Animal (2013), **7:8**, pp 1239–1245 © The Animal Consortium 2013
doi:10.1017/S1751731113000347

animal

Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens

A. M. Fouad[1,2], H. K. El-Senousey[1,2], X. J. Yang[1] and J. H. Yao[1†]

[1]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China;* [2]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

(Received 29 September 2012; Accepted 7 February 2013; First published online 11 March 2013)

This study investigated the effects of different levels of dietary L-arginine (L-Arg) supplementation on the abdominal fat pad, circulating lipids, hepatic fatty acid synthase (FAS) gene expression, gene expression related to fatty acid β-oxidation, and the performance of broiler chickens. We tested whether the dietary L-Arg levels affected the expression of genes related to lipid metabolism in order to reduce body fat deposition. A total of 192 broiler chickens (Cobb 500) aged 21 days with an average BW of 920 ± 15 g were randomly assigned to four groups (six broilers per replicate and eight replicates per treatment). The control group was fed a basal diet, whereas the treatment groups were fed basal diets supplemented with 0.25%, 0.50%, or 1.00% L-Arg for 3 weeks. The average daily feed intake, average daily gain and feed : gain ratio were not affected by the dietary L-Arg levels. However, chickens supplemented with L-Arg had lower abdominal fat content, plasma triglyceride (TG), total cholesterol (TC) concentrations, hepatic FAS mRNA expression and increased heart carnitine palmitoyl transferase1 (CPT1) and 3-hydroxyacyl-CoA dehydrogenase (3HADH) mRNA expression. These findings suggest that the addition of 0.25% L-Arg may reduce the plasma TC concentration by decreasing hepatic 3-hydroxyl-3-methylglutaryl-CoA reductase mRNA expression. This may lower the plasma TG and abdominal fat content by suppressing hepatic FAS mRNA expression and enhancing CPT1 and 3HADH (genes related to fatty acid β-oxidation) mRNA expression in the hearts of broiler chickens.

Keywords: L-arginine, chickens, fatty acid β-oxidation, fatty acid synthase

Implications

Modern commercial chickens have a high capacity for fatty acid biosynthesis and fat accumulation. Supplementation with L-arginine (L-Arg) is effective for reducing unfavorable fat levels in pigs, meat ducks and chickens. However, the dietary L-Arg levels that may reduce fat deposition in broilers were unknown. It was also not known whether supplementation with L-Arg would improve fatty acid oxidation as a possible mechanism for reducing fat deposition. This study answered both questions. The findings of this study help to understand the fat-reducing effects of dietary L-Arg supplementation.

Introduction

L-arginine (L-Arg) is a critical amino acid for chickens because, like other birds, they are unable to obtain Arg from endogenous sources because of the absence of most of the enzymes involved in the urea cycle. It has also been shown that chickens have the highest Arg requirement of all animals studied (Ball *et al*., 2007). L-Arg is a precursor required for the biosynthesis of many molecules, including proteins, nitric oxide, creatine, ornithine, glutamate, polyamines, proline, glutamine, agmatine and dimethylarginine, hence it has multiple physiological functions in poultry (Khajali and Wideman, 2010). Experimental studies have shown that L-Arg supplementation alleviated oxidative stress and improved the antioxidant capacity (Atakisi *et al*., 2009), attenuated pulmonary hypertension syndrome, reduced ascites mortality (Tan *et al*., 2006), and enhanced innate, cellular and humoral immune responses in broiler chickens (Tayade *et al*., 2006; Munir *et al*., 2009).

Modern commercial broilers have a high capacity for lipid biosynthesis (Cui *et al*., 2012) because selection strategies have focused on improved BW gain, breast yield and feed efficiency, whereas they have ignored fat deposition in the chicken abdomen (Havenstein *et al*., 2003). Choct *et al*. (2000) demonstrated that modern strains of broilers contain 15% to 20% fat and >85% of this fat is not physiologically required for body function. Therefore, the excessive accumulation of fat in the abdomens of modern broiler chickens is one of the major problems facing the broiler industry

† E-mail: yaojunhu2004@sohu.com

(Le Mignon *et al*., 2009) because it is considered to be wasted dietary energy and a waste product with low economic value, which also reduces the carcass yield and affects consumer acceptance (Emmerson, 1997).

