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Effects of Dietary Alpha-lipoic Acid on Anti-oxidative Ability and Meat Quality in Arbor Acres Broilers*

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ABSTRACT : An experiment was conducted to evaluate the effects of dietary alpha-lipoic acid (LA) on growth performance, carcass characteristics and meat quality in Arbor Acres broilers. A total of 240 1-d-old male Arbor Acres broilers were randomly allocated to 4 dietary treatments (0, 300 ppm, 600 ppm, and 900 ppm dietary LA supplementation, respectively). Birds were slaughtered at 42 days old. Live body weight (BW), average daily gain (ADG), average feed intake (AFI), feed conversion ratio (FCR), dressing percentage, breast muscle percentage, thigh muscle percentage, abdominal fat percentage, muscle color (L*, a*, b*), pH values at 24 h postmortem, meat shear force value (SFV) and anti-oxidative ability were measured. Results showed that addition of 600 ppm or 900 ppm LA decreased BW ($p<0.01$), ADG ($p<0.01$) and AFI ($p<0.05$) compared with other diets. FCR was not affected by dietary LA content. LA had no marked effect on dressing percentage, breast muscle percentage or thigh muscle percentage. Abdominal fat percentage was lower ($p<0.05$) in the 900 ppm LA supplementation group than the control group. Dietary 900 ppm LA increased ($p<0.05$) breast and thigh muscle pH value at 24 h postmortem compared with the control treatment. Dietary LA increased thigh muscle a* value, though no significant difference was found in thigh muscle a* value among the treatments. Dietary LA significantly decreased breast muscle L* value ($p<0.05$), breast muscle b* value ($p<0.01$) and thigh muscle b* value ($p<0.05$). Broilers fed LA had higher breast muscle a* value ($p<0.05$) and thigh muscle L* value ($p<0.05$). All test groups had lower ($p<0.05$) breast muscle SFV than the control group. Dietary 600 ppm or 900 ppm LA both decreased ($p<0.01$) thigh muscle SFV compared with the control treatment. Dietary 900 ppm LA significantly increased ($p<0.05$) TAOC, SOD and GSHPx compared with no LA treatment. Broilers fed LA had lower ($p<0.01$) MDA compared with the control treatment. These results suggested that dietary LA enhanced the anti-oxidative ability and oxidative stability, and contributed to the improvement of meat quality in broilers. (**Key Words :** Alpha-lipoic Acid, Carcass Traits, Meat Quality, Anti-oxidative Ability, Broiler)

INTRODUCTION

Alpha-lipoic acid (LA) and its reduced form, dihydrolipoic acid (DHLA), have received widespread attention as antioxidants with both preventative and therapeutic uses in humans and laboratory animals. LA is an antioxidant and exogenously supplied LA can be NADH and NADPH-dependently reduced in mitochondria and cytosol (Haramaki et al., 1997). LA has been shown to act as an NADH oxidase inhibitor to block oxidant production and decrease phagocytosis of myelin by macrophages (Goes et al., 1998). The thiol compound lipoic acid and its reduced form dihydrolipoic acid are able to scavenge various reactive oxygen species. Moreover, the lipoic acid redox couple is capable of recycling other antioxidants such as vitamin E, vitamin C, and GSH, thus forming a socalled "antioxidant defense network". In addition, LA has been shown to be effective in upregulation of the intracellular GSH content of lymphocytes (Sen et al., 1997). LA is a coenzyme involved in mitochondrial metabolism. The reduced form of LA, dihydrolipoic acid, is a powerful mitochondrial antioxidant (Packer et al., 1997a; b; Moini et al., 2002). Dietary administration of LA elicits fatty acid mobilization in β-adrenergic response to isoproterenol when the basal level of plasma glucose is maintained (Hamano et al., 2000). LA is a mitochondrial nutrient and it is also one

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of the mostly studied mitochondrial nutrients on mitochondrial function in cellular and animal models related to brain aging and neurodegeneration (Liu, 2008).

LA is a strong antioxidant occurs widely in foods (Packer et al., 1995). Very recently, it was reported that LA has anti-obesity effects by suppressing the activity of hypothalamic AMPK, while at the same time activating AMPK in skeletal muscle (Kim et al., 2004). Shen and Du has reported that dietary LA supplementation is a potential way to reduce the incidence of pale, soft, exudative (PSE) meat (Shen and Du, 2005).

The studies above mostly focused on the effects of LA supplementation in the diets of human, mice or other animals. The trials illuminated that dietary LA did promote antio-xidation and health. But whether or not the similar responses to dietary LA could be observed in broilers has not been confirmed. Furthermore, whether dietary LA contributes to meat quality has not been reported, either.

