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Influence of *Ligustrum lucidum* and *Schisandra chinensis* Fruits on Antioxidative Metabolism and Immunological Parameters of Layer Chicks

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ABSTRACT : The experiment was conducted to evaluate the effects of *Ligustrum lucidum* (LL) and *Schisandra chinensis* (SC) on the growth, antioxidative metabolism and immunity of laying strain male chicks. The results showed that diets supplemented with 1% of either LL or SC had no effects on the growth performance of chicks compared with the control. Furthermore, both LL and SC significantly reduced malondialdehyde (MDA) concentration of serum and heart of chicks ($p<0.05$). In addition, superoxide dismutase (SOD) activity of serum of the birds was significantly elevated by supplementation with SC ($p<0.05$). Glutathione reductase (GR) activity of heart and serum of the birds was significantly elevated by supplementation with LL or SC ($p<0.05$). LL supplementation significantly elevated antibody values against Newcastle Disease virus (NDV)($p<0.05$) and lymphoblastogenesis ($p<0.05$) of the birds. The results suggest that diets supplemented with 1% of either LL or SC may improve immune function and antioxidant status of chicks.
(**Key Words :** *Ligustrum lucidum*, *Schisandra chinensis*, Chicks, Antioxidative Metabolism, Immunity)

INTRODUCTION

In poultry production, it is very important to improve immunity to prevent infectious diseases. A variety of factors can induce immunodeficiency, such as vaccination failure, infection by immune suppressive diseases, and abuse of antibiotics. Particularly, under intensive conditions birds contribute to infection by pathogen; therefore, prevention of infection through effective vaccination and administration medicines are vital to minimize disease in poultry. Feeding antibiotics have been used extensively to overcome infection and to maintain the health status of animals (Iovine and Blaser, 2004). However, the long-term use of feeding antibiotics, particularly those used in human medicine, has raised many issues by consumers regarding the emergence of antibiotics resistant bacteria and the link to humans via the food chain (Salisbury et al., 2002). As the result, alternatives to feeding antibiotics in commercial chickens have become important. Chinese herbal medicines are one kind of many such classes of alternatives that have aroused great interest because of their natural origin, enriching in biologically active constituents, such as polysaccharides, essential oil, and etc. Furthermore, most of them are lack of drug residue and low side effects (See review by Tian and Feng, 1994). In a previous study, effects of administration eleven Chinese herbal medicines including Ligustrum lucidum (LL) and Schisandra chinensis (SC) on growth and antibody titers against Newcastle Disease virus (NDV) of chicks were evaluated. Among the eleven Chinese herbal medicines, we found that both LL and SC significantly increased antibody titers against NDV in chicks (Ma et al., 2003). Furthermore, in a recent study, it was found that diets supplement with 1% of LL and SC significantly increased laying performance, immunity and antioxidant of laying hens during heat stress (Ma et al., 2005). Moreover, in another study, it was found that diets supplement with 1% of LL and SC significantly increased immunity and antioxidant of broilers (Ma et al., 2006).

LL is the fruit of plant of LL, enriched in oleanolic acid (OLA) (7-15 mg/g LL), D-mannanoligosaccharides (D-MOS) (6-9 mg/g LL), and specnuezhenide (0.6-1.2 mg/g LL) (Shi et al., 1998). In traditional Chinese medicine, LL was considered to serve functions as nourishing liver and kidney, and brightening eyes (Zang, 2000). Studies from modern medicine showed that LL or its main effective constituents are effective immunostimulants and excellent

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Table 1. Composition of the basal diet

Ingredient	Diets (g/kg)
Corn	613
Soybean meal	230
Wheat bran	100
Fish meal	20
L-lysine	1
DL-methionine	1.5
Limestone	14
$CaHPO_4$	13.3
NaCl	3
Vitamin premix[1]	0.2
Mineral premix[2]	3
50% choline chloride	1
Total	1,000
Calculated composition[3]	
ME (MJ/kg)	11.8
CP (g/kg)	185
Lysine (g/kg)	10.0
Methionine (g/kg)	4.5
Ca (g/kg)	10.0
Available phosphorus (g/kg)	4.0

[1] Provided per kg diet: Vitamin A, 10,000 IU; Vitamin D, 1,500 IU; Vitamin E, 20 mg; Vitamin K, 2.2 mg; Vitamin B_1, 1.1 mg; Vitamin B_2, 8 mg; Vitamin B_{12}, 0.02 mg; folic acid, 0.8 mg; niacin, 50 mg; pantothenic acid, 14 mg.