Studies with mice (Clemmensen *et al*., 2012), obese rats (Jobgen *et al*., 2009), growing-finishing pigs (Tan *et al*., 2009), White Pekin ducks (Wu *et al*., 2011) and chickens (Corzo *et al*., 2003) suggest that dietary L-Arg supplementation is effective for reducing body fat deposition. L-Arg supplementation at a level of 1.00% reduced body fat deposition in meat-type ducks by suppressing the activities of malate dehydrogenase, glucose-6-phosphate dehydrogenase and fatty acid synthase (FAS; Wu *et al*., 2011). Similarly, 1.00% L-Arg reduced the mRNA levels of the acetyl-coenzyme A carboxylase-α gene in the abdominal adipose tissue of finishing pigs (Tan *et al*., 2011). Therefore, dietary L-Arg supplementation reduces fat accumulation by modulating *de novo* lipogenesis, although theoretically it might also decrease the expression of lipogenesis genes and/or increase the expression of lipolysis genes to reduce fat accumulation. However, the effects of L-Arg supplementation on lipolysis, blood lipid profiles and the expression of genes related to lipid metabolism have not been investigated, and only one study reported a reduction in the abdominal fat pad at 42 to 56 days after increasing the L-Arg concentration in broiler chicken diets (Corzo *et al*., 2003). Thus, we investigated the effects of supplementation with different levels of L-Arg in the diets of broiler chickens on fat deposition, plasma lipid profiles, FAS gene expression in the liver (a key indicator used to monitor lipogenesis), and the expression levels of the carnitine palmitoyl transferase1 (CPT1) and 3-hydroxyacyl-CoA dehydrogenase (3HADH) genes in the heart (the main indicators used to evaluate lipolysis levels), as well as the growth performance.

Material and methods

Birds, diets and management

A total of 256 1-day-old Cobb 500 male broiler chicks were obtained from a commercial hatchery (Hua Dacheng, Xianyan, China). The chicks were placed in cages, exposed to continuous light and fed a commercial starter diet (22% CP and 3000 kcal ME/kg; Hua Qing, Yanling, China). The temperature was maintained at 35°C during the first 3 days and then reduced weekly by 3°C until it reached 22°C. Before the experiment, all chickens were weighed individually after withdrawal of feed overnight (12 h) and some of the chickens with the lowest or highest weights were eliminated. A total of 192 21-day-old broiler chickens with similar average BWs (920 $\pm$ 15 g) were selected and assigned randomly to four dietary treatments, that is, eight replicate cages (length 100 cm $\times$ width 50 cm $\times$ height 45 cm) with six birds per replicate. Broiler chickens were fed *ad libitum* with the same basal diets (in mash form), which were supplemented with 0 (control), 0.25%, 0.50% or 1.00% Arg in the form of L-arginine monohydrochloride (99.3% L-Arg, Binhai Hanhong Bio-Chemical Co. Ltd, Shanghai, China) from 21 to 42 days of age. The control diet (Table 1) was prepared to meet or exceed the National Research Council (NRC, 1994) requirements for broiler chickens and the dietary treatments were analyzed (Table 2) in duplicate to determine the amino acid contents (Beijing Zhongxu Yangguang Animal Husbandry Technology Development Co. Ltd, China).

Table 1 *Ingredients and chemical composition of the basal diet*

Ingredients	%
Corn	57.55
Soybean meal (43% CP)	29.36
Soy oil	6.00
Fish meal (60% CP)	4.00
Dicalcium phosphate	1.20
Limestone	1.30
Salt	0.30
DL-Methionine	0.15
Vitamin–mineral premix[1]	0.14
Nutritional value	
Metabolizable energy (Mcal/kg)	3.17
CP (%)	19.92 (20.00)[2]
Arginine (%)	1.25
Methionine (%)	0.47
Lysine (%)	1.07
Methionine + cystine (%)	0.65
Calcium (%)	0.83
Total phosphorus (%)	0.67
Available phosphorus (%)	0.38

[1]Supplied per kilogram of diet: vitamin A, 12 000 IU; vitamin D3, 4000 IU; vitamin E, 30 IU; vitamin K3, 3 mg; thiamine, 2.2 mg; riboflavin, 10 mg; D-pantothenic acid, 7 mg; folic acid, 0.55 mg; pyridoxine, 4 mg; niacin, 37 mg; cobalamin, 0.02 mg; biotin, 0.20 mg; choline chloride, 400 mg; iron, 85 mg; copper, 8 mg; manganese, 75 mg; zinc, 69 mg; iodine, 0.4 mg; and selenium, 0.3 mg.
[2]Analyzed.

Performance parameters

BWs were recorded and the feed intake was observed daily and weighed on a replicate basis at the start and the end of the 3-week test period to determine the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR). Mortalities were recorded daily throughout the experimental period (3 weeks). All experimental procedures were reviewed and approved by the Animal Care Committee of the Northwest Agriculture & Forestry University (Shaanxi, China).