In this study, we aimed to evaluate the effects of LA supplementation in diets on carcass traits, meat quality and anti-oxidative ability in Arbor Acres broilers, and to seek for the relationship between dietary LA and meat quality.

MATERIALS AND METHODS

Birds and diets

A total of 240 1-d-old male Arbor Acres broilers were selected to evaluate the effects of dietary LA on anti-oxidative ability and meat quality. The birds were randomly allocated to 4 dietary treatments with 6 replicates of 10 birds per replicate pen, which was equipped with raised-wire floor. Birds were vaccinated for Newcastle disease and infectious bronchitis disease at hatchery, on 7 and 21 d of age, respectively. A 24 h lighting regime was carried out during the first 3 days, and 23 h lighting with 1 h darkness from 4 d of age was used. Mean air temperature of animal chamber maintained at approximately 35°C during the first week, then decreased gradually to get a constant temperature of 25°C during the rest of the trial. All birds had access to feed and water *ad libitum*, the whole experiment period lasted 42 days.

In the present study, nutrient contents met or exceeded the NRC (1994) recommendations. The supplements of LA for the four groups were 0 (group A), 300 ppm (group B), 600 ppm (group C), 900 ppm (group D). The composition and nutrient content of basal diet formulated for broilers in different growing phases are shown in Table 1.

Table 1. Composition and nutrient content of basal diets in different growing phases

Diets	Age (d)	
	1 to 21	22 to 42
Ingredients (%)		
Corn	46.90	55.50
Corn gluten meal	5.70	2.40
Extruded soybean	20.00	16.00
Soybean meal	20.00	19.00
Limestone	1.20	1.30
Dicalcium phosphate	1.70	1.20
Salt	0.30	0.30
Corn oil	2.70	2.80
Premix[1]	1.00	1.00
Bentonite	0.50	0.50
Total	100	100
Nutrient composition		
ME (MJ/kg)	13.24	13.24
CP (%)	22.7	19.5
Ca (%)	0.98	0.88
Total phosphorus (%)	0.64	0.54
Available phosphorus (%)	0.44	0.35
Met (%)	0.50	0.38
Met+cystine (%)	0.90	0.73
Lys (%)	1.12	1.00

[1] The vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 15,000 IU; cholecalciferol, 3,000 IU; vitamin E, 20 IU; vitamin K_3, 2.18 mg; thiamine, 2.15 mg; riboflavin, 8.00 mg; pyridoxine, 4.40 mg; vitamin B_{12} 0.02 mg; calcium pantothenate, 25.60 mg; nicotinic acid, 65.80 mg; folic acid, 0.96 mg; biotin, 0.20 mg; Fe, 109.58 mg; Cu, 8.14 mg; Zn, 78.04 mg; Mn, 105.00 mg; I, 0.34 mg; Se, 0.14 mg; choline chloride, 1,500 mg.

Sample collection

On 42 d of age of the experiment, two birds from each pen with body weights close to the average were selected. Feed and water were withdrawn 12 h prior to slaughter. The birds were humanely slaughtered and carcasses were collected. Breast and thigh muscle from both sides of the carcass were skinned and deboned for the measurements of carcass traits, muscle color (L*, a*, b*), pH value and tenderness. Blood samples were collected from the wing vein. Then, serum were prepared and stored at -30°C until using for determination of anti-oxidative ability.

Measurements

Carcass characteristics : Live body weight, carcass weight, and weight of breast and thigh muscle from both sides of the carcass were weighed. Carcass weight was defined as the weight with feather-scalded, eviscerated carcass (with head, neck, blood, and hocks removed) (Dilger et al., 2006). Carcass was weighed prior to deboning, breast and thigh muscle were removed from each carcass within 30 min postmortem, trimmed, weighed and chilled on ice. The breast and thigh muscle from the right side of each carcass was used to determine pH, muscle color and shear force value.

