability ($p<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

Results of Table 3 clarified the effect of dietary arginine on some physical blood traits which included RBC count, PCV and Hb concentration in broiler fed diet supplemented with different levels of arginine. Supplementary arginine has significantly increased ($p<0.05$) each of RBC count, PCV and Hb concentration in 2nd, 4th and 6th weeks of age and with relation to the total means of these traits. However, the total mean values of RBC count were 2.22, 2.43, 2.54 and 2.61 x 10^6/µl of blood for C, T1, T2 and T3, respectively. Total mean values of PCV were 26.71, 29.41, 30.93 and 32.05% for C, T1, T2 and T3, respectively. Whereas, the total mean values for the total average of Hb concentration were 6.91, 8, 8.52 and 9.07 g/dl for C, T1, T2 and T3, respectively. Moreover, results of Table 3 also indicated that the highest total mean values of RBC, PCV and Hb concentration were recorded in the bird group that received the highest level of arginine (T3). Effect of dietary supplementation of arginine on MCV, MCH and MCHC is showed in Table 4. It was noticed that arginine treatments (T1, T2 and T3) significantly surpass

Table 3: Effect of dietary arginine on RBC, PCV and Hb concentration (Mean±SE) of broiler chickens at different weeks of age

	RBC (x 10^6/µl)				PCV (%)				Hb (g/dl)			
	Weeks				Weeks				Weeks			
Trt.	2	4	6	Total average	2	4	6	Total average	2	4	6	Total average
C	2.01±0.08^b	2.10±0.03^c	2.56±0.09^c	2.22±0.071^c	22.50±0.66^b	27.3±2.23^c	30.3±0.99^c	26.71±1.29^c	4.37±0.23^b	6.07±0.56^c	10.29±0.10^c	6.91±0.30^c
T1	2.26±0.03^a	2.22±0.04^b	2.81±0.05^b	2.43±0.04^b	23.80±0.78^a	31.2±0.37^b	33.2±0.93^b	29.41±0.69^b	5.72±0.14^a	7.27±0.55^b	11.02±0.33^b	8.00±0.34^b
T2	2.27±0.02^a	2.36±0.05^a	3.00±0.02^{ab}	2.54±0.03^a	24.30±0.87^a	33.0±0.96^a	35.5±0.61^a	30.93±0.81^a	5.95±0.20^a	7.75±0.08^a	11.88±0.19^a	8.52±0.16^b
T3	2.35±0.02^a	2.44±0.08^a	3.04±0.06^a	2.61±0.05^a	24.95±0.47^a	35.0±0.68^a	36.2±0.77^a	32.05±0.64^a	6.17±0.18^a	8.56±0.20^a	12.50±0.15^a	9.07±0.18^a

C: Control group; T1, T2 and T3: adding arginine to the diet of broiler chicken at levels of 0.02, 0.04 and 0.06%, respectively.

^{a,b,c}Mean values having different superscript in column differ significantly ($p<0.05$). Trt. = Treatment

Table 4: Effect of dietary supplementation of arginine on MCV, MCH and MCHC (Mean ± SE) of broiler chickens at different weeks of age

	MCV (femtoliter)				MCH (picogram)				MCHC (g/dl)			
	Week				Week				Week			
Trt.	2	4	6	Total average	2	4	6	Total average	2	4	6	Total average
C	104.17±5.33^c	130.00±08.56^b	116.55±10.04^b	116.90±10.53^c	21.74±1.33^b	28.90±5.50^c	37.19±2.98^c	29.27±7.04^c	19.42±3.15^b	21.23±2.60^b	31.90±2.97^b	24.18±4.10^b
T1	104.17±5.33^c	140.54±09.23^a	118.14±12.21^a	121.90±12.26^b	25.30±2.21^a	32.74±6.08^b	39.21±6.03^b	32.41±5.33^b	23.98±2.19^a	23.30±3.45^a	33.19±3.23^a	26.82±5.09^a
T2	108.53±8.52^b	139.83±11.13^a	118.33±09.99^a	121.23±09.87^b	26.21±4.05^a	32.83±5.59^b	39.60±4.76^b	32.88±6.34^b	24.48±2.98^a	23.48±2.89^a	33.46±3.77^a	27.14±3.99^a
T3	111.94±7.92^a	143.44±10.76^a	119.27±10.55^a	124.88±09.67^a	26.25±3.32^a	35.08±4.48^a	41.11±3.91^a	34.14±6.65^a	24.72±4.56^a	24.45±3.98^a	34.47±3.25^a	27.88±3.76^a

