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Effect of Feedingarginine on the Growth Performance, Carcass Traits, Relative Expression of Lipogenic Genes, and Blood Parametersof Arian Broilers

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ABSTRACT

The aim of this study was to investigate the effects of different dietary levels of L-arginine on the growth performance, blood parameters, and lipogenic gene expression of Arian broiler chickens. For this purpose, 168 Arian broiler chicks (40.33±1.7 g) were assigned to four treatments with three replicates of14 birds each, according to completely randomized design. The experimental treatments consisted of 100, 124, 139, and 154% dietary arginine levels relative to the published requirements of Arian broilers. On 42 d of the experiment, blood samples were collected from two birds (six birds per treatment) for blood metabolite measurements. These birds were then euthanized for carcass evaluation and collection of tissue samples. Increasing dietary arginine levels reduced (p<0.05) the gene expression of fatty acid synthase, acetyl-coenzyme A carboxylase, and malic enzyme in the liver and lipoprotein lipase in the abdominal fat tissue, as well as abdominal fat relative weight. Increasing dietary arginine levels significantly increased (p<0.05) body weight, feed efficiency, carcass yield, breast and thigh relative weights, and glucose and HDL (high-density lipoprotein) blood levels, and reduced cholesterol, triglyceride, and LDL (low-density lipoprotein) blood levels. Since almost similar performance and carcass trait results were obtained both with 124 and 139% arginine levels, supplying Arian broiler diets with 124% arginine is suggested.

INTRODUCTION

Genetic selection of broiler chickens has resulted in the development of excessive body adiposity along with high muscle mass (Collin *et al*., 2009). However, excessive fat in broilers are not desirable by producers due their lower feed efficiency, neither by consumers due to health concerns (Ebrahimi *et al*., 2014). Therefore, finding novel means of improving feed efficiency and stimulating muscle growth without increasing carcass fatness is essential.

Some studies reported positive effects of arginine on broiler weight gain, muscle growth, feed efficiency, and meat quality (Fernandes *et al*., 2009; Jiao *et al*., 2010; Al-Daraji & Salih, 2012a; Ebrahimi *et al*. 2014, 2016). Al-Daraji & Salih (2012a,b) observed higher weight gain, body weight, feed intake, and feed conversion ratio, as well as increasing carcass yield and weight, as well as breast and thigh yields in broilers fed diets with 0.02, 0.04, and 0.06% arginine compared with the diet no arginine addition. Previous studies with broilers also obtained lower abdominal fat and abdominal adipocyte size, and higher breast muscle fat content and meat production by feeding high levels of arginine (Wu *et al*., 2011; Ebrahimi *et al*. 2014).

These effects may indicate the mobilization of energy from the adipose tissue to the skeletal muscle (Tan *et al*., 2011; Ebrahimi *et al*., 2014). Tan *et al.* (2011) showed that the supplementation of 1.0% arginine in pig diets increased the gene expression of fatty acid synthase in the muscle and of hormone sensitive lipase in the adipose tissue, while it decreased gene expression of glucose transporter-4, lipoprotein lipase, and acetyl-coenzyme A carboxylase-α in the adipose tissue. In an attempt to understand the mode of action of arginine on the fat metabolism of poultry, Ebrahimi *et al*. (2014) reported that, when Ross broiler chickens were fed high arginine levels, the expression of lipogenic genes was upregulated in the muscle (fatty acid synthase and lipoprotein lipase),and down regulated in the adipose tissue (fatty acid synthase and lipoprotein lipase) and liver (acetyl-coenzyme A carboxylase, fatty acid synthase, and malic enzyme). These results suggest that the addition of arginine to broiler diets may be used to reduce carcass fat content. According to Collin *et al*. (2009), there is an interaction between genotype and responses to the composition of the diet.

Arian is a native broiler strain of Iran and enhancing its production and carcass quality may aid the country's economy. Accordingly, the arginine requirements of this genotype need to be researched aiming at improving its meat production and quality, reducing carcass fatness. Therefore, the objective of this study was to investigate the effects of different dietary L-arginine levels on the expression of lipogenic genes in the liver and adipose tissue, growth performance, carcass traits, and blood metabolites of Arian broiler chickens.