Muscle pH : Muscle pH was measured at 24 h after slaughter by using a testo 205 pH meter (Testo Instrument Co. LTD., Germany). The pH meter was standardized by using a two-point method against standard buffers of pH 4.0 and pH 7.0. Three measurements were recorded and

Table 2. Effects of dietary alpha-lipoic acid on the growth performance of broilers

Parameter	Group[1]				Pooled SEM[2]
	A	B	C	D	
Start BW (g)[2]	42.9	42.6	42.6	42.7	0.121
Final BW (g)[2]	2198.3[A]	2150.5[A]	1992.3[B]	1989.7[B]	22.703
ADG (g)[2]	51.3[A]	50.2[A]	46.4[B]	46.0[B]	0.540
AFI (g)[2]	93.5[a]	93.2[a]	88.3[b]	87.9[b]	0.834
FCR (g:g)[2]	1.82	1.86	1.88	1.89	0.014

[1] Group A (control); group B (300 ppm LA); group C (600 ppm LA); group D (900 ppm LA).
[2] BW = Body weight; ADG = Average daily gain; AFI = Average feed intake; FCR = Feed conversion ratio; SEM = Standard error of mean.
[a, b] Means with different superscripts differ significantly ($p<0.05$).
[A, B] Means with different superscripts differ significantly ($p<0.01$).

averaged for each breast and thigh muscle.

Muscle color : Hunter L* (lightness), a* (redness), and b* (yellowness) values were generated from breast and thigh muscle at the time of deboning, using a hand-held color difference meter (SC-80C, Kangguang apparatus Co. LTD., Beijing, China), with an illuminant D65 and 10° standard observer. An average of three reading values from the medial surface of the muscle free from color defects, bruising and hemorrhages were taken for color evaluation (Fletcher, 1999).

Shear force value : Fillets (12.7 mm, diameter) were removed from the anterior end of each fillet with attached sampler. Thereafter, they were cooked in the 80°C water-bath for several minutes until to an internal temperature of 75°C. Fillets were then cooled, and as soon as the fillets reached the room temperature, they were prepared to measure shear force. Each sample was sheared perpendicular to grain of the muscle fiber using a 25-kg load cell and crosshead speed of 200 mm/min with a Digital Meat Tenderness Meter of Model C-LM3 (Northeast Agricultural University, Harbin, China). The shear force is expressed in Newton (N) and used as a criterion for tenderness of the chicken meat. For each cooked muscle, the core was sheared in 3 locations, and the average of the maximum forces was used for data analysis (Tang et al., 2007).

Anti-oxidative ability : The TAOC, SOD, MDA and GSHPx analyses were conducted using the assay kits purchased from Nanjing Jiancheng Bioengineering Insititute (Nanjing, China) according to manufacturer's instructions. The absorption values were determined with a Vis Spectrophotometer (Model 722N, Shanghai Precision & Scientific Instrument Co., Ltd. Shanghai, China).

Statistical analysis

Data was conducted using a completely randomized block design. The statistical analyses were performed using the GLM analysis of variance procedure of Statistical Analysis System (SAS 8.2). Significant effects were further explored using ANOVA to ascertain differences among treatment means. A significance level of $p<0.05$ was used during analysis and other significance levels were also specified whenever necessary.

RESULTS

Table 2 described the effects of dietary LA on growth performance. Addition of LA to broiler's feed had significant impact on live body weight, average daily gain and average feed intake. Broilers fed 600 ppm or 900 ppm LA had lower live body weight ($p<0.01$), average daily gain ($p<0.01$) and average feed intake ($p<0.05$) than those of other treatments. There was no significant difference on feed conversion ratio among treatments during the entire experiment period.

The effects of dietary LA on carcass traits are presented in Table 3. Dietary LA had no significant effects on dressing percentage, breast muscle percentage and thigh muscle percentage, though them increased with increasing LA except dressing percentage. Abdominal fat percentage was lower ($p<0.05$) from 900 ppm LA supplementation group than that from control group.

Table 4 shows the effects of dietary LA on pH value, muscle color (L*, a*, b*) and shear force value. Dietary

Table 3. Effects of dietary alpha-lipoic acid on carcass characteristics of broilers

Parameter	Group[1]				Pooled SEM[2]
	A	B	C	D	
Dressing percentage (%)	76.54	77.98	77.77	76.70	0.402
Breast muscle percentage (%)	23.45	24.26	24.33	24.41	0.337
Thigh muscle percentage (%)	19.66	20.59	20.75	20.93	0.268
Abdominal fat percentage (%)	1.55[a]	1.45[ab]	1.42[ab]	1.37[b]	0.024

[1] Group A (control); group B (300 ppm LA); group C (600 ppm LA); group D (900 ppm LA).
[2] SEM = Standard error of mean.
[a, b] Means with different superscripts differ significantly ($p<0.05$).