[2] Provided per kg diet: copper (as cupric sulfate $5H_2O$), 20 mg; iron (as ferrous sulfate $7H_2O$), 100 mg; zinc (as zinc sulfate $7H_2O$), 100 mg; selenium (as sodium selenite), 0.3 mg; iodine (potassium iodate), 1 mg; manganese (as manganese sulfate H_2O), 120 mg.

[3] Based on a dry matter content of 86%.

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).

SC is the fruit of plant of SC. The main effective constituents of SC are essential oil (approximately 2 g/100 g SC), lignans (schisandrin A: 0.026-0.083 g/100 g SC, deoxyschizandrin: 0.007-0.945 g/100 g SC, schisandrin B: 0.002-0.121 g/100 g SC, and schisandrin C: 0.010-0.038 g/100 g SC) (Zang, 2000; Zhang et al., 2002; Gao et al., 2003). SC has been used for nourishing heart and stomach, and strengthening immune function in traditional Chinese medicine (Zang, 2000). Reports from recent studies demonstrated that SC exhibited strong protective effect on phase I oxidative metabolism in the liver damaged by oxidative stress (Zhu et al., 1999; Zhu et al., 2000) and modulate cellular immune response, especially in the activation of macrophages and lymphocytes (Din, 1995).

The objectives of the present study were to investigate the effects of LL and SC supplementation on growth, lipids stability and immunity of chicks.

MATERIALS AND METHODS

Birds and experimental design

Two hundred ten 1-d-old laying strain cockerels were used for 7 wks experimental period. Birds were randomly assigned to 3 groups, which are control group fed the basal diet (Table 1), LL group fed the basal diet supplemented with 1% LL, and SC group fed the basal diet supplemented with 1% SC. Each group consisted of 5 replicates of 14 chicks per pen, stratified by BW to minimize the variability in pen weight. The basal diet was corn-soybean meal based and formulated to meet or exceed the minimum requirements of chicks based on NRC (1994). The birds had free access to feed and water during the experiment. The lighting schedule and temperature of the environment are controlled according to introduction of laying strain chicks.

Both LL and SC used in this study were originated from Shiyitand pharmaceutical factory of Harbin pharmaceutical group. The main active constitutes of LL:OLA (10-12 mg/g LL), D-MOS (5-9 mg/g LL), and specnuezhenide (0.8-1.0 mg/g LL). The main active constitutes of SC: essential oil (2.0-2.3 g/100 g SC), lignans (0.05-1.2 g/100 g SC, including schisandrin A, schisandrin B, deoxyschizandrin, and schisandrin C), and schisandra polysacchride (3-5 g/100 g SC). Both LL and SC were grinded into powder before added to diets. The birds were vaccinated 0.2 ml against Newcastle Disease (ND) with La Sota vaccine (containing mineral oil as adjuvant, product of Institute of Harbin Veterinary Research, Chinese Academy of Agricultural Sciences, Harbin, P. R. China, 150001.) by i.m. inoculation on 21 d old.

Samples

The birds were weighed, and feed consumption and mortality were recorded on 1 d, 7 d, 14 d, 21 d, 28 d, 35 d and 49 d old by pen. Blood samples were taken via wing vein from two birds per cage (a total of 10 birds per treatment) on the day receiving vaccination (day 0) and at 7 d, 14 d, 21 d and 28 d after vaccination (day 7, 14, 21 and 28**)**. Then, serum were separated and stored at -20°C for analysis. At the end of the experiment (49 d old), two birds per cage (a total of 10 birds per treatment) were randomly selected and euthanized by cervical disclocation, and spleen samples were immediately obtained to measure lymphocyte proliferation under bacterium free conditions. At the same time, 10ml blood samples were taken and sera samples were separated and stored at -20°C for analysis. Heart, liver, and kidney tissues were removed and homogenized in ice-cold 1% Triton X-100 in 0.01 M phosphate-buffered saline solution, pH 7.2, and filtered through double-layered cheesecloth according to Asikainen et al. (1980).

Assay of malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity, and glutathione reducetase (GR) activity

Protein levels, MDA concentration, GR and SOD activity in serum and tissues homogenates were determined using commercial protein, MDA, GR, and SOD detection

Table 2. The effect of ligustrum lucidum and schisandra chinensis on growth performance of chicks[1]

Treatment	Body weight (g/bird)[2]		Daily body weight gain[2] 1-42 d (g/bird d)	Daily feed consumption[2] (g/bird d)	FCR[2]	Mortality[2] (%)
	1 d old	49 d old				
Control	38.7±0.73	621.8±10.3	11.9±1.1	32.4±2.3	2.6	4.2
Ligustrum lucidum	39.2±1.09	626.7±13.5	12.0±0.9	30.5±2.5	2.4	5.1
Schisandra chinensis	38.8±0.67	637.2±8.9	12.2±0.7	35.1±1.6	2.8	3.1

[1] Data represents the mean value for each treatment (Mean±SD, 70 birds per treatment).
[2] There were no significant effects of treatment ($p>0.05$).