MATERIALS AND METHODS

Birds and Housing

This study was conducted at the department of Animal Science, Sari Agricultural and Natural Resources University, Iran. The experimental protocol was approved by the Animal Care Committee of Sari Agricultural and Natural Resources University.

Birds were managed according to the Arian management guide (Corporation Support of Animal Affairs, 1062539). A total of 168 one-day-old Arian broiler chicks were assigned according to completely randomized design to four treatments with three replicates of 14 birds each. The experimental treatments consisted of diets containing 100, 124, 139, or 154% of the arginine requirements of Arian broilers (Corporation Support of Animal Affairs, 2008). Before diet formulation, the chemical composition (AOAC, 1984) and the amino acid contents (Andrews & Baldar, 1985) of all protein-containing ingredients were analyzed. The basal diets were formulated to supply the nutrient requirements of Arian broilers (Corporation Support of Animal Affairs, 2008), except for arginine, during the starter (d 1 to 10), grower (d 10 to 24), and finisher (d 24 to 42) periods. Arginine was added to the experimental diets as L-arginine (Aldrich, W381918) at the expense of sand (Table 1). Feed and water were provided *ad libitum*.

Live performance and carcass composition

Feed intake and body weight were measured at the end of starter (d 10), grower (d 24), and finisher (d 42) in order to calculate feed efficiency. On day 42, two birds per replicate (six birds per treatment) were selected, individually weighed after four hours of feed fasting, and euthanized. Eviscerated carcass, abdominal fat, breast, and thigh were weighed, and their weights relative to live body weight were calculated.

Blood metabolite analysis

Blood samples were collected by heart puncture in non heparinized tubes before the birds were euthanized for carcass evaluation and stored at 4°C for approximately 5h. Samples were then centrifuged (2,500*g for 15min), and the sera separated and storedat -20°C formetabolite analyses. Glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) serum levels were analyzed byenzymatic and colorimetric method using reagents supplied by Sigma Diagnostics (Zist Shimi Co, Iran) by spectrophotometric assays (Ebrahimi *et al*., 2014).

Tissue sample collection, RNA extraction and Real-time PCR

Liver and abdominal fat samples were collected from the birds euthanized for carcass evaluation and stored at -80°C for the evaluation of relative gene expression of the lipogenic enzymes fatty acid synthase (FAS), acetyl-coenzyme A carboxylase (ACC), and malic enzyme (MAE) in the liver, and lipoprotein lipase (LPL) in the abdominal fat. The β-Actin gene was considered as a housekeeping gene, and RNA extraction was performed according to Ebrahimi *et al*. (2014) using the same materials. All specific primers for real-time PCR reactions (Methabion, Germany) are shown in Table 2.

Table 1 – Ingredient and nutritional composition of experimental diets (on DM1 basis) supplied to Arian broilers during the starter (0-10 d), grower (11-21 d), and finisher (25-42 d) periods.

	100% arginine			124% arginine			139% arginine			154% arginine		
	0-10	11-24	25-42	0-10	11-24	25-42	0-10	11-24	25-42	0-10	11-24	25-42
Basal diet ingredients (%)												
Corn grain [2]	40.37	47.75	55.16	40.37	47.75	55.16	40.37	47.75	55.16	40.37	47.75	55.16
Soybean meal [2]	39.71	29.74	15.63	39.71	29.74	15.63	39.71	29.74	15.63	39.71	29.74	15.63
Canola meal [2]	4.80	12.39	20.00	4.80	12.39	20.00	4.80	12.39	20.00	4.80	12.39	20.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dicalcium phosphate	2.13	1.53	1.43	2.13	1.53	1.43	2.13	1.53	1.43	2.13	1.53	1.43
Limestone	1.16	0.90	0.96	1.16	0.90	0.96	1.16	0.90	0.96	1.16	0.90	0.96
Common salt	0.40	0.39	0.39	0.40	0.39	0.39	0.40	0.39	0.39	0.40	0.39	0.39
Vitamin premix [3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix [4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Met	0.26	0.15	0.13	0.26	0.15	0.13	0.26	0.15	0.13	0.26	0.15	0.13
L-Lys-HCl	0.07	0.00	0.17	0.07	0.00	0.17	0.07	0.00	0.17	0.07	0.00	0.17
L-threonine	5.00	1.05	0.03	5.00	1.05	0.03	5.00	1.05	0.03	5.00	1.05	0.03
Inert material (sand)	1.50	1.50	1.50	1.13	1.16	1.22	0.90	0.95	1.05	0.67	0.74	0.88
L-Arg addition	0	0	0	0.37	0.34	0.28	0.60	0.55	0.45	0.83	0.76	0.62
Total Arg (basal diet+added Arg)	1.53	1.41	1.15	1.90	1.75	1.43	2.13	1.96	1.60	2.36	2.17	1.77