Sample collection

At 42 days of age, the live BWs were recorded after an overnight fast (12 h) and 16 chickens per treatment (two chickens per replicate) with a BW close to the replicate average BW were selected and slaughtered. First, eight chickens per treatment (one chicken per replicate) were slaughtered to collect blood samples for lipid analysis, liver and heart samples for RNA isolation, the abdominal fat pad to calculate the abdominal fat pad percentage, and breast muscles to determine the intramuscular fat content (IMF). Second, eight chickens per treatment were slaughtered to

Table 2 *Concentrations of amino acids determined in the experimental diets*

Item	L-Arginine (%)			
	Control	0.25	0.50	1.00
Essential amino acid				
Arginine	1.25	1.49	1.76	2.24
Histidine	0.56	0.56	0.55	0.57
Isoleucine	0.83	0.82	0.84	0.86
Leucine	1.65	1.64	1.65	1.66
Lysine	1.11	1.11	1.11	1.12
Methionine	0.49	0.49	0.47	0.48
Phenylalanine	0.93	0.93	0.93	0.93
Threonine	0.76	0.76	0.75	0.76
Valine	0.94	0.94	0.97	0.97
Nonessential amino acid				
Alanine	0.99	1.01	1.01	1.02
Aspartic acid	1.93	1.95	1.94	1.97
Glutamic acid	3.37	3.38	3.39	3.42
Glycine	0.85	0.85	0.86	0.86
Proline	1.12	1.11	1.12	1.12
Serine	0.92	0.95	0.92	0.93

collect only the abdominal fat pad and breast muscles. After bleeding, the abdominal fat pad was removed from the proventriculus surrounding the gizzard down to the cloaca and weighed. The right breast muscles were removed from the sternum, minced after removing any obvious fat, and stored at −20°C. The IMF of the breast muscle was determined in triplicate, according to the method described by Zhao *et al.* (2007), and expressed as the percentage total fat (dry weight). Blood samples were collected using a 5-ml anticoagulant syringe (Shanghai K & G International Co. Ltd, Shanghai, China) via the wing vein, centrifuged at 4°C and 5900 × **g** for 10 min to obtain the plasma, and stored in 1.5-ml Eppendorf tubes at −70°C until analysis. Liver and heart samples were collected rapidly in 1.5-ml RNase-free centrifuge tubes, frozen immediately in liquid nitrogen, and stored at −80°C until RNA isolation.

Assay of lipid parameters

The levels of plasma triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and nonesterified fatty acids (NEFA) were estimated spectrophotometrically using commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, China; Chen *et al.*, 2011). Each sample was analyzed in triplicate.

RNA isolation and real-time PCR

Total RNA was extracted from the liver and heart samples using RNAiso Plus (TaKaRa, Biotechnology Co. Ltd, Dalian, China), according to the manufacturer's protocol. The total RNA was dissolved in 0.1% DEPC-treated water and the integrity of the total RNA was estimated using agarose gel electrophoresis, whereas the concentration and purity of the RNA were evaluated spectrophotometrically at 260/280 nm. The RNA concentrations of all samples were 1.9 to 2.0, according to estimates made by measuring the A_{260}. The total RNA was reverse transcribed to cDNA using a PrimeScript RT Reagent Kit (Perfect Real Time; TaKaRa, Biotechnology Co. Ltd). Briefly, 2 μg of RNA was reacted with 4 μl of 5× PrimeScript, 1 μl of PrimeScript RT Enzyme Mix, 1 μl of oligo dT primer (50 μM), 1 μl of random 6-mers (100 μM) and RNase-free water (TaKaRa, Biotechnology Co. Ltd) to produce a final volume of 20 μl. Reverse transcription was performed at 37°C for 15 min, followed by heat inactivation for 5 s at 85°C. The chicken 3HADH primer used for real-time PCR was prepared as reported by Mujahid and Furuse (2008) and synthesized by TaKaRa Biotechnology Co. Ltd. The other primers were designed based on chicken mRNA gene sequences in GenBank and were also synthesized by TaKaRa Biotechnology Co. Ltd (Supplementary Table S1).

The gene expression levels in all samples were measured in triplicate using three individual preparations of reverse-transcribed cDNA by real-time PCR with the Bio-Rad MiniOpticon quantitive system (Bio-Rad, Hercules, CA, USA) and SYBR® Premix Ex Taq™ (Perfect Real Time; TaKaRa Biotechnology Co. Ltd), according to the manufacturer's instructions. Briefly, 2 μl of cDNA was mixed with 12.5 μl of SYBR® Premix Ex Taq®, 1 μl of each primer (10 μM) and sterile distilled water to produce a final volume of 25 μl. The thermal cycle was as follows: 30 s at 95°C for one cycle, 5 s at 95°C and 34 s at 60°C for 40 cycles. The mRNA expression of β-actin was used as an internal control (Chen *et al.*, 2011), which was not significantly different between the two experimental groups. The values were normalized against the mRNA expression of β-actin and calculated as the percentage of the control value.