C: Control group; T1, T2 and T3: adding arginine to the diet of broiler chicken at levels of 0.02, 0.04 and 0.06%, respectively.

^{a,b,c}Mean values having different superscript in column differ significantly ($p<0.05$). Trt. = Treatment

Table 5: Effect of dietary arginine on WBC count, H/L ratio and Thr. count (Mean ± SE) of broiler chickens at different weeks of age

	WBC (×10^3/µl)				H/L ratio				Thr. (×10^3/µl)			
	Week				Week				Week			
Trt.	2	4	6	Total average	2	4	6	Total average	2	4	6	Total average
C	21.36±0.06^b	21.15±0.05^b	22.76±0.05^b	21.75±0.04^b	0.250±0.014^a	0.320±0.09^a	0.426±0.01^a	0.332±0.01^a	28.67±0.08^b	c31.58±0.02	31.84±0.02^b	30.69±0.03^b
T1	21.78±0.08^a	23.70±0.03^a	25.35±0.05^a	23.61±0.05^a	0.245±0.07^b	0.268±0.07^b	0.246±0.04^b	0.253±0.04^b	30.20±0.05^a	b32.62±0.02	35.98±0.01^a	32.93±0.03^a
T2	21.81±0.07^a	23.96±0.03^a	25.46±0.06^a	23.74±0.05^a	0.240±0.06^b	0.249±0.08^b	0.244±0.05^b	0.245±0.03^{bc}	30.44±0.04^a	a33.98±0.01	36.08±0.03^a	33.50±0.03^a
T3	21.82±0.02^a	24.08±0.04^a	25.64±0.04^a	23.84±0.06^a	0.236±0.06^c	0.235±0.06^c	0.236±0.06^c	0.236±0.04^c	30.52±0.03^a	a34.24±0.02	36.32±0.02^a	33.69±0.04^a

C: Control group; T1, T2 and T3: adding arginine to the diet of broiler chicken at levels of 0.02, 0.04 and 0.06%, respectively.

^{a,b,c}Mean values having different superscript in column differ significantly ($p<0.05$). Trt. = Treatment

($p<0.05$) C group during all periods of experiment and in total average values with respect to MCV, MCH and MCHC with one exception for 2nd week when there was no significant differences were found between C and T1 groups for the MCV trait. However, total averages of MCV were 116.9, 121.9, 121.23 and 124.88 femtoliter (fl) for C, T1, T2 and T3, respectively and for MCH were 29.27, 32.41, 32.88 and 34.14 picogram (pg) for (C, T1, T2 and T3, respectively. While total averages of MCHC were 24.18, 26.82, 27.14 and 27.88 g/dl for C, T1, T2 and T3, respectively. Table 4 also reveals that the highest value of these three traits belongs to the highest level of supplemented arginine (T3). The results of MCV, MCH and MCHC are in accordant with the results of RBC, PCV and hemoglobin concentration since MCV, MCH and MCHC determination mainly depended on the basis of RBC, PCV and hemoglobin values. Results from Table 5 indicated that supplementing arginine to the diet of broiler chickens significantly increased ($p<0.05$) thrombocyte count, WBC count and decreased ($p<0.05$) H/L ratio. All arginine treated groups (T1, T2 and T3) had significantly higher ($p<0.05$) means of WBC than C group during all weeks of experiment (2nd, 4th and 6th) and respecting the total mean values of this trait which being 21.75, 23.61, 23.74 and 23.84 x 10^3/µl of blood for C, T1, T2 and T3, respectively. It is also denoted from Table 5 that H/L ratio significantly decreased ($p<0.05$) in all arginine treatment groups (T1, T2 and T3) in all weeks of experiment (2nd, 4th and 6th) and in the total mean values of this trait which being 33.2, 25.7, 24.5 and 23.6 for C, T1, T2 and T3, respectively. However, adding arginine to broiler diet (T1, T2 and T3) significantly increased ($p<0.05$) Thr. count during all weeks of experiment and in the total average of this trait which being 30.69, 32.93, 33.50 and 33.69 x 10^3/µl of blood for C, T1, T2 and T3, respectively. It is worth mentioning that the highest value of WBC and Thr. counts in all weeks of experiment was recorded for the highest level of arginine treatment (T3). In contrast, the lowest values of H/L related to the highest level of arginine treatment (T3). The results of this study clearly indicated that RBC, PCV, Hb, WBC, Thr., MCV, MCH and MCH were significantly improved as a result of dietary supplementation with arginine.

