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Effects of Dietary Acetyl-L-Carnitine on Meat Quality and Lipid Metabolism in Arbor Acres Broilers

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ABSTRACT : An experiment was conducted to evaluate the effects of dietary acetyl-L-carnitine (ALC) on growth performance, carcass characteristics, meat quality and lipid metabolism in broilers. A total of 240 one-day-old male Arbor Acres broilers were randomly allocated to 4 dietary treatments (0, 300, 600, and 900 mg/kg dietary ALC supplementation, respectively). Compared with the control treatment, addition of ALC resulted in lower (linear effect, $p<0.05$) ADG and AFI. Abdominal fat percentage decreased (linear effect, $p<0.05$) as dietary ALC was increased, but there was no effect on dressing percentage, breast muscle percentage or thigh muscle percentage. Breast muscle pH value 24 h post-mortem increased (linear effect, $p<0.05$), but there were no significant differences among treatments. However, thigh muscle pH value increased (linear effect, $p<0.05$) as dietary ALC was increased. Breast and thigh muscle a* values increased (linear effect, $p<0.05$), and breast and thigh muscle b* values decreased (linear effect, $p<0.05$) with increased ALC in the diet. In addition, breast and thigh muscle shear force value decreased (linear effect, $p<0.05$) as dietary ALC was increased. Total cholesterol, triglyceride, low-density lipoprotein cholesterol and lipoprotein lipase decreased (linear effect, $p<0.05$) and free fatty acid and lipase in serum increased (linear effect, $p<0.05$) with increased ALC in diets. (**Key Words :** Acetyl-L-carnitine, Carcass Characteristics Meat Quality, Lipid Metabolism, Broiler)

INTRODUCTION

Acetyl-L-Carnitine (ALC) is an acetylated form of L-carnitine which is derived from lysine and methionine. The major metabolic role of L-carnitine appears to be the transport of long-chain fatty acids into the mitochondria for β-oxidation (Coulter, 1995). L-Carnitine supplementation increased weight gain, reduced carcass fat and improved feed conversion in weaning pigs (Weeden et al., 1990) and broiler chickens (Lettner et al., 1992; Rabie et al., 1997a; Rabie et al., 1997b). In addition, Rabie et al. (1997a) reported that supplementing 50 mg L-carnitine/ kg diet to broiler chickens increased breast muscle and leg meat yields and content of fat in breast muscle ($p<0.05$).

ALC is the acetyl derivative of L-carnitine, which is required for the transport of long-chain fatty acids into mitochondria for β-oxidation, ATP production and for the removal of excess short- and medium-chain fatty acids. ALC treatment was also found to restore mitochondrial function and ambulatory activity (Hagen et al., 1998), to reduce serum cholesterol levels (Ruggiero et al., 1990), and to normalize changes of heart mitochondrial lipid composition in old rats (Paradies et al., 1992). In addition, ALC is an acetyl-group donor in oxidative glycolysis and has antioxidant activity (Tesco et al., 1992; Colucci et al., 1988).

Previous studies mostly focused on the effects of L-carnitine in the diets of broilers, pigs, humans and rats, but the response to dietary ALC in broilers has not been confirmed. Whether dietary ALC has the same effects on lipid metabolism of broilers as L-carnitine, and whether dietary ALC contributes to meat quality is not known.

This study aimed to determine the effects of dietary ALC on fat metabolism in broilers, and to establish the relationship between dietary ALC and meat quality.

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MATERIALS AND METHODS

Birds and diets

A total of 240 one-d-old male Arbor Acres broilers were randomly allocated to 4 dietary treatments with 6 replicates of 10 birds per replicate pen, which was equipped with a raised-wire floor. There were no significant differences in initial BW across treatments Birds were vaccinated against Newcastle disease and infectious bronchitis disease on 7 and 21 d of age, respectively. A 24 h lighting regime was maintained during the first 3 days, and 23 h lighting with 1 h darkness was used from 4 d of age. All birds had access to feed and water *ad libitum* and the experiment lasted 42 days.

All nutrient contents met or exceeded the recommendations of NRC (1994). ALC was added at 0, 300, 600, 900 mg/kg. The ingredient composition and nutrient content of the basal diet formulated for broilers in the starter and finisher phases are shown in Table 1.

Sample collection

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

Table 1. Composition and nutrient content of basal diets in the starter and finisher 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
Vitamin-trace mineral premix[1]	1.00	1.00
Bentonite	0.50	0.50
	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.