Calculated nutritional composition (%)	Starter	Grower	Finisher
ME (Kcal/kg)	3050	3150	3200
Total protein	23.00	22.00	20.00
Calcium	1.00	0.88	0.85
Available phosphorus	0.50	0.45	0.40
Lysine	1.25	1.20	1.12
Methionine	0.57	0.50	0.45
Methionine+cysteine	0.90	0.83	0.81
Threonine	0.76	0.81	0.58
Isoleucine	1.06	1.01	0.72
Total arginine	1.53	1.41	1.15
Tryptophan	0.25	0.21	0.19
Leucine	1.69	1.73	1.32
Valine	1.08	0.92	0.80

[1] Dry matter.
[2] The chemical composition and amino acid content of these ingredients were analyzed, and the actual amounts were used for diet formulation basal diet.
[3] Each kg of the vitamin premix contained: 9,000,000 IU vit A, 2,000,000 IU vit D3, 18,000 IU vit E, 1,800 mg vit B1, 6,600 mg vit B2, 10,000 mg vit B3, 3,000 mg vit B6, 156 mg vit B12, 2,000 mg vit K3, 1,000 mg vit B9, 30,000 mg vit B5, 100 mg vit H2, 500,000 mg choline chloride and 1,000 mg Antioxidant.
[4] Each kg of the mineral premix contained: 100,000 mg/kg Manganese, 50,000 mg/kg Iron, 85,000 mg/kg Zinc, 10,000 mg/kg Copper, 1,000 mg/kg Iodine, 200 mg/kg Selenium.

Table 2 – Primer Sequences used for Real-Time PCR.

Gene[1]	Primer sequence (5′ - 3′)	Orientation	GenBank accession number	Product size (bp)
β-Actin	TGCGTGACATCAAGGAGAAG TGCCAGGGTACATTGTGGTA	Forward Reverse	L08165	300
LPL	CAGTGCAACTTCAACCATACCA AACCAGCCAGTCCACAACAA	Forward Reverse	NM_205282	150
ACC	CACTTCGAGGCGAAAAACTC GGAGCAAATCCATGACCACT	Forward Reverse	J03541	447
MAE	ATGAAGAGGGGCTACGAGGT CCCATTCCATAACAGCCAAG	Forward Reverse	AF408407	470
FAS	GGAGTCAAACTAGTTATCCATGGCC AAAGGAGATTCCAGCATCGTGCAGC	Forward Reverse	J04485	423

[1]Abbreviations: LPL, lipoprotein lipase; ACC, acetyl-coenzyme A carboxylase; MAE, malic enzyme; FAS, fatty acid synthase.

Statistical analyses

Data were analyzed by the General Linear Model procedures of SAS (SAS 9.2, SAS Inst. Cary, NC) and based on a completely randomized design. Initial body weight was considered as covariate in all analyses. Means were compared by Duncan's test and the effects were considered significant at $p<0.05$. Results are presented as least square means ± SEM.

RESULTS AND DISCUSSION

Expression of lipogenic genes and abdominal fat deposition

Beta-actin gene expression was not influenced by the treatments. Dietary arginine levels significantly decreased ($p<0.01$) ACC, FAS, and MAE gene expression in the liver. The lowest expression of the mentioned genes was observed in broilers fed 154% arginine (Table 3). Moreover, LPL gene expression in the abdominal fat significantly decreased ($p<0.01$) as arginine levels increased, with the lowest expression observed when the diet with 154% arginine was fed (Table 3). Furthermore, dietary arginine treatments significantly reduced ($p<0.01$) relative abdominal fat weight, with the lowest weight observed in the birds fed 154% arginine (Table 4).