Statistical analysis

The experimental data were analyzed by one-way ANOVA using the GLM procedure in SAS (version 8.02; SAS Institute Inc., Cary, NC, USA) where the cage was the experimental unit for growth performance and the individual bird served as the experimental unit for plasma lipids and gene expression. The linear and quadratic effects of L-Arg among treatments were analyzed using a contrast statement. Significant differences among treatments were tested using Duncan's multiple-range test at a significance level of $P < 0.05$.

Results and discussion

All chickens were healthy and no mortality occurred throughout the entire experimental period (data not shown). The slaughter weight and growth performance (ADG, ADFI, and FCR) were not significantly affected ($P > 0.05$) by L-Arg supplementation in the diet, as shown in Table 3. Similarly, Fernandes *et al.* (2009) reported that the average live BW of broiler chickens at 42 days was not affected by L-Arg supplementation. Tan *et al.* (2006) also found that supplementing broiler chicken diets with 1.00% L-Arg did not affect

Table 3 *Growth performance, abdominal fat content and fat deposition in the breast muscle of broiler chickens fed diets with or without L-arginine supplementation*

Item	L-Arginine (%)				s.e.m.[3]	*P*-value[4]	
	Control	0.25	0.50	1.00		Linear	Quadratic
Slaughter weight (kg)[1]	2.646	2.651	2.655	2.657	0.025	0.88	0.97
Weight gain (g/day) [2]	84.8	85.4	85.0	85.3	0.6	0.87	0.89
Feed intake (g/day) [2]	164.4	163.1	162.6	164.6	0.5	0.96	0.09
Feed : gain ratio[2]	1.94	1.91	1.92	1.93	0.01	0.84	0.49
Abdominal fat (%)[1]	1.98[a]	1.57[b]	1.71[ab]	1.46[b]	0.07	0.02	0.54
Intramuscular fat (%)[1]	5.70	6.00	5.83	5.86	0.18	0.87	0.73

[1]Values are means with $n = 16$ chickens per treatment (two chickens/replicate).
[2]Values are means with $n = 8$ cages per treatment (six chickens/cage).
[3]Pooled s.e.m.
[4]Linear and quadratic effects of L-arginine levels.
[a,b]Means within a row that do not share a common superscript are significantly different ($P < 0.05$).

the BW gain, whereas Lee *et al.* (2002) showed that increasing the L-Arg level from 100% to 300% NRC (1994) did not affect the BW gain in White Leghorn chickens. D'Amato and Humphrey (2010) reported that the addition of L-Arg at 2 g/kg did not affect the BW, feed intake or FCR compared with chickens fed a basal diet. A study using White Pekin ducks found that diet supplementation with 1.00% L-Arg did not affect the ADFI or feed efficiency (Wu *et al.*, 2011). By contrast, Emadi *et al.* (2011) reported that feeding chicks from 1 to 49 days with diets supplemented with 2.5% L-Arg above their requirements according to NRC (1994) recommendations improved the ADG, ADFI and FCR compared with those fed the control diet. These differences may have been due to the period and/or levels of supplementation.

The results showed that IMF of breast muscle was not significantly ($P > 0.05$) affected by L-Arg supplementation (Table 3). We also found that the abdominal fat content expressed as the percentage BW was significantly ($P < 0.02$; Table 3) lower with the 0.25% and 1.00% L-Arg treatments compared with the control, whereas they did not differ significantly from each other. IMF is one of the major factors that affects meat quality because of its effects on other traits such as the flavor, juiciness and tenderness of meat (Zhao *et al.*, 2007). The abdominal fat tissue grows faster compared with other fat tissues (Butterwith, 1989) and it reaches its maximum rate of growth at 42 days of age (Havenstein *et al.*, 2003*)*. It is the key indicator used to judge total body fat (Chen *et al.*, 2011) because it is linked directly to the total body fat content in broiler chickens (Sonaiya, 1985). Our results agreed with previous studies of broiler chickens, pigs and White Pekin ducks (Corzo *et al.*, 2003; Tan *et al.*, 2009; Wu *et al.*, 2011). Corzo *et al.* (2003) detected a significant reduction in the abdominal fat content after increasing the L-Arg concentration in the diet of broiler chickens between 42 and 56 days of age. Tan *et al.* (2009) also showed that dietary L-Arg supplementation led to a significant reduction in total body fat deposition in pigs. Wu *et al.* (2011) reported that supplementing the diet of meat-type ducks with 1.00% L-Arg significantly decreased the abdominal fat pad percentage by reducing the activities of malate dehydrogenase, glucose-6-phosphate dehydrogenase and FAS (lipogenic enzymes) in the liver.