Measurements

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

Muscle pH : Muscle pH was measured 24 h after slaughter using a pH meter (Testo Instrument Co. Ltd., Germany). The pH meter was standardized against standard buffers of pH 4.0 and pH 7.0. Three measurements were recorded and 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 were taken for color evaluation from the medial surface of the muscle free from color defects, bruising and hemorrhages (Fletcher, 1999).

Shear force value : Muscle samples were packed and sealed in a boilable pack. Thereafter, they were cooked in an 80°C water-bath for several minutes until reaching an internal temperature of 75°C. Samples were cooled to room temperature, and then a fillet (12.7 mm, diameter) was removed from each sample parallel to the myofibre orientation. Each fillet was sheared perpendicular to the 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 was 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).

Lipid analysis : Triglyceride, total cholesterol, free fatty acid, lipoprotein lipase, low-density lipoprotein cholesterol and lipase analyses were conducted using assay kits

Table 2. Effects of dietary acetyl-L-carnitine on the growth performance of broilers

Parameter	ALC (mg/kg)				Pooled SEM[1]	P	
	0	300	600	900		L	Q
ADG (g)[1]	51.3[a]	49.9[a]	48.7[a]	46.5[b]	0.513	0.0001	0.244
AFI (g)[1]	93.5[a]	88.5[b]	87.9[b]	86.6[b]	0.826	0.002	0.176
FCR (g:g)[1]	1.82	1.80	1.81	1.84	0.011	0.396	0.357

[1] 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 ($p<0.05$).

purchased from Nanjing Jiancheng Bioengineering Institute (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 were analyzed by the GLM ANOVA procedures of Statistical Analysis System (SAS 8.2). Significant effects were further explored using Duncan's multiple range test to ascertain differences among treatment means. The significance level was designated as 0.05, whereas $p<0.10$ was considered a tendency.

RESULTS AND DISCUSSION

Table 2 shows the effects of dietary ALC on growth performance. ADG and AFI decreased (linear effect, $p<0.05$) with increased ALC in the diets. The diet with 900 mg/kg ALC decreased ADG compared to other treatments. Broilers fed diets with ALC had lower AFI than the control group. No significant difference among treatments was found in FCR. Dietary ALC decreased average daily gain and feed intake. These results are in agreement with those of Tanaka et al. (2004), who observed that three-month administration of ALC to aged rats (initially 19 months old) decreased mean body weight from 439±19 g to 417±16 g.

The effects of dietary ALC on carcass traits are presented in Table 3. The abdominal fat percentage decreased (linear effect, $p<0.05$) with increased dietary ALC; diets with either 600 mg/kg or 900 mg/kg ALC decreased abdominal fat percentage compared with the control group. No differences in dressing percentage and breast or thigh muscle percentages were observed in the current study. Studies on the effects of dietary ALC on carcass characteristics of broilers have been scarce. Lien and Horng (2001) reported that supplementary carnitine did not significantly influence carcass characteristics of broilers. In the current study, dietary ALC at 600 mg/kg or 900 mg/kg significantly decreased abdominal fat percentage of broilers which is in agreement with the findings of Xu et al. (2003), who found a decrease ($p<0.05$) in abdominal fat percentage with the addition of L-carnitine in the diet. Zhou and Liu (1996) considered that inhibition of β-oxidization due to decreased concentration of L-carnitine *in vivo* could result in raised level of serum FFA. Likewise, Wang et al. (2000) concluded that L-carnitine lead to decreased abdominal fat through increased oxidation of FFA. In this study, we found that ALC increased serum FFA, and thus minimized the deposition of fat in subcutaneous fat.

Table 4 shows the effects of dietary ALC on pH value, muscle color (L*, a*, b*) and shear force value. Breast muscle pH value 24 h post-mortem increased (linear effect, $p<0.05$), but there were no significant differences among treatments. However, thigh muscle pH value increased (linear effect, $p<0.05$) as dietary ALC was increased and the diet with 900 mg/kg ALC induced a higher thigh muscle pH value 24 h post-mortem compared to the control. In addition, thigh muscle L* values decreased (linear effect, $p<0.10$), although no significant differences were found among the treatments. Breast and thigh muscle a* increased (linear effect, $p<0.05$) and breast and thigh muscle b* decreased (linear effect, $p<0.05$) as dietary ALC was increased. Broilers fed a diet with 900 mg/kg ALC had higher breast muscle a* value, whereas those fed diets with either 600 mg/kg or 900 mg/kg ALC had lower breast muscle b* values than the control treatment. Diets with either 600 mg/kg or 900 mg/kg ALC increased thigh muscle