Table 3. The effect of ligustrum lucidum and schisandra chinensis on MDA concentration, SOD activity, and GR activity in serum and tissues of chicks[1]

Treatment	MDA concentration				SOD activity				GR activity			
	Serum (nmol/ml)	Heart (nmol/mg prot)[2]	Liver (nmol/mg prot)[2]	Kidney (nmol/mg prot)[2]	Serum (NU/ml)[3]	Heart (NU/mg pro)[4]	Liver (NU/mg pro)[4]	Kidney (NU/mg pro)[4]	Serum (U/ml)[5]	Heart (U/mg pro)[6]	Liver (U/mg pro)[6]	Kidney (U/mg pro)[6]
Control	9.18±0.86[a]	0.86±0.10[a]	0.50±0.09	0.40±0.12	85.12±4.28[a]	59.90±4.23	38.67±2.45	42.11±4.63	10.29±1.63[a]	2.74±1.09[a]	4.82±3.10	5.59±1.49
Ligustrum lucidum	7.55±0.27[b]	0.63±0.11[b]	0.44±0.03	0.38±0.05	90.18±5.03[ab]	60.06±8.05	39.77±3.22	41.05±4.12	14.02±2.58[b]	5.30±2.00[b]	5.48±1.52	6.72±2.16
Schisandra chinensis	8.13±0.68[ab]	0.60±0.14[b]	0.47±0.03	0.31±0.08	96.44±3.22[b]	61.32±6.12	41.04±2.45	44.13±5.02	14.41±2.97[b]	5.48±1.48[b]	5.55±1.33	7.14±1.18

[a-b] Means within a column with no common subscript differ significantly ($p<0.05$).
[1] Data represents the mean value for each treatment (Mean±SD, n = 10).
[2] Means nmol per milligram protein. [3] Means nitrite unit per ml.
[4] Means nitrite unit per milligram protein. [5] Means unit of activity per ml.
[6] Means unit of activity per milligram of protein.

kits, respectively (product of Nanjin Jiancheng Bioengineering Institute, Nanjin, P. R. China, 210000) according to the manufacturer's instructions, respectively. The MDA concentration was expressed as nmol per ml (nmol/ml) or nmol per milligram of protein (nmol/mg protein). The SOD activity in serum or tissues was expressed as a nitrite unit per ml (NU/ml) or NU per milligram of protein (NU/mg.protein), respectively. One NU was defined as the amount of SOD that inhibited the rate of cytochrome c reduction by 50%. The GR activity in serum or tissues was expressed as unit of activity per ml (U/ml) or unit per milligram of protein (U/mg protein), respectively. One unit of the GR was defined as the oxidation of 1 μmol of NADPH/min.

Assay of serum antibody titers

Serum antibody titers were determined by means of a hemagglutinin assay-microhemagglutination-inhibition (HA -HI) test. The titers were expressed as $\log_2 X$ of the highest dilution giving total agglutination (Liu, 1999). In brief, dilution series of sera were incubated with four HAU (haemagglutinating units) of NDV-Ulster at room temperature for 60 min. The HAU were titrated before each assay. Thereafter chicken erythrocytes were added and agglutination was scored after incubation at 4°C for 60 min. The HI titre is the reciprocal of the highest serum dilution completely inhibiting agglutination.

Assay of spleen lymphoblastogenesis

The spleen samples were processed to be 2×10^7 cells per ml single lymphocyte suspension (Liu, 1998) to measure lymphocyte proliferation. A modified lymphoblastogenesis microassay with spleencytes (Maslak and Reynolds, 1995; Liu, 1998) was conducted to evaluate the induction of *in vitro* lymphocyte proliferation by concanavalin A (ConA). In brief, 100 μl of single lymphocyte suspension was added to each well of a 96-well, flat-bottomed tissue culture plate containing RPMI1640 and ConA (products of Sigma Chemical Co., St. Louis, MO 63178-9916), with three replicates per sample. Plates were incubated at 39.6°C under 5% CO_2 for 72 h and then 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) (ICN Biomedicals Inc., Aurora, OH 44202) was added to each well. Plates continued to incubate for 3 h at 39.6°C under 5% CO_2 to allow the cleavage of MTT by lymphocytes to produce dark-blue formazan crystals. Samples were reacted with 10% saponin to lyse the cells, and then HCl-isopropanol solution was added to dissolve the formazan crystals to produce a solution suitable for measurement of absorbance. The optical density (OD) of supernatant from each well was read by a microtiter plate reader (Dynatech MR5000 Dynatech Laboratories, Inc., Chantilly, VA 22021) at wave length 550 nm.

