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Effects of *Ligustrum lucidum* Fruits on Growth Performance, Antioxidation and Meat Quality in Arbor Acres Broilers

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ABSTRACT : This study was conducted to evaluate the effects of *Ligustrum lucidum* (LL) on growth performance, antioxidation, and meat quality in broilers. 270 birds (1 d old) were allotted to 3 treatments with 6 replicates per treatment. The feeding program included a starter diet from d 1 to 21 of age and a grower diet from d 22 to 42 of age. The birds were given a basal corn-soybean meal diet supplemented with 0 (the control), 5 or 10 g of LL/kg, respectively. The results showed that in the starter, grower and overall phase, broilers fed with 5 or 10 g of LL/kg had better ($p<0.01$) average daily gain, but there were no differences in feed: gain between treatments. In the starter and overall phase, average daily feed intake of LL groups was greater ($p<0.05$) than that of the control, but this difference was not observed in the grower phase. Supplementation of LL significantly increased ($p<0.05$) superoxide dismutase activity and total antioxidant capability in serum of chickens. In breast muscle, birds fed 5 g of LL/kg had an increase ($p<0.05$) in superoxide dismutase activity. The LL supplementation significantly decreased ($p<0.05$) malondialdehyde contents. Adding 5 or 10 g of LL/kg to the diet significantly increased pH value and reduced drip loss of meat ($p<0.05$). The results of this study indicated that dietary LL could improve growth performance, increase pH value and reduce drip loss of meat by decreasing lipid peroxidation and by improving antioxidative status in broilers. (**Key Words :** Broilers, *Ligustrum lucidum*, Performance, Antioxidation, Meat Quality)

INTRODUCTION

Oxidative rancidity represents one of the major causes of deterioration in food for human consumption. Besides producing unpleasant odors, it is responsible for losses in flavor, texture, consistency, appearance and nutritional value of the meat (Gray et al., 1996; Valenzuela and Nieto, 1996). In a similar way, in living animals, oxidative stress constitutes an important mechanism that leads to biological damage, and is regarded as one of the causes of several pathologies that affect poultry growth (Avanzo et al., 2001). Great concern is being given for improving meat quality with antioxidant additives in broiler chickens. Synthetic antioxidants have an established admissible daily ingest, but their innocuousness has been questioned (Iverson, 1995), due to the possibility that at high doses some of these agents may exert carcinogenic or mutagenic effects. Natural antioxidants are currently receiving considerable attention in human and animal nutrition because of their association with food quality characteristics (Marsh et al., 1981; Ashgar et al., 1989). For example, green tea (Yang et al., 2003), containing high concentrations of antioxidants has been demonstrated to reduce lipid peroxidation in chicken muscle. Besides that, Chinese natural medicinal products as feed supplements have been used as growth and health promoter in farm animals in China for centuries. *Ligustrum lucidum* (LL) is considered to be the one having immunoactive and antioxidative properties. It has been proved that LL and its effective component oleanolic acid could improve the lipid stability by removing free radicals and inhibiting the formation of lipid peroxidation. A study from our laboratory (Ma et al., 2007) showed that supplementation with LL significantly decreased malondialdehyde concentration of serum and heart in chicks.

LL is the fruit of the plant of LL and is rich in oleanolic acid (OLA) (7-15 mg of LL/g), D-mannanoligosaccharides (D-MOS) (6-9 mg of LL/g), and specnuezhenide (06-1.2 mg of LL/g). In traditional Chinese medicines, LL is considered to serve functions as nourishing liver and kidney, and brightening eyes. Study from modern medicine shows that LL or its main effective constituents are excellent biological antioxidants which protect cells from damage of lipid peroxidation stimulated by oxidative stress (Jin et al., 1995; Luo and Luo, 1999; Yim et al., 2001).

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Table1. Ingredients and nutrient composition of the basal diet

Items	Starter phase	Grower phase
Ingredients (%)		
Corn	53.70	59.11
Soybean meal	33.80	28.42
Fish meal	4.00	4.00
Soybean oil	4.22	4.68
Salt	0.35	0.35
Dicalcium phosphate	1.29	1.07
Limestone	1.28	1.18
L-lysine·HCl	0.08	0.04
DL-methionine	0.28	0.15
Premix[1]	1.00	1.00
Nutrients by calculation		
ME (Mcal/kg)	3.00	3.10
CP (%)	22.0	20.00
Lysine (%)	1.31	1.15
Methionine (%)	0.65	0.50
Calcium (%)	1.00	0.90
Available phosphorus (%)	0.45	0.40

[1] The premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,750 IU; vitamin E, 30 IU; vitamin K_3, 2 mg; vitamin B_{12} 12 μg; riboflavin, 6 mg; pantothenic acid, 12 mg; nicotinic acid, 40 mg; choline chloride 800 mg; biotin, 0.2 mg; folic acid, 0.5 mg; pyridoxine, 3.0 mg. iron (as ferrous sulfate $7H_2O$), 95 mg; zinc (as zinc sulfate $7H_2O$), 60 mg; manganese (as manganese sulfate H_2O), 80 mg; iodine (as potassium iodate), 0.35 mg; copper (as cupric sulfate $5H_2O$), 10 mg; selenium (as sodium selenite), 0.3 mg.