Table 3. Effects of dietary acetyl-L-carnitine on carcass characteristics of broilers

Parameter	ALC (mg/kg)				Pooled SEM[1]	P	
	0	300	600	900		L	Q
Dressing percentage (% BW)	76.54	76.94	77.60	77.74	0.424	0.296	0.881
Breast muscle percentage (% BW)	23.45	24.24	24.61	24.91	0.314	0.104	0.695
Thigh muscle percentage (% BW)	19.66	19.97	20.97	21.27	0.289	0.027	0.997
Abdominal fat percentage (% BW)	1.55[a]	1.44[ab]	1.37[b]	1.33[b]	0.026	0.001	0.408

[1] SEM = Standard error of mean.
[a,b] Means with different superscripts differ significantly ($p<0.05$).

Table 4. Effects of dietary acetyl-L-carnitine on the meat quality of broilers

Parameter	ALC (mg/kg)				Pooled SEM[1]	P	
	0	300	600	900		L	Q
Breast $pH_{24\ h}$	5.61	5.65	5.69	5.71	0.017	0.027	0.849
Thigh $pH_{24\ h}$	5.82[b]	5.91[ab]	5.93[ab]	6.01[a]	0.022	0.002	0.917
Breast L*	42.25	41.81	41.30	40.97	0.271	0.141	0.480
Breast a*	6.71[b]	6.96[ab]	7.05[ab]	7.28[a]	0.083	0.016	0.931
Breast b*	14.62[a]	14.10[ab]	13.76[b]	13.60[b]	0.132	0.003	0.418
Thigh L*	45.36	44.55	43.99	43.43	0.356	0.055	0.859
Thigh a*	8.30[b]	8.69[ab]	8.90[a]	9.08[a]	0.106	0.007	0.602
Thigh b*	18.45[a]	17.66[ab]	17.20[ab]	16.98[b]	0.196	0.004	0.402
Breast shear force (N)	25.2[a]	24.7[ab]	24.1[b]	23.8[b]	0.175	0.003	0.741
Thigh shear force (N)	22.1[a]	21.8[a]	21.3[ab]	20.5[b]	0.218	0.004	0.514

[1] SEM = Standard error of mean.
[a,b] Means with different superscripts differ significantly ($p<0.05$).

a* value, whereas the diet with 900 mg/kg ALC decreased thigh muscle b* value compared to the control.

Shear force values of breast and thigh muscles of broilers fed diets containing ALC were significantly affected (linear effect, $p<0.05$) among treatments. Diets with either 600 mg/kg or 900 mg/kg ALC decreased shear force value of breast muscle compared to the control group. Furthermore, the diet containing 900 mg/kg ALC decreased thigh muscle shear force value compared to control and 300 mg/kg treatments.

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. Le Bihan-Duval et al. (2001) reported a strong negative correlation between ultimate pH and drip loss. In the present experiment, breast and thigh muscle pH values 24 h postmortem increased with incremental dietary ALC levels. Muscle pH decrease during the progression of rigor mortis is due to ATP hydrolyzation and accumulation of lactic acid (Calkins et al., 1982). Glycogen content and its depletion rate post-mortem determines pH decline of muscle (Lister et al., 1970), and glycogen in muscle can be manipulated by dietary composition (Rosenvold et al., 2003). The results indicated that dietary ALC was effective in maintaining a relatively higher pH value of meat.

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* indicate paler, redder and yellower meat, respectively. Yellowness (b*) value is mainly affected by the forms of myoglobin present (Lindahl et al., 2001). Lightness (L*) value was negatively correlated with water-holding capacity (Woelfel et al., 2002). Boulianne and King (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 ALC increased both breast and thigh muscle a* values, whereas it decreased breast and thigh muscle b* values. For breast muscle, the reduction of lightness (L*) and yellowness (b*) values and the increase of redness (a*) value contribute to the acceptability of meat. Therefore, the result demonstrated that dietary ALC improved meat color to some extent.