However, Fernandes *et al.* (2009) reported that increasing the dietary L-Arg concentration from 1.49% to 1.79% during the starter phase had no effect on the abdominal fat content of broiler chickens on day 42. However, Fernandes *et al.* (2009) supplemented the diet with L-Arg from 1 to 21 days of age, whereas all chickens consumed the same diet without Arg supplementation from 22 to 42 days of age. Therefore, the difference between these two results may be attributed to the timing of the supplementation because the starter phase is associated with a rapid growth rate, but it is not linked to excessive fat deposition in broiler chickens (Tzeng and Becker, 1981).

Table 4 shows the TC, HDL-C, LDL-C, TG and NEFA concentrations in the plasma of broilers fed diets containing different levels of L-Arg. The plasma concentrations of TC with the L-Arg treatments were significantly ($P < 0.0002$) lower than those of the control, whereas they did not differ significantly from each other. The concentration of HDL-C was not affected by the dietary treatments, whereas the concentration of LDL-C was significantly ($P = 0.0219$) lower with 1.00% L-Arg at compared with the control group. Our results agree with Emadi *et al.* (2011), who demonstrated that supplementation of L-Arg in the basal diet significantly reduced the blood TC concentration in broiler chickens. Similar findings were also reported in rats by El-Kirsh *et al.* (2011), who found that an oral dose of L-Arg three times per week did not significantly affect the plasma HDL-C, whereas the plasma TC and LDL-C were lower. Our results and previous studies suggest that L-Arg supplementation could reduce the blood TC concentration. To the best of our knowledge, no information is available about the mechanism underlying the cholesterol-lowering effect of dietary L-Arg supplementation. In chickens, the TC content of muscle is associated with the blood cholesterol level and both are controlled by hepatic hydroxyl-3-methylglutaryl-CoA reductase (HMGR) gene

Table 4 *Plasma lipid concentrations of broiler chickens fed diets with or without L-arginine supplementation*

Item	L-Arginine (%)				s.e.m.[1]	*P*-value[2]	
	Control	0.25	0.50	1.00		Linear	Quadratic
TC (mmol/l)	3.63[a]	3.41[b]	3.35[b]	3.26[b]	0.04	0.0002	0.3470
HDL-C (mmol/l)	2.48	2.41	2.43	2.39	0.02	0.0973	0.6202
LDL-C (mmol/l)	1.0[a]	0.88[ab]	0.79[ab]	0.75[b]	0.04	0.0219	0.6047
TG (mmol/l)	0.72[a]	0.63[bc]	0.66[ab]	0.58[c]	0.01	0.0005	0.7177
NEFA (μmol/l)	657.8[b]	726.4[ab]	692.0[ab]	773.8[a]	14.7	0.0154	0.8137

TC = total cholesterol; HDLC = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglyceride; NEFA = nonesterified fatty acid.
[1]Pooled s.e.m., $n = 8$ (eight chickens/treatment).
[2]Linear and quadratic effects of L-arginine levels.
[a,b,c]Means within a row that do not share a common superscript are significantly different ($P < 0.05$).

Table 5 *mRNA expression[1] levels of genes related to lipid metabolism in the tissues of broiler chickens fed diets with or without L-arginine supplementation*

Item ($\times 10^{-2}$)	L-Arginine (%)				s.e.m.[2]	*P*-value[3]	
	Control	0.25	0.50	1.00		Linear	Quadratic
Liver							
HMGR	1.08[a]	0.61[b]	0.50[b]	0.42[b]	0.07	0.0001	0.0521
FAS	1.04[a]	0.70[b]	0.84[ab]	0.63[b]	0.05	0.0045	0.6460
Heart							
CPT1	1.00[c]	1.48[ab]	1.15[bc]	1.62[a]	0.08	0.0186	0.9698
3HADH	1.02[c]	2.19[ab]	1.55[bc]	2.69[a]	0.17	0.0005	0.9324

HMGR = 3-hydroxyl-3-methylglutaryl-CoA reductase; FAS = fatty acid synthase; CPT1 = carnitine palmitoyl transferase1; 3HADH = 3-hydroxyacyl-CoA dehydrogenase.
[1]Values are expressed as percentages of the control value.
[2]Pooled s.e.m., $n = 8$ (eight chickens/treatment).
[3]The linear and quadratic effects of L-arginine levels.
[a,b,c]Means within a row that do not share a common superscript are significantly different ($P < 0.05$).

expression (Cui *et al.*, 2010). Thus, we analyzed HMGR expression in the liver of chickens as a possible mechanism for explaining the plasma TC reduction. HMGR is a crucial enzyme during cholesterol biosynthesis (Cui *et al.*, 2010). In the present study, L-Arg inclusion in the diets of broilers significantly ($P < 0.0001$; Table 5) reduced HMGR mRNA expression, which agreed with Jobgen *et al.* (2006) who reported that L-Arg was involved in the regulation of cholesterol metabolism via nitric oxide. However, it is still unknown whether L-Arg inhibited the expression of HMGR directly or indirectly, therefore further studies are required to elucidate how L-Arg affects hepatic HMGR mRNA expression.