The present study was conducted to evaluate the effects of LL on growth performance, meat quality and antioxidative activity in broilers.

MATERIALS AND METHODS

Birds and management

Two hundred and seventy 1-d-old Arbor Acres male broilers were randomly allocated to 3 treatments, each with 6 replicates consisting of 15 chicks. All birds were placed in wire cages (length 100 cm×width 50 cm×height 45 cm) in a temperature-controlled house. The temperature was maintained at 34±1°C on 1 d of age up to 7 d of age and then gradually decreased to 26±1°C by 21 d of age, after which the chicks were maintained at room temperature. Lighting was continuous and water and feed were available *ad libitum*. Body weights and feed intake were recorded weekly. Mortality was recorded daily. Any bird that died was weighted and the weight was used to adjust the feed: gain ratio. Feed: gain radios were calculated by dividing total fed intake by weight gain of live plus dead birds.

Experimental design and diets

The birds were given a basal corn-soybean meal diet supplemented with 0 (the control), 5 or 10 g of LL/kg, respectively. Starter and grower diets were offered to the birds from 0 to 3 wk of age and from 4 to 6wk of age, respectively. All essential nutrient contained in the basal diet (Table 1) met or slightly exceeded nutrient requirements recommended by NRC (1994). All diets were fed in mash form.

LL used in this study was originated from Shiyitang pharmaceutical factory of Harbin pharmaceutical group. The main active constitutes of LL: OLA (10-12 mg of LL/g), D-MOS (5-9 mg of LL/g), and specnuezhenide (0.8-1.0 mg of LL/g). LL was grounded to power before being added to diets.

Sampling

At 21 d and 42 d of age, birds were deprived of feed for 12 h and then weighed to get average daily gain (ADG), average daily feed intake (ADFI) and feed : gain data. At 42 d of age, twelve broilers per treatment group (2 birds per replicate) were randomly selected and killed for sampling. Blood was collected (5 ml) by cardiac puncture using a 10-ml anticoagulant-free Vacutainer tube, centrifuged at 3,000 ×g for 10 min to obtain the serum, and stored at -20°C until analysis. Some muscles from the breast were removed and immediately stored at -80°C for measuring the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capability (T-AOC) and the contents of malondialdehyde (MDA). Further muscle samples were stored individually in plastic bags at 4°C for analysis of meat quality.

Assay of antioxidant indices in serum and muscle

For biochemical assays, forty milligrams of frozen muscle tissues in 4 ml of homogenization buffer (0.05 M Tris-HCL, pH 7.4, 1 mM EDTA, 0.25 M sucrose) was homogenized on ice with an Ultra-Turrax homogenizer (T8, IKA-Labortechnik, Staufen, Germany) for 5s at 13,500 rpm. The homogenate was centrifuged at 3,000 rpm for 10 min. The assays were conducted using the assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China) and followed the instructions of the kits. The T-AOC was measured by the method of ferric reducing -antioxidant power assay (Benzie and Strain, 1996) and detected at 520 nm with the spectrophotometer. Activity of SOD was measured by the xanthine oxidase method, which monitored the inhibition of reduction of nitro blue tetrazolium by the sample (Winterbourn et al., 1975). Activity of GSH-Px was detected with 5, 5'-dithiobis-p-nitrobenzoic acid, and the change of absorbance at 412 nm was monitored using a spectrophotometer (Hafeman et al., 1974). The MDA level was analyzed with 2-thiobarbituriz acid, monitoring the change of absorbance at 532 nm with the spectrophotometer (Placer et al., 1996). Enzyme activity was expressed as units per milligram of protein for tissues

Table 2. Effects of diary *Ligustrum lucidum* (LL) supplement on growth performance of broilers[1]