Possible mechanisms for the improvement in these traits may be account for that arginine stimulate GH secretion and GH induces Insulin-like Growth Factor (IGF)-1 (Le Roith *et al*., 2001), which in turn counteracts apoptosis similarly to Erythropoietin (EPO) and fosters proliferation and differentiation of Burst- and Colony-Forming Units-Erythroid (BFU-E, CFU-E) and myeloid progenitor and peripheral blood cells (Deicher and Walter, 2005). Erythropoietin (EPO) is a hematopoietic growth factor produced by kidney, acts directly on certain RBC progenitors and precursors in the bone marrow and controls the proliferation, differentiation and maturation of RBCs. The expression of erythropoietin is markedly increased in kidneys during hypoxic state, a condition mediated by the transcription factor Hypoxia Inducible Factor-1 (HIF-1). The ultimate effect is to increase erythropoiesis in an attempt to maintain oxygen delivery to vital organs (Westenfelder, 2002). The stimulating effect of arginine to GH and GH on erythropoiesis could be explained, at least partly, by anabolic action rather than a direct effect. The anabolic effect of GH induces an increase in metabolic activity and necessity for oxygen transport to peripheral tissue, resulting in an increase of oxygen transportation and Hb levels (Jepson and Mcgarry, 1972). It has been reported that GH has a direct stimulating effect on hematopoietic cells *in vitro* (Golde *et al*., 1977). It has also been reported that GH stimulates erythropoiesis via Insulin-like Growth Factor-I (IGF-I), a GH dependent growth factor (Merchav *et al*., 1988). A study by Dara and Jamal (2009) denoted that subcutaneous injection of GH in rats significantly increased RBC, WBC, Hb, thrombocyte, reticulocyte count and PCV. Kurtz *et al*. (1988) speculated that the increase of kidney mass during growth causes an increase in renal oxygen consumption and in consequence, a relative renal deficiency of oxygen. In turn, an enhanced rate of EPO production would lead to stimulation of erythropoiesis and thus adapt RBC mass to body growth. Ziemann *et al*. (2010) reported that in human arginine ingestion has been marked increase in White Blood Cell (WBC) count and arginine increases the size of the thymus and stimulates the production of lymphocytes by the thymus and restores the production of thymic hormones to youthful levels (Dean, 1999). Since growth hormone have important interactions with thyroxine hormone (Harvey, 1993), thyroxine regulate the cell cycle, proliferation and apoptosis of different types of human cells (Hara *et al*., 2000) and they play an important role in development. Thyroxine has been known to be important regulators of bone development and metabolism (Varga *et al*., 1997). It was well known that thyroxine influenced proliferation and differentiation of human Hematopoietic Stem/ Progenitor Cells (HSPC). And thyroid hormone is required in nearly all tissues, with major effects on oxygen consumption and metabolic rate. Therefore, erythropoiesis which provides the necessary oxygen capacity of the blood and the control of erythropoiesis by EPO has always been closely linked with the effects of thyroid hormones (Brenner *et al*., 1994). Adaptation to this increase in metabolic demands is partly achieved by potent effects of thyroid hormone on erythropoiesis and thus blood oxygen capacity. Thyroid hormones directly increase the proliferation of erythroid progenitors (Dame *et al*., 1998) and thyroid hormone receptors were identified on nucleated erythroid cells isolated from hypoxic hamsters (Boussios *et al*., 1982). In addition, tissue perfusion may be increased through the