Statistical analyses

Data are presented as the mean±SD. The data of the experiment were analyzed by one-way analysis of variance (ANOVA) for effects of diet. Data for body weight (BW), daily bodyweight gain (BWG), FCR, and percentage of mortality were collected and analyzed per cage. MDA contents, SOD activity, GR activity, antibody titers against NDV, and OD of lymphoblastogenese were collected and

Table 4. The effect of ligustrum lucidum, and schisandra chinensis on serum antibody titers against NDV (Log_2X) and proliferation of spleen lymphocytes (optical density, OD) of chicks[1]

Treatment	Day 0[2]	Day 7[2]	Day 14[2]	Day 21	Day 28	Means[3]	OD[4]
Control	3.4±0.2	3.5±0.3	4.0±0.6	5.1±0.2[a]	5.0±0.3[a]	5.1[a]	0.18±0.03[a]
Ligustrum lucidum	2.8±0.1	3.6±0.6	5.0±0.3	7.0±0.2[b]	6.8±0.3[b]	6.9[b]	0.33±0.02[c]
Schisandra chinensis	3.0±0.2	3.3±0.5	4.9±0.4	6.2±0.2[ab]	6.4±0.5[ab]	6.3[ab]	0.21±0.05[a]

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

[1] Data represents the mean value for each treatment (Mean±SD, n = 10).

[2] There were no significant effects of treatment ($p>0.05$).

[3] Mean of days 21 and 28.

[4] The optical density read by a microtiter plate reader at wave length 550 nm. The optical density is expressed without correcting for basal change absorbance.

analyzed with individual chick. The statistical analyses were accomplished using the general liner models procedure of SAS software (SAS Institute, 1996).

RESULTS

The results showed that no significant differences were found in growth performance, including BW, BWG, FCR or percentage of mortality among treatments ($p>0.05$) (Table 2).

Antioxidant of chicks is shown in Table 3. Compared to the control, feeding LL and SC significantly decreased MDA content in heart and serum of the birds ($p<0.05$). Whereas, LL and SC had no significant effect on MDA content in live and kidney of birds ($p>0.05$). In addition, both LL and SC have beneficial effect on SOD and GR activity in serum and heart of the birds. SC significantly enhanced SOD activity of serum of the birds ($p<0.05$). Moreover, LL and SC significantly increased GR activity in serum and heart of the birds. Little effects of LL and SC on liver and kidney of the birds were found.

The chick immune system responded similarly between LL and SC, as shown in Table 4. The antibody value against NDV was increased after NDV vaccination in all of groups in the present study. The antibody titers of the birds reached the highest on the Day 21. Supplementation with 1% of either LL or SC elevated the antibody titers of the birds on the Day 21 and 28. However, only LL significantly enhanced the antibody titers of the birds, compared with the control group. In addition, LL elevated spleen lymphoblastogenese of the birds significantly ($p<0.05$), compared with the control group (no treatment). It is also found that SC had advantageous effect on spleen lymphoblastogenese of the birds, yet, no significant difference were found ($p>0.05$), compared with the control group.

DISCUSSION

The current study indicated no effect of supplementing diets with 1% of either LL or SC on the growth performance of laying strain male chicks. There are a few reports on effect of Chinese herbal medicines, including LL and SC on growth or production of animals. Similar to the present study, previous studies from us shown that diets supplemented eleven Chinese herbal medicines including LL and SC has no effect on growth performance of chicks or broilers (Ma et al., 2003; Ma et al., 2006), respectively. Chen et al. (2003) reported diets supplemented with Chinese herbal polysaccharides (astragalan or achyranthan) had no effect on the growth performance of broiler. However, Kim et al. (2004) reported that diet supplemented with 0.2% plant extract can improved body weight gain of weaned pigs without negative affects. It has been proposed that immunostimulation may have adverse effects on growth, because antibodies and more nutrients will be repartitioned to synthesize and develop immune organs, there by decreasing the nutrients available for growth (Hevener et al., 1999; Takahashi et al., 2000). In contrast, Klasing (1998) proposed that immunostimulation would not necessarily decrease growth, because the immune system needs a relatively small amount of nutrients in relation to the nutrients needed for growth. In the current study, immunostimulation may have been limited to the immune system, and not accompanied with the whole-body reactions and the series of physiological and metabolic changes such as fever, decreased feed intake, acute phage protein synthesis, and increased nutrient catabolism.