Item[2]	Treatments		
	Control	5 g of LL/kg	10 g of LL/kg
0-3 wk			
ADG (g)	29.8±1.28[b]	33.1±1.47[a]	32.9±1.01[a]
ADFI (g)	43.9±2.24[b]	47.4±2.27[a]	47.5±2.85[a]
Feed:gain	1.48±0.08	1.44±0.11	1.44±0.08
4-6 wk			
ADG (g)	62.1±2.31[b]	67.7±1.31[a]	67.2±2.47[a]
ADFI (g)	117.9±9.08	126.3±6.52	125.0±4.71
Feed:gain	1.90±0.12	1.86±0.07	1.86±0.02
0-6wk			
ADG (g)	46.0±0.94[b]	50.4±2.40[a]	50.1±1.67[a]
ADFI (g)	80.9±4.53[b]	86.9±4.04[a]	86.3±2.41[a]
Feed:gain	1.76±0.09	1.72±0.07	1.72±0.07

[a,b] Means within a ROW with no common superscript differ significantly ($p<0.05$).
[1] Data represents the mean value for each treatment (Means±SD, n = 6).
[2] ADG = Average daily gain, ADFI = Average daily feed intake.

and units per milliliter for serum.

Meat quality measurements

Muscle pH : The breast muscle pH value was measured at 45 min after slaughter with a portable pH meter (HI8424, Beijing Hanna Instruments Science & Technology Co. Ltd., Beijing, China) equipped with an insertion electrode calibrated in buffers at pH 4 and 7 at ambient temperature.

Drip loss measurements : Drip loss was determined as described by Remmignon et al. (1996). The breast muscle was excised, weighed and then placed in plastic bags and freely suspended using steel wire hook at 4°C. Muscle contact with the inside surface of the bag was kept to a minimum. Muscle samples were wiped and weighted 24 h later to evaluate the drip loss, which was expressed as a percentage of the initial muscle weight.

Shear force measurements : Shear force was measured using a universal Warner-Brazler testing machine (G. R. Electric manufacturing Co., Manhattan, KS). Muscle samples were stored in a water bath at 4°C for 24 h and were then individually cooked in a water bath at 80°C in plastic bags to an internal temperature of 70°C. The samples then were removed and chilled to room temperature. Strips (1.0 cm (width)×0.5 cm (thickness)×2.5 cm (length)) parallel to the muscle fiber were prepared from the medial portion of the meat and sheared vertically (Molette et al., 2003). Shear force was expressed in kilograms.

Statistical analysis

Data were analyzed by one-factorial ANOVA. Duncan's multiple test was used to determine whether means were significantly different ($p<0.05$). Values were expressed as means±SD. All the statistical analyses were performed using SPSS statistical software (Ver.11.5 for windows, SPSS). Replicate was considered as the experimental unit for performance determined. The experimental unit was a bird for the other parameters. Number (n) used for statistics is noted in the tables.

RESULTS

Growth performance

The effects of treatments on growth performance of broilers are shown in Table 2.

During the starter phase, dietary supplementation of 5 or 10 g of LL/kg improved ADG ($p<0.01$), ADFI ($p<0.05$) compared with birds fed the control diet. The ADG of birds fed 5 and 10 g of LL/kg increased by 11.1%, 10.4%, and ADFI was enhanced by 8.0%, 8.2%, respectively, compared with the control. There were no differences in feed: gain radios among treatments.

During the grower phase, the ADG of birds fed 5 and 10 g of LL/kg were 9.0 and 8.2% greater than that of the control ($p<0.01$). Dietary supplementation of LL did not affect ADFI and feed: gain radio.

Table3. Effects of dietary *Ligustrum lucidum* (LL) supplement on antioxidant induces of serum and breast muscle in broilers[1]

Item[2]	Treatments		
	Control	5 g of LL/kg	10 g of LL/kg
Serum			
SOD (U/ml)	126±10.3[b]	153±11.3[a]	162±18.4[a]
T-AOC (U/ml)	12±1.1[b]	20±1.4[a]	19±1.1[a]
GSH-Px (U/ml)	173±10.2	175±11.8	177±12.0
MDA (nmol/ml)	5.78±0.962	5.13±0.685	5.04±0.827
Breast			
SOD (U/mg of protein)	124±9.9[b]	150±10.6[a]	160±16.2[a]
T-AOC (U/mg of protein)	0.91±0.082	0.94±0.067	0.98±0.041
GSH-Px (U/m of protein)	1.66±0.423	1.71±0.122	1.75±0.275
MDA (nmol/mg of protein)	3.58±0.363[b]	3.17±0.234[a]	3.11±0.096[a]

[a,b] Means within a ROW with no common superscript differ significantly ($p<0.05$).
[1] Data represents the mean value for each treatment (Mean±SD, n = 12).
[2] SOD = Superoxide dismutase, T-AOC = Total antioxidant capability, GSH-Px = Glutathione peroxidase, MDA = Malondialdehyde.