Table 4. Effects of dietary alpha-lipoic acid on the meat quality of broilers

Parameter	Group[1]				Pooled SEM[2]
	A	B	C	D	
Breast $pH_{24\,h}$	5.61^{b}	5.66^{ab}	5.70^{ab}	5.76^{a}	0.022
Thigh $pH_{24\,h}$	5.82^{b}	5.90^{ab}	5.93^{ab}	5.95^{a}	0.021
Breast L*	42.25^{a}	40.89^{ab}	40.59^{ab}	39.93^{b}	0.312
Breast a*	6.71^{b}	7.16^{ab}	7.27^{a}	7.04^{ab}	0.084
Breast b*	14.62^{A}	13.91^{AB}	13.58^{B}	13.48^{B}	0.135
Thigh L*	45.36^{b}	45.69^{ab}	46.08^{a}	47.59^{a}	0.314
Thigh a*	8.30	8.60	8.83	8.75	0.119
Thigh b*	18.45^{a}	17.74^{ab}	17.42^{b}	17.09^{b}	0.183
Breast shear force (N)	25.2^{a}	24.0^{b}	23.8^{b}	23.9^{b}	0.193
Thigh shear force (N)	22.1^{A}	21.1^{AB}	20.3^{B}	20.0^{B}	0.248

[1] Group A (control); group B (300 ppm LA); group C (600 ppm LA); group D (900 ppm LA).
[2] SEM = Standard error of mean.
[a, b] Means with different superscripts differ significantly ($p<0.05$). [A, B] Means with different superscripts differ significantly ($p<0.01$).

with 900 ppm LA had higher ($p<0.05$) breast and thigh muscle pH values at 24 h postmortem than those of no LA treatment.

Dietary LA had no significant effect on thigh muscle a* value among the treatments. However, significant difference was observed in breast muscle L*, a*, b* values and thigh muscle L*, b* values. Broilers fed 600 ppm LA had higher ($p<0.05$) breast muscle a* value, whereas broilers fed 900 ppm LA had lower ($p<0.05$) breast muscle L* value than that of control treatment. Dietary with 600 ppm or 900 ppm LA both decreased breast muscle b* value ($p<0.01$) and thigh muscle b* value ($p<0.05$), and increased ($p<0.05$) thigh muscle L* value than those of no LA group.

Not only breast muscle but also thigh muscle of broilers fed LA had significantly effects on shear force value among treatments. Dietary LA decreased ($p<0.05$) breast muscle shear force value than that of control group, dietary with 600 ppm or 900 ppm LA both decreased ($p<0.01$) thigh muscle shear force value than that of control treatment.

The effects of dietary LA on the anti-oxidative ability of broilers were presented in Table 5. Significant difference was found in TAOC, SOD, MDA and GSHPx among the treatments, respectively. Dietary with 900 ppm LA increased TAOC ($p<0.05$) and SOD ($p<0.05$) than those of no LA treatment, and dietary with 900 ppm LA increased ($p<0.05$) GSHPx than that of no LA and 300 ppm LA treatments. Broilers fed with LA had lower ($p<0.01$) MDA than that of control treatment.

DISCUSSION

Dietary LA decreased live body weight, average daily gain and feed intake. The result is in agreement with the results of Shen et al. (2005), who stated that dietary LA decreased ($p<0.05$) weight gain in mice during the second and third weeks compared with no LA treatment. Dietary LA significantly decreased ($p<0.05$) ADG and ADFI of pigs, and these results might be caused by inhibition of feed intake of disulfide bone in LA molecule (Maddock et al., 2001). Dietary LA supplementation decreased feed consumption in mice as a response to the inhibition of AMPK activity in the hypothalamus (Kim et al., 2004). Several reports showed that inhibition of hypothalamic AMPK activity has an anorexigenic effect (Andersson et al., 2004; Minokoshi et al., 2004). The decrease in feed consumption following LA supplementation can not be attributed to taste aversion to diets because an intraperitoneal injection of LA caused a similar decrease in feed consumption (Shen et al., 2005).

The effect of dietary LA on carcass characteristics of broilers in this study was consistent with Schmidt et al. (2005), who found that LA supplementation did not affect ($p>0.10$) carcass weight, dressing percentage. Whereas dietary LA decreased abdominal fat percentage of broilers

Table 5. Effects of dietary alpha-lipoic acid on serum anti-oxidative ability of broilers

Parameter	Group[1]				Pooled SEM[2]
	A	B	C	D	
TAOC (U/ml)[2]	18.73^{b}	21.35^{ab}	21.63^{ab}	22.90^{a}	0.540
SOD (U/ml)[2]	126.56^{b}	130.46^{ab}	136.90^{ab}	140.65^{a}	2.251
MDA (nmol/ml)[2]	5.51^{A}	4.76^{B}	4.63^{B}	4.39^{B}	0.115
GSHPx (μmol/L)[2]	421.85^{b}	426.13^{b}	442.21^{ab}	448.65^{a}	4.093

[1] Group A (control); group B (300 ppm LA); group C (600 ppm LA); group D (900 ppm LA).
[2] TAOC = Total antioxidant capability; SOD = Superoxide dismutase; MDA = Maleic dialdehyde; GSHPx = Glutathione peroxidase; SEM = Standard error of mean.
[a, b] Means with different superscripts differ significantly ($p<0.05$). [A, B] Means with different superscripts differ significantly ($p<0.01$).

than that of control treatment in the present experiment. The result was supported by Reed (1973), who thought that lipoamide dehydrogenase was the flavoprotein component of the α-keto acid, and which was involved in Krebs cycle promoting energy metabolism.