Shear force value is an important index relating to meat tenderness. Besides sex and muscle size, the changes in histochemistry during rigor mortis directly affect tenderness (Calkins and Seideman, 1988; Wheeler and Koohmaraie, 1994). In the present experiment, both breast and thigh muscle of birds fed with ALC had a lower shear force value. Rabie and Szilagyi (1998) reported that L-carnitine increased intramuscular fat of breast muscle of broilers, which supports the lower shear force value observed in this experiment.

The effects of dietary ALC on lipid metabolism of broilers are presented in Table 5. Significant differences (linear effect, $p<0.05$) were found in triglyceride, total cholesterol, free fatty acid, lipoprotein lipase, low-density lipoprotein cholesterol and lipase among the treatments. Broilers fed a diet with 300 mg/kg ALC had lower levels of low-density lipoprotein cholesterol, lipoprotein lipase and a higher level of lipase than the control. The diet with 600 mg/kg ALC decreased total cholesterol, low-density lipoprotein cholesterol, triglyceride and lipoprotein lipase and increased free fatty acid and lipase compared with the control group. Broilers fed the diet with 900 mg/kg ALC had lower levels of total cholesterol, triglyeride, low-density lipoprotein cholesterol and lipoprotein lipase and higher levels of free fatty acid and lipase than the control treatment. Moreover, broilers fed the diet with 900 mg/kg ALC also had lower total cholesterol and higher free fatty acid than the 300 mg/kg treatment.

Carnitine supplementation has long been known to ameliorate lipid metabolism in patients with type IV hyperlipoproteinemia (Maebashi et al., 1978). With ALC treatment, levels of triacylglycerol and total cholesterol in

Table 5. Effects of dietary acetyl-L-carnitine on lipid metabolism in serum of broilers

Parameter	ALC (mg/kg)				Pooled SEM[1]	P	
	0	300	600	900		L	Q
Total cholesterol (mmol/L)	4.87[a]	4.32[ab]	4.02[bc]	3.67[c]	0.132	0.001	0.611
Triglyceride (mmol/L)	0.69[a]	0.61[ab]	0.56[b]	0.51[b]	0.020	0.001	0.653
Free fatty acid (μ mol/L)	122.50[c]	141.39[bc]	152.21[ab]	164.34[a]	4.578	0.001	0.635
Low density lipoprotein-cholesterol (mmol/L)	1.58[a]	1.29[b]	1.18[b]	1.04[b]	0.059	0.001	0.408
Lipoprotein lipase (U/ml)	1.36[a]	1.24[b]	1.20[b]	1.15[b]	0.025	0.002	0.352
Lipase (U/L)	26.94[b]	32.77[a]	34.01[a]	35.86[a]	1.144	0.004	0.320

SEM = Standard error of mean.
[a,b ,c] Means with different superscripts differ significantly ($p<0.05$).

aged rats were reduced to those of young rats (Tanaka et al., 2004). It was also shown that administration of L-carnitine to rats decreased plasma triacylglycerol, cholesterol, phospholipids, nonesterified fatty acid and very low density lipoprotein concentrations (Maccari et al., 1987). It is possible that supplemental carnitine may increase the rate of fatty acid transportation in broilers, and hence reduce serum nonesterified fatty acid and triacylglycerol contents (Lien and Horng, 2001). L-carnitine is the only factor that can transfer FFA into mitochondria for their oxidization, so it plays a key role in the metabolism of fat. L-carnitine in the diet of chickens obviously decreased the triaclyglyerol content of sera and liver and abdominal fat percentage, which is related to L-carnitine promotion of β-oxidization of FFA (Wang et al., 2003). Feeding ALC increased activity of lipase ($p<0.01$) and decreased ($p<0.05$) activity of lipoprotein lipase, thereby leading to a higher concentration of fatty acid in serum by accelerating hydrolysis of TG to glycerol and fatty acid, while reducing the concentration of TG in sera. Lipoprotein lipase, which catalyzes the conversion of TG to glycerol and fatty acids, showed a decrease in activity which signified an increased hydrolysis of VLDL. VLDL play a major role in regulating fat deposition, leading to minimization of subcutaneous fat deposition (Griffin and Whitehead, 1982). Our data suggest that ALC supplementation is beneficial to lipid metabolism.

In conclusion, the results presented in this study show that dietary ALC obviously contributes to muscle pH and meat color and tenderness of broilers, and thus ameliorated meat quality. ALC supplementation in diets improved lipid metabolism in broilers.

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