Table 4. Effects of diary *Ligustrum lucidum* (LL) supplement on meat quality of broilers[1]

Item	Treatments		
	Control	5 g of LL/kg	10 g of LL/kg
pH value	5.79±0.26[b]	6.05±0.20[a]	6.01±0.10[a]
Drip loss (%)	3.28±1.31[b]	2.45±0.89[a]	2.49±0.92[a]
Shear force (kfg)	3.61±0.50	3 .63±0.52	3.87±0.35

[a,b] Means within a ROW with no common superscript differ significantly ($p<0.05$).

[1] Data represents the mean value for each treatment (Means±SD, n = 12).

For the overall period, the ADG of birds fed 5 and 10 g of LL/kg was 9.6 and 9.0% greater ($p<0.01$) than the control. The ADFI in the 5 and 10 g of LL/kg treatments were 7.4 and 6.7% greater ($p<0.05$) than that of the control. Dietary supplementation of LL did not also affect feed:gain radio.

Lipid peroxidation and antioxidant enzyme activities

Table 3 shows the result of antioxidant indices in serum and breast muscle of broilers. In serum, LL birds had greater ($p<0.05$) SOD and T-AOC than the control birds. However, no differences were observed in the MDA content. In breast muscle, treatment of birds with 5 and 10 g of LL/kg caused an increase of SOD activity by 20.97% ($p<0.05$) and 29.03% ($p<0.05$). The MDA content was significantly decreased in breast muscle of LL supplemented chickens ($p<0.05$).

Meat quality

The results of meat quality of breast muscles are presented in Table 4. Adding 5 or 10 g of LL/kg to the diet significantly increased pH value and reduced drip loss of meat ($p<0.05$). Adding 5 or 10 g of LL/kg to the diet significantly increased the pH value of meat by 4.50 and 3.80%. Meanwhile, the drip loss meat was significantly improved by 25.30 and 24.09% with the 5 and 10 g of LL/kg addition, respectively. The difference in shear force of breast muscle was not significant among treatments ($p>0.05$).

DISCUSSION

The results of the growth performance trial indicated: compared with the control diets, feeding 5 or 10 g of LL/kg didn't result in an improvement in feed: gain, which agreed with our previous finding. But in contrast to his findings, the current study observed that there was beneficial effect of feeding LL on ADG and the LL birds had an increased ADFI in starter and overall phase. Figure 1 and 2 represent the relationship between ADFI and ADG in starter period ($R^2 = 0.98$), in grower period ($R^2 = 0.982$). This illustrated the fact that the increased ADG caused by the addition of LL had a high liner correlation with the increased ADFI. Previous studies showed that dietary supplementation of LL couldn't increase the nutrient digestibility. Therefore, the improved ADG was not due to improved nutrient digestibility but due to the increased feed intake.

Reactive oxygen species (ROS) is a family of oxygen derivatives including superoxide, hydroxyl radical, hydrogen peroxide, and nitric oxide. In normal conditions,

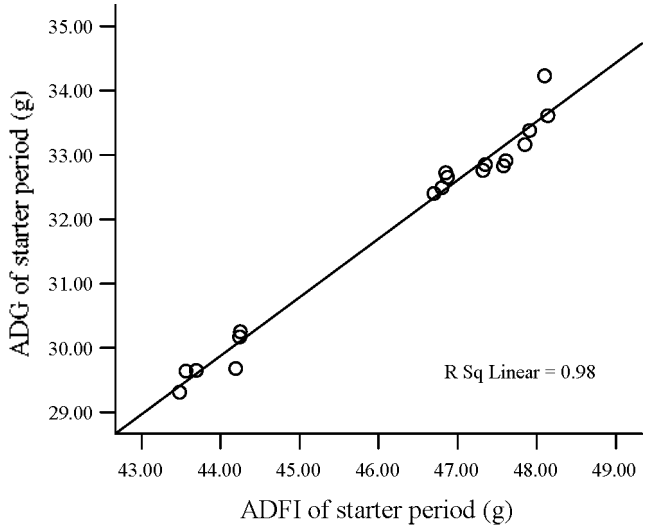

Figure 1. The relationship between ADFI and ADG in starter period. ADG = Average daily gain, ADFI = Average daily feed intake.