The results of the present study are consistent with the findings of Ebrahimi *et al*. (2014), who reported a decrease in the gene expression of FAS and LPL in the abdominal fat, and of ACC, FAS, and MAE in the liver, accompanied by lower abdominal fat deposition, as well as increased gene expression of FAS and LPL in the muscle of broilers fed diets with high arginine levels. Ducks fed10 g L- arginine/kg diet presented low fat deposition and small adipocytes, as well as low activity of the hepatic lipogenic enzymes malic dehydrogenase, glucose-6-phosphate dehydrogenase, and FAS (Wu *et al*. 2011). Jiao *et al*. (2010) also reported a linear decrease in the abdominal fat yield of broilers fed increasing dietary levels of arginine (80, 100, 120, and 140% of the NRC recommendation).

Table 3 – Effects of different dietary arginine levels on the expression of fat-metabolic genes in the liver and abdominal fat tissue of Arian broilers.

Variable[1, 2, 3]	100% arginine	124% arginine	139% arginine	154% arginine	SEM	P-Value
FAS - liver	1.33[a]	1.03[b]	0.97[b]	0.44[c]	0.06	<0.01
ACC - liver	2.74[a]	2.24[b]	2.13[b]	0.77[c]	0.10	<0.01
MAE - liver	1.04[a]	0.76[ab]	0.57[ab]	0.29[b]	0.14	0.03
LPL - abdominal fat	1.39[a]	1.00[b]	0.55[c]	0.21[d]	0.05	<0.01

[1] Data are presented as least square means.

[2] mRNA levels were detected using real-time RT-PCR. The comparative Ct value method was also employed for quantifying the expression levels of the target genes relative to β-actin expression levels.

[3] Abbreviations: FAS, fatty acid synthase; ACC, acetyl-coenzyme A carboxylase; MAE, malic enzyme; LPL, lipoprotein lipase.

[a, b, c, d] Data with different superscripts within the same row significantly differ ($p<0.05$).

It was demonstrated that the liver is the primary site of fatty acid synthesis in birds, and that fat tissue development relies on the blood availability of triglycerides, which are transported as LDL (Hermier, 1997). The main enzymes involved in fatty acid synthesis in birds' liver are MAE and FAS (Hermier, 1997), while, ACC is responsible for the carboxylation of acetyl-CoA into malonyl-CoA (Tan *et al*., 2011). Accordingly, low gene expression of these enzymes in the liver reduces fat deposition in the carcass of poultry (Hermier, 1997; Saez *et al*., 2009; Ebrahimi *et al*., 2014). As

Table 4 – Effects of different dietary arginine levels on the growth performance, carcass traits, and blood metabolites of Arian broilers.

Variable[1]	100% arginine	124% arginine	139% arginine	154% arginine	SEM	P-Value
Body weight (g), (d 10)	131.17[b]	150.33[a]	156.00[a]	156.50[a]	5.94	0.03
Feed efficiency (d 1-10)	0.60	0.66	0.65	0.59	0.02	0.16
Body weight (g), (d 24)	626.67[ab]	640.88[a]	646.83[a]	595.00[b]	10.75	0.02
Feed efficiency (d 11-24)	0.55[b]	0.65[a]	0.65[a]	0.57[ab]	0.02	0.03
Body weight (g), (d 42)	1836.66[b]	1958.33[a]	1966.67[a]	1811.67[b]	34.03	0.01
Feed efficiency (d 25-42)	0.52[c]	0.63[ab]	0.64[a]	0.55[bc]	0.02	0.01
Carcass yield (%), (d 42)	57.62[b]	68.17[a]	67.53[a]	58.43[b]	2.40	0.01
Relative breast weight (%), (d 42)	20.61 [c]	23.29 [ab]	23.40 [a]	21.62[bc]	0.55	<0.01
Relative thigh weight (%), (d 42)	16.14[c]	18.23[a]	18.08[ab]	16.39[bc]	0.55	0.03
Relative abdominal fat tissue weight (%), (d 42)	1.78[a]	1.38[b]	1.20[b]	1.08[b]	0.09	<0.01
Metabolites						
Glucose (mg/dL)	195.33[b]	202.16[ab]	218.50[a]	223.33[a]	6.86	0.04
Cholesterol (mg/dL)	128.33[a]	118.01[ab]	112.50[b]	109.33[b]	4.01	0.03
Triglyceride (mg/dL)	109.67[a]	103.83[a]	82.33[b]	80.83[b]	5.91	<0.01
HDL [2] (mg/dL)	61.83 [b]	58.33 [b]	73.50 [a]	74.50 [a]	3.44	0.01
LDL [3] (mg/dL)	40.27 [a]	27.33 [b]	23.40 [b]	13.27 [c]	1.75	<0.01