It is well known that muscle pH is associated with numerous meat quality attributes, such as meat color, tenderness, water-holding capacity and other characteristics of muscle. LA supplementation resulted in the higher pH at 45 min of pig ($p = 0.029$) (Berg et al., 2003). Dietary LA increased pH value in postmortem mice muscle (Shen and Du, 2005). Our study supports the former report. In the present experiment, breast muscle and thigh muscle pH values at 24 h postmortem increased with incremental dietary LA content. Muscle pH decreased during the progression of rigor mortis was due to ATP hydrolyzation and accumulation of lactic acid (Calkins et al., 1982). Glycogen content and its depletion rate *post mortem* determined pH decline of muscle (Lister et al., 1970), and glycogen in muscle could be manipulated by dietary composition (Rosenvold et al., 2003).

The color of meat is one of the most important quality attributes of meat product for consumer acceptance. Higher values of L*, a* and b* indicated paler, redder and yellower meat, respectively. Yellowness (b*) value is mainly affected by the forms of presented myoglobin (Lindahl et al., 2001). Lightness (L*) value was identified to negatively correlated with water-holding capacity (Woelfel et al., 2002). Boulianne and King (1995, 1998) reported that pale fillet had significantly greater lightness value, less redness and greater yellowness, whereas dark fillets had lower lightness and yellowness. In the present study, dietary LA increased breast muscle a * value and thigh muscle *L value, whereas decreased breast muscle L* and b* values, thigh muscle b* value. For breast muscle, reduction of lightness (L*) and yellowness (b*) values and increase of redness (a*) value contribute to the acceptability of meat. In the report of Young et al. (2003), higher pH value of pectoralis major from stressed broilers was associated with lower L* value which was in agreement with the measurements of breast muscle in the present experiment. But, the change trend of thigh muscle is opposite to the breast muscle. Summary of the possible reasons for discordant results: In the present experiment, broilers were not in stressed status. Moreover, the difference of myofiber types or fatty acid composition between these two muscles may cause variation in the results. Further detailed research into the reason is required. Although we can not adequately account for the enhancement of thigh muscle L* value, the results accords with Rentfrow et al. (2004), who reported supplemental LA had higher ($p<0.05$) L* value of beef.

Shear force value was an important index relating to meat tenderness. Besides sex and muscle size, the changes of histochemistry during rigor mortis directly affected tenderness (Goll et al., 1964; Calkins and Seideman, 1988; Wheeler and Koohmaraie, 1994). The decrease of breast and thigh shear force is consistent with Schmidt et al. (2005), who reported dietary LA decreased ($p<0.01$) Warner-Bratzler shear force values of steaks. Berg et al. (2003) also stated similar effect on shear force value in swine finishing diets.

Feeding poultry a higher level of dietary antioxidants provides the poultry industry with a simple and practical method for improving oxidative stability (Jiang et al., 2008; Lin et al., 2008; Tsai et al., 2008), shelf life of poultry meat (Ryu et al., 2005) and meat quality of broilers (Jiang et al., 2007). Rybak et al. (1999) reported LA supplementation increased SOD, CAT and GSHPx activity. Kowluru et al. (2005) also found that lipoic acid regulated the mitochondrial SOD activity (Mn-SOD) in the retina of diabetic rats. The accumulated studies supported the present study, in which dietary LA increased TAOC, SOD, GSHPx activity and decreased MDA content in serum in different extent as expected. So this might be the reason that dietary LA improved meat quality via their anti-oxidative function.

In conclusion, the results presented in this study indicated that dietary LA had significant effects on growth performance, carcass traits, meat quality and anti-oxidative ability. These parameters showed that dietary LA ameliorated meat quality of broilers by decreasing shear force value, and enhancing the meat tenderness. Dietary LA supplementation is a potential way to reduce the incidence of PSE meat by increasing pH value 24 postmortem. The results also indicated that dietary LA enhanced the anti-oxidative ability and oxidative stability. Therefore, LA partially ameliorated meat quality in broilers.

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