The level of TG was significantly lower ($P < 0.0005$; Table 4) in broiler chickens fed diets containing 0.25% or 1.00% L-Arg compared with those fed the control diet, whereas the NEFA level was significantly ($P = 0.0154$; Table 4) increased by 1.00% L-Arg supplementation compared with the control group. These findings suggest that L-Arg promotes the conversion of TG to glycerol and free fatty acids. Previous studies also showed that L-Arg dietary supplementation reduced the blood TG concentration in obese rats (Jobgen *et al.*, 2009), growing-finishing pigs (Tan *et al.*, 2009), laying quail (Atakisi *et al.*, 2009) and broiler chickens (Emadi *et al.*, 2011).

To acquire a comprehensive understanding of the molecular mechanisms underlying the fat-reducing effect of dietary L-Arg supplementation, we quantified the gene expression of FAS in the liver, and CPT1 and 3HADH in the heart of broiler chickens (Table 5). Our results showed that FAS gene expression in the liver was significantly ($P = 0.0045$) lower, whereas the expression levels of CPT1 and 3HADH in the heart were increased significantly ($P < 0.05$) with 0.25% or 1.00% L-Arg supplementation compared with the control. However, there were no significant differences between L-Arg supplementation at levels of 0.25% and 1.00%. FAS is an essential enzyme in the final step of the lipogenic pathway (Pitel *et al.*, 1998) and the liver is the most important vital organ for fatty acid synthesis in avian species, in which almost 85% of the fat accumulated by growing birds is produced in the liver (Molette *et al.*, 2012). Therefore, we estimated the FAS mRNA expression in the liver as a biomarker of fatty acid synthesis. Our results showed that dietary L-Arg supplementation reduced fatty acid synthesis by downregulating hepatic FAS gene expression. The downregulation of hepatic FAS gene expression will reduce the availability of fatty acids for esterification into TGs and storage in adipose tissue. Thus, our results partly explain why dietary L-Arg supplementation reduced the

abdominal fat content and lowered the plasma TG level. Santoso *et al*. (1995) noted that the blood TG level was correlated with fatty acid synthesis in chickens. Tan *et al*. (2011) and Wu *et al*. (2011) also reported that dietary L-Arg supplementation inhibited *de novo* fatty acid synthesis in the adipose tissue of pigs by downregulating the expression of the acetyl-coenzyme A carboxylase-α gene and by reducing the activities of malate dehydrogenase, glucose-6-phosphate dehydrogenase and FAS (lipogenic enzymes) in the livers of meat-type ducks.

Significant increases were also noted in the CPT1 and 3HADH mRNA expression levels of the control and 0.25% and 1.00% L-Arg treatments, which are summarized in Table 5. Our study is not the first experiment to investigate the effects of dietary L-Arg supplementation on fatty acid β-oxidation, but it is the first experiment designed specifically to investigate the fat-reducing effects of amino acids on the β-oxidation of fatty acids. A previous report suggested that dietary L-Arg supplementation improved fatty acid oxidation via nitric oxide due to the elevation of CPT1A (Jobgen *et al*., 2006). However, it is very difficult to compare our findings with other studies. CPT1 is an integral outer mitochondrial membrane enzyme that catalyzes the first step in the β-oxidation of fatty acids. In broiler chickens, L-CPT1 (liver-type CPT1) and M-CPT1 (muscle-type CPT1) are the main CPT1 isoforms involved with transporting fatty acids into the mitochondria and they control the flux of fatty acids entering the β-oxidation pathway (Skiba-Cassy *et al*., 2007), whereas 3HADH is involved with the oxidation phases of fatty acid β-oxidation (Eaton, 2002). Thus, the mRNA expression levels of CPT1 and 3HADH in the hearts of chickens were used as biomarkers of fatty acid β-oxidation because Mujahid and Furuse (2008) used the CPT1 and 3HADH mRNA levels in the hearts of chickens as reliable parameters for detecting changes in the mitochondrial fatty acid oxidation level. For the first time, our study suggested that dietary supplementation with 0.25% L-Arg increased the mRNA expression of CPT1 and 3HADH in the hearts of chickens, which could help to explain the lower fat accumulation in chickens at 42 days of age. This is because the balance of the fatty acid uptake, hepatic *de novo* fatty acid synthesis (anabolism), and fatty acid oxidation via β-oxidation (catabolism) determines the amount of fat that can be deposited in the body.