[1] Data are presented as least square means

[2] High-density lipoprotein.

[3] Low-density lipoprotein.

[a, b, c] Data with different superscripts within the same row significantly differ (p<0.05).

LPL is the primary enzyme responsible for the uptake of fatty acids by the adipose tissue (Zechner, 1997), a decrease in LPL gene expression in the abdominal fat tissue causes reduced the entry of fatty acids into the adipocytes and adipose tissue growth (Hermier, 1997;Ebrahimi *et al*., 2014).

Nitric oxide (NO) is synthesized from L-arginine in almost all cells and tissues by different isoforms of NO synthase (Jobgen *et al*, 2006). Studies have demonstrated the role of NO in stimulating lipolysis and fatty acid oxidation in adipose tissue, and also that feeding L-arginine induced the same results by stimulating NO production (Jobgen *et al*, 2006; Jobgen, 2007). Moreover, NO affects lipid synthesis in the hepatocytes by decreasing ACC activity (Jobgen, 2007). Therefore, it seems that the reduced expression of lipogenic genes observed in the present study by feeding L-arginine may be mediated by the roles of NO on lipid metabolism.

Growth performance and carcass composition

Dietary arginine levels significantly increased (p<0.05) the bodyweight (BW) of 10-, 24-, and 42-d-old broilers, as well as the feed efficiency (FE) measured on days 24 and 42 (Table 4). The highest BW and FE were observed in the broilers fed 139% and124% arginine, while statistically similar BW and FE when the diets with 154% and100% arginine were fed, except for BW on day 10 (Table 4). Furthermore, arginine levels significantly influenced (p<0.05) carcass yield, and breast and thigh relative weights (Table 4). The highest carcass yield and thigh relative weight were obtained with 124% arginine, while the highest breast relative weight was observed in the 139% arginine treatment group (Table 4).

These results are in agreement with Ebrahimi *et al*. (2014), who fed Ross broilers with increasing digestible arginine levels up to 168% relative to their requirements, and observed improvements in growth performance (higher BW, average daily weight gain, and feed efficiency) and carcass composition (higher breast and thigh weights). Murakami *et al*. (2012) reported that dietary arginine supplementation increased the live weight and feed conversion ratio of broilers. Emadi *et al*. (2010) showed that high dietary arginine levels increased the body weight gain and feed intake of Cobb broiler chickens. In another study, the addition of0.04 and 0.06 % arginine to broiler diets increased carcass weight, carcass yield, and breast, thigh, and drumstick yields (Al-Daraji & Salih, 2012a). Khajali *et al*. (2013) demonstrated that addition of 15 g arginine/kg diet (120% of the NRC recommendation) promoted the best broiler performance. Jiao *et al*. (2010), evaluating increasing dietary arginine levels (80, 100, and 120% of the NRC recommendation), reported a linear increase in breast and leg meat yields. Munir *et al*. (2009) found that adding 2% arginine to the diet promoted higher broiler body weight. Fernandes *et al*. (2009), adding increasing

digestible arginine levels to a starter diet, observed a linear increase in breast and breast fillet weights, as well as in myofiber diameter. Kidd *et al*. (2001) fed broilers with120% of the NRC arginine and lysine recommendations and did not find any interaction between dietary Lys and Arg; however, when Arg, but not Lys, levels were increased to up 120% of the NRC recommendations, body weight gain increased. Moreover, Gorman *et al*. (1997) suggested that high dietary arginine to lysine ratio is required to optimize breast meat yield of broilers reared at thermoneutral temperatures. The different optimal level of arginine to stimulate growth observed in the present study may be attributed to the broiler strain evaluated (Arian), which may have specific requirements, as well as to the different feed formulation and experimental design applied compared with the mentioned studies.