stimulated expression of the potent vasodilator by thyroid hormones (Murakami *et al*., 1998). In the current experiment, the arginine treated groups recorded low values of H/L ratio compared to the control group which may be refers to the role of arginine in alleviating the impact of stress (Tong and Barbul, 2004). Since this experiment was conducted during summer months and the recorded subsequence temperature degree during the whole experiment period were 31.8-36.83°C. There is a direct relationship between this trait (H/L ratio) and concentration of stress hormone (corticosterone) in the blood (Broom and Johnson, 1993). Physiological responses to acute stress include the rapid secretion of catecholamines from the adrenal medulla and glucocorticoids from the adrenal cortex (Sapolskyr, 1992) and their levels have often been used to assess the level of stress (Wingfield, 1994). In birds, epinephrine levels can increase within seconds of exposure to stress and glucocorticoids rise within minutes (Le Maho *et al*., 1992). In the field, plasma levels of glucocorticoids can increase by an order of magnitude within 20-30 min of capture, so unless handling time is very short, the effect of these investigator-imposed stressors may swamp other inputs. Elevation of corticosterone, the major glucocorticoid in birds, leads to a series of events that can enhance short-term survival, including redirected behavior and mobilization of energy reserves (Wingfield *et al*., 1998). The half-life of these hormones is short (minutes to hours), so their levels drop and their effects disappear if the stressor is removed. This is functionally important because chronic stress and chronically elevated glucocorticoids can result in stress-related disease (Sapolskyr, 1992). The high rate of H/L ratio refers to the high level of stress hormone (Al-Daraji *et al*., 2008). Leukocyte numbers change more slowly (30 min to 20 hr.) in response to stress than does corticosterone (Cunnick *et al*., 1994). These changes are less variable and longer lasting than the corticosterone response and multiple stressors usually have an additive affect (Mckee and Harrison, 1995). It was clearly noticed from the results of the present study that arginine has major role in reducing heat stress on birds as evidenced by the improvement in blood traits especially low H/L ratio in particular. Gross and Siegel (1983) reported that H/L ratio measures a physiological changes, whereas the concentration of corticosteroids in the blood is affected by many factors before physiological changes occur. The H/L ratio should be a better measure of long-term changes in the environment and the concentration of corticosteroids in the blood should be a better measure of short-term changes. Therefore, the H/L ratio is a good measure of the chicken's perception in its environment and increasing H/L ratio indicated that the birds were under acute stress (Al-Daraji *et al*., 2002). There are many studies that confirm the efficiency of arginine in reducing the impact of heat stress. Likewise, inspection of the results of Brake *et al*. (1994) and Mendes *et al*. (1997) indicated that arginine alleviate the effects of heat stress in broiler that was raised in hot climate. Therefore, the reason of low H/L ratio in the arginine supplemented groups compared to control group, may be explained by the effects of arginine in alleviating the influence of heat stress. However, Nitric Oxide (NO) as a product of arginine was found to be work as an inhibitor of corticosteroid secretion during stress (Wimalawansa *et al*., 1996; Wimalawansa *et al*., 1997).

Conclusion: In conclusion feeding diets containing L-arginine resulted in significant improvement in blood traits included in this study. Therefore, arginine could be used as an efficient feed additive for enhance physiological performance of birds.

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