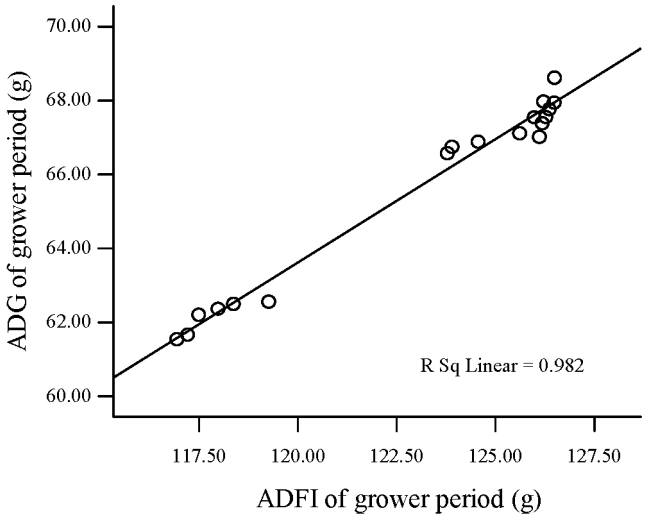

Figure 2. The relationship between ADFI and ADG in grower period. ADG = Average daily gain, ADFI = Average daily feed intake.

excessive oxidative radicals are eliminated by antioxidant systems including nonenzymatic components and series of antioxidant enzymes. Several enzymatic factors such as SOD and GSH-Px can scavenge formed ROS to function as antioxidants. In the current study, LL increased SOD levels in serum and muscle but GSH-Px levels were not altered. Supplementation with LL, therefore, may enhance the ROS scavenging by elevating the SOD level rather than the GSH-Px level. A similar result was found in a study of our laboratory, in which LL was demonstrated to increase serums SOD level in broilers. Furthermore, a previous experiment using 2 g/kg oleanolic acid (OLA) extracted from LL produced improvement in the level of SOD and T-AOC. We also assayed T-AOC levels in serum and muscle to evaluate total antioxidative capacity: when the diet was supplemented with LL, T-AOC levels in serum were elevated. The results indicated that the LL enhancement on body total antioxidant capacity may include nonenzymatic and enzymatic antioxidant systems.

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and meat products. Oxidative damage to lipids occurs in the living animal because of an imbalance between the production of ROS and the animal's defense mechanism. MDA is a soluble degraded product of lipids and the extent of lipid peroxidation can be monitored by MDA levels (Uuganbayar et al., 2005; Wang et al., 2006). Our previous study showed the contents of MDA in serum and tissues were significantly decreased by 10 g of LL/kg treatment in broilers. In the present study, MDA production of the breast muscle was decreased by adding LL in diet in broilers. This finding suggested that LL supplementation in broilers may protect tissues against attack by lipid oxidation products.

Previous studies showed that the aqueous extract of LL had free hydroxyl radicals scavenging activities. Yim et al. (2001) demonstrated that OLA, a main effective constituent of LL, had hepatoprotective action, which may be mediated by the enhancement of hepatic-glutathione regeneration capacity, particularly under conditions of carbon tetrachloride-induced oxidative stress. The previous studies from our studies showed that 0.2% aqueous extract of LL could improve the antioxidant status. All of these studies had the implication that LL improved antioxidant status of broilers.

Generally, pH value is a direct reflection of muscle acid content, and affects shear force, drip loss and color in meat. In this study, dietary supplication with LL at 5 or 10 g /kg increased the pH value. Tenderness is the most important textural characteristic of meat and has the greatest influence on consumer preference. Meat tenderness can be estimated by measuring the shear force; lower shear force indicates tenderer meat. Our study suggested that the dietary supplementation of LL couldn't improve meat tenderness of broilers. Drip loss or water loss percentage is a widely investigated approach for measuring water-holding capacity, by which savor, tenderness, color, fragrance, and nutrient content in muscle can be influenced. Lower water-holding capacity in muscles can induce liquid outflow, loss of soluble nutrients and flavor. Therefore, the muscle becomes dry, hard and tasteless, and meat quality is decreased. A study from our laboratory reported that the addition of 2 or 4 g of OLA/kg to diets of broilers could significantly influence the drip loss in breast muscle, which was similar to the results from the current study. The previous study showed that improvements were in drip loss and pH development postmortem were due to increased anti-oxidative status in the chickens (Young et al., 2003). Therefore, the presence of supplemental LL would be expected to reduce meat oxidation and improve meat quality.

In conclusion, dietary supplementation of 5 or 10 g of LL/kg improved the growth performance, increased the pH value and reduced drip loss of meat. This effect was likely mediated through the effects of LL on elevating the antioxidative enzymes activities and oxidative stability of lipid. These results demonstrate that LL shows good potential as an antioxidant in broilers. However, further study is needed to elucidate the mechanism by which LL improves the antioxidative activity, and the antioxidant effects of LL observed in this study on chicken meat is also needed to be proved on other food-producing species.

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