We detected linear effects of L-Arg levels but the addition of 0.50% L-Arg did not appear to significantly affect the abdominal fat content or the mRNA expression of genes involved with lipid metabolism compared with the control, unlike the other L-Arg treatments. This may have been due to the interaction between L-Arg and other nutrients when the diet was supplemented with 0.50% L-Arg. It is possible that the diets supplemented with 0.50% L-Arg did not modulate the mRNA expression of genes involved with lipid metabolism sufficiently to reduce the abdominal fat content in the same way as the other Arg treatments. Indeed, this confirmed that supplementation with 0.25% L-Arg was sufficient to modulate the lipid metabolism, whereas supplementation with 1.00% L-Arg led to a reduction in the abdominal fat content and modulated the lipid metabolism, but not significantly compared with 0.25% L-Arg supplementation, although the L-Arg concentration was four times higher in the 1.00% L-Arg treatment.

In conclusion, this study showed that 0.25% L-Arg supplementation in the diets of broiler chickens reduced the blood plasma TC and TG concentrations and abdominal fat deposition. The lower abdominal fat deposition may have been attributable to reduced hepatic FAS gene expression and enhanced fatty acid β-oxidation via increased expression of the CPT1 and 3HADH genes. These findings suggest that dietary L-Arg supplementation is effective for reducing unfavorable fat deposition (abdominal fat) and it had no negative effects on the favorable fat (IMF) content. We also proposed a novel biochemical basis to explain the beneficial effects of L-Arg in reducing fat accumulation in avian species. Further studies are required to determine whether dietary L-Arg supplementation in high-energy diets produces the same results.

Acknowledgments

This study was supported by the Science & Technological Project of Shaanxi Province, China (2010ZDGC-02, 2011KTCQ02-02, and 2011KTDZ02-01-01).

Supplementary materials

For supplementary material referred to in this article, please visit http://dx.doi.org/10.1017/S1751731113000347

References

Atakisi O, Atakisi E and Kart A 2009. Effects of dietary zinc and L-arginine supplementation on total antioxidants capacity, lipid peroxidation, nitric oxide, egg weight, and blood biochemical values in Japanese quails. Biological Trace Element Research 132, 136–143.

Ball RO, Urschel KL and Pencharz PB 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. Journal of Nutrition 137, 1626–1641.

Butterwith SC 1989. Contribution of lipoprotein lipase activity to the differential growth of three adipose tissue depots in young broiler chickens. British Poultry Science 30, 927–933.

Chen J, Wang M, Kong Y, Ma H and Zou S 2011. Comparison of the novel compounds creatine and pyruvate on lipid and protein metabolism in broiler chickens. Animal 5, 1082–1089.

Choct M, Naylor A, Hutton O and Nolan J 2000. Increasing efficiency of lean tissue composition in broiler chickens. A Report for the Rural Industries Research and Development Corporation. Publication No 98/123. Retrieved August 20, 2010, from https://rirdc.infoservices.com.au/downloads/98-123 .

Clemmensen C, Madsen AN, Smajilovic S, Holst B and Brauner-Osborne H 2012. L-Arginine improves multiple physiological parameters in mice exposed to diet-induced metabolic disturbances. Amino Acids 43, 1265–1275.

Corzo A, Moran ET Jr and and Hoehler D 2003. Arginine need of heavy broiler males: applying the ideal protein concept. Poultry Science 82, 402–407.

Cui HX, Zheng MQ, Liu RR, Zhao GP, Chen JL and Wen J 2012. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. Molecular Biology Reports 39, 3479–3484.

Cui HX, Yang SY, Wang HY, Zhao JP, Jiang RR, Zhao GP, Chen JL, Zheng MQ, Li XH and Wen J 2010. The effect of a mutation in the 3-UTR region of the HMGCR gene on cholesterol in Beijing-you chickens. Animal Biotechnology 21, 241–251.

D'Amato JL and Humphrey BD 2010. Dietary arginine levels alter markers of arginine utilization in peripheral blood mononuclear cells and thymocytes in young broiler chicks. Poultry Science 89, 938–947.

Eaton S 2002. Control of mitochondrial beta-oxidation flux. Progress in Lipid Research 41, 197–239.

El-Kirsh AA, Abd El-Wahab HM and Abd-Ellah Sayed HF 2011. The effect of L-arginine or L-citrulline supplementation on biochemical parameters and the vascular aortic wall in high-fat and high-cholesterol-fed rats. Cell Biochemistry & Function 29, 414–428.

Emadi M, Jahanshiri F, Kaveh K, Hair-Bejo M, Ideris A and Alimon AR 2011. Nutrition and immunity: the effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. Avian Pathology 40, 63–72.