Under intensive conditions, birds are subjected to a number of stressful agents, including the constant exposure to various microorganisms and vaccination. Free radicals could be generated by disease or stress. Increased presence of free radicals can cause oxidation of biomolecules, leading to cell death and tissue injury involved in pathogenesis of several disease states (Kehrer, 1993). Toxic effects of free radicals may be reduced by the presence of antioxidants. In the last few years much emphasis has been given to natural antioxidants. Herbs are important natural antioxidants, among which LL and SC have been demonstrated to have strong antioxidant activities, and both of them are natural oxidation inhibitors (IP et al., 1996; Zhu et al., 2000). The current study showed that diets

supplementation with either 1% LL or SC can decrease MDA concentration of serum and tissues of chicks by enhancement SOD and GR activity. MDA is the main product of lipid peroxidation, and was considered to be an indicator for lipid peroxidation (Halliwell, 1995). Consistent with the present study, the previous studies from our studies showed that diet supplement with 1% of either LL or SC can decrease MDA concentration of serum and tissues of laying hens during heat stress or broilers by enhancement GR activity (Ma et al., 2005; Ma et al., 2006). A recent report from Li and Wang (2004) revealed that the aqueous extract of LL has free hydroxyl radicals scavenging activities. Yim et al. (2001) demonstrated that oleanolic acid, a main effective constituent of LL, has hepatoprotective action, which may be mainly mediated by the enhancement of hepatic-glutathione regeneration capacity, particularly under conditions of carbon tetrachloride (CCl_4)-induced oxidative stress. It was also shown that the ethanol extract of LL has inhibitory effects on the hemolysis of red blood cells induced by 2, 2'-azo-bis-(2-amidinopropane) dihydrochloride (He et al., 2001). Chiu et al. (2002) reported that a lignan-enriched extract of SC has a dose-dependent protection against CCl_4 hepatotoxicity. Ohsugi et al. (1999) evaluated the active-oxygen scavenging activity of 70 traditional herbal medicines used in China and Japan as nourishing tonics, and found that the water extract of SC reveal strong scavenging activity against hydroxyl radical. In addition, Zhu et al. (1999, 2000) reported that SC exhibited strong protective effect on Phase I oxidative metabolism in the liver damaged by CCl_4, suggesting SC to be a promising agent for the improvement of phase I oxidative metabolism in the liver damaged by CCl_4. The above studies with other species are agreement with the present study with chickens. All of these studies suggest that both LL and SC be excellent antioxidants that scavenge the free radicals generated by stress in cell membranes, maintaining cellular metabolic functions.

It had been shown that, utilization of immunostimulants is one solution to improve the immunity of poultry and to decrease their susceptibility to infectious diseases (Liu, 1999). Results from the current study revealed that both LL and SC showed immunostimulating effects including increasing antibody titers against NDV and spleen lymphocyte proliferation of chicks in the present study, which was agreement with the previous studies from us (Ma et al., 2003; Ma et al., 2005; Ma et al., 2006). Consistent with our results, Sun et al. (1983) demonstrated that the aqueous extracts of LL could augment the spontaneous $^{[3H]}$thymidine incorporation in the mononuclear cells of normal subjects and patients. In addition, it could also augment the proliferation of lymphocytes from both normal subjects and patients induced by ConA or phytohemaaglutinin. Jin (1995) reported that Shi-ka-pon (a CHM decoction) and its constituent Lithospermum erythrorhizon and LL could resist immunosuppression induced by the antitumor angent mitomycin C and suggested that its mechanisms might be correlated with stimulation of the reticuloendothelial system, activation of T cell blastogenesis, and NK cell cytotoxicity. SC is a traditional Chinese herb clinically used to treat viral and chemical hepatitis. Schisandra polysacchride is one of effective constituents of SC, which has been found to modulate cellular immune response, especially in the activation of macrophages and lymphocytes (Din, 1995). Recent study showed that haemoglobin values was significantly improved in broilers due to feeding of Growell (polyherbal feed supplement) (Kalorey et al., 2005). The present results with chicks were confirmed with previous studies with other species.

In conclusion, the current results showed that diets supplement with either 1% LL or SC enhance lipids stability of chicks by increasing antioxidant enzymes including SOD and GR, or delete MDA directly. In addition, LL and SC could improve immunity including antibody titers against NDV and spleen lymphocyte proliferation of chicks. The results suggested that LL and SC have more potential to be utilized as a feed additive to improve the immunity and lipids stability of chicks. In the future research, it needs to be ascertained whether the extracts of the two CHMs has improvements on immunity and antioxidant of chicks.

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