Arginine may enhance broiler growth through several pathways. Arginine stimulates secretion of insulin and of growth hormone to promote growth (Jahanian, 2009; Fernandes *et al*., 2014). Birds are not able to endogenously synthesize arginine due to the absence of a functional urea cycle, and therefore, arginine is considered an essential amino acid that should be supplied in the feed. It was shown that insufficient dietary arginine directly affects protein synthesis in birds (Fernandes *et al*., 2009). Part of the effects of arginine may be exerted through the formation of polyamines (putrescine, spermidine, and spermine), which promote growth through their role in the synthesis of DNA, RNA, and proteins, and by improving the uptake of amino acids by the cells (Jahanian, 2009; Khajali & Widerman, 2010, Basoo *et al*., 2012). Arginine is also a substrate for the synthesis of nitric oxide (NO) by activating NO synthase in all cell types; NO has multiple roles, including muscle growth and fat reduction, enhancement of the immune system, and regulation of cardio-pulmonary blood flow (Jobgen *et al*., 2006; Khajali & Widerman, 2010). Therefore, the favorable effects of arginine on broiler growth performance and carcass traits may be mediated by any of the above-mentioned mechanisms. According to the results of the present study that showed no performance or carcass differences in broilers fed either 124 and 139% arginine, the supply of 124% arginine relative to the published requirement of Arian broilers, is recommended.

Blood metabolites

Dietary arginine levels influenced glucose and HDL serum levels (p<0.05), with the highest levels obtained with 139 and 154% arginine (Table 4). On the other hand, increasing arginine levels reduced cholesterol, triglyceride, and LDL serum levels (p<0.05). The lowest cholesterol and triglyceride levels were observed in birds fed 139 and 154% arginine, and the lowest LDL level was obtained with the 154% arginine treatment (Table 4).

Ebrahimi *et al*. (2014) did not find any effects of higher dietary arginine levels on glucose serum levels, but observed reduced urea, cholesterol, and triglyceride levels. Emadi *et al*. (2010, 2011) reported that increasing arginine levels in the diet of broiler chickens increased albumin, total protein, and glucose serum levels, and reduced cholesterol and triglyceride levels. On the other hand, Ma *et al*. (2010) did not find any influence of dietary arginine supplementation on the serum levels of cholesterol, free fatty acids, glucose, urea, LDL, triglycerides or insulin in finishing pigs. Tan *et al* (2009), adding 1.0% L-arginine ingrowing-finishing pigs' diet, reported lower triglyceride levels, but no effect on LDL, HDL, glucose, or cholesterol levels. Jobgen (2007) observed reduced glucose and triglyceride serum levels in diet-induced obese rats supplemented with 1.51% L-arginine HCl. The addition of 1.0% L-arginine in the diet of growing pigs increased glucose serum levels, tended to increase HDL levels, and reduced lipid, urea, VLDL, and triglyceride levels (He *et al*. 2009).

As the *de novo* synthesis of fatty acids mainly takes place in the liver of birds, the main transporter of triglycerides is VLDL (Hermier, 1997). The results of the present study showed a reduction in the expression of lipogenic genes in the liver, which resulted in low flow of lipids in the blood (low serum levels of cholesterol, triglyceride, and LDL). In addition, arginine can change lipid metabolism by synthesizing nitric oxide (Jobgen *et al*., 2006) and increasing thyroid hormone blood concentrations (Ebrahimi *et al*., 2014), consequently reducing lipid blood concentrations (Jobgen *et al*., 2006; Hall, 2015). On the other hand, the protein synthesis promoted by arginine through the mentioned mechanisms may explain the high HDL results of the present study. The observed high glucose levels with increasing dietary arginine level maybe attributed to the glycogenic properties of arginine (Foye *et al*., 2006).

The overall results of this study indicated that supplying 124 and 139%arginine in the diet of Arian broiler chickens reduced the expression of lipogenic genes in the liver and abdominal fat tissue, and improved the growth performance and the profile of blood metabolites. Since almost similar performance and carcass trait results were obtained both with the

124 and 139%arginine treatments, supplying Arian broiler diets with124% arginine is suggested.

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