Emmerson DA 1997. Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. Poultry Science 76, 1121–1125.

Fernandes JI, Murakami AE, Martins EN, Sakamoto MI and Garcia ER 2009. Effect of arginine on the development of the pectoralis muscle and the diameter and the protein:deoxyribonucleic acid rate of its skeletal myofibers in broilers. Poultry Science 88, 1399–1406.

Havenstein GB, Ferket PR and Qureshi MA 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82, 1509–1518.

Jobgen W, Meininger CJ, Jobgen SC, Li P, Lee MJ, Smith SB, Spencer TE, Fried SK and Wu G 2009. Dietary L-arginine supplementation reduces white fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. Journal of Nutrition 139, 230–237.

Jobgen WS, Fried SK, Fu WJ, Meininger CJ and Wu G 2006. Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. Journal of Nutritional Biochemistry 17, 571–588.

Khajali F and Wideman RF 2010. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. World's Poultry Science Journal 66, 751–766.

Le Mignon G, Pitel F, Gilbert H, Le Bihan-Duval E, Vignoles F, Demeure O, Lagarrigue S, Simon J, Cogburn LA, Aggrey SE, Douaire M and Le Roy P 2009. A comprehensive analysis of QTL for abdominal fat and breast muscle weights on chicken chromosome 5 using a multivariate approach. Animal Genetic 40, 157–164.

Lee JE, Austic RE, Naqi SA, Golemboski KA and Dietert RR 2002. Dietary arginine intake alters avian leukocyte population distribution during infectious bronchitis challenge. Poultry Science 81, 793–798.

Molette C, Theron L, Marty-Gasset N, Fernandez X and Remignon H 2012. Current advances in proteomic analysis of (fatty) liver. Journal of Proteomics 75, 4290–4295.

Mujahid A and Furuse M 2008. Central administration of corticotropin-releasing factor induces thermogenesis by changes in mitochondrial bioenergetics in neonatal chicks. Neuroscience 155, 845–851.

Munir K, Muneer MA, Masaoud E, Tiwari A, Mahmud A, Chaudhry RM and Rashid A 2009. Dietary arginine stimulates humoral and cell-mediated immunity in chickens vaccinated and challenged against hydropericardium syndrome virus. Poultry Science 88, 1629–1638.

National Research Council (NRC) 1994. Nutrient requirements of poultry, 9th edition. National Academy Press, Washington, DC, USA.

Pitel F, Fillon V, Heimel C, Le Fur N, el Khadir-Mounier C, Douaire M, Gellin J and Vignal A 1998. Mapping of FASN and ACACA on two chicken microchromosomes disrupts the human 17q syntenic group well conserved in mammals. Mammalian Genome 9, 297–300.

Santoso U, Tanaka K and Ohtani S 1995. Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. British Journal of Nutrition 74, 523–529.

Skiba-Cassy S, Collin A, Chartrin P, Medale F, Simon J, Duclos MJ and Tesseraud S 2007. Chicken liver and muscle carnitine palmitoyltransferase 1: nutritional regulation of messengers. Comparative Biochemistry and Physiology Part B 147, 278–287.

Sonaiya EB 1985. Abdominal fat weight and thickness as predictors of total body fat in broilers. British Poultry Science 26, 453–458.

Tan B, Yin Y, Liu Z, Li X, Xu H, Kong X, Huang R, Tang W, Shinzato I, Smith SB and Wu G 2009. Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. Amino Acids 37, 169–175.

Tan B, Yin Y, Liu Z, Tang W, Xu H, Kong X, Li X, Yao K, Gu W, Smith SB and Wu G 2011. Dietary L-arginine supplementation differentially regulates expression of lipid-metabolic genes in porcine adipose tissue and skeletal muscle. Journal of Nutritional Biochemistry 22, 441–445.

Tan X, Sun WD, Li JC, Pan JQ and Wang XL 2006. Changes in pulmonary arteriole protein kinase c-alpha expression associated with supplemental L-arginine in broilers during cool temperature exposure. British Poultry Science 47, 230–236.

Tayade C, Koti M and Mishra SC 2006. L-Arginine stimulates intestinal intraepithelial lymphocyte functions and immune response in chickens orally immunized with live intermediate plus strain of infectious bursal disease vaccine. Vaccine 24, 5473–5480.

Tzeng R and Becker WA 1981. Growth patterns of body and abdominal fat weights in male broiler chickens. Poultry Science 60, 1101–1106.

Wu LY, Fang YJ and Guo XY 2011. Dietary L-arginine supplementation beneficially regulates body fat deposition of meat-type ducks. British Poultry Science 52, 221–226.

Zhao GP, Chen JL, Zheng MQ, Wen J and Zhang Y 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poultry Science 86, 2309–2314.