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Original Research Article

Effects of sporoderm-broken spores of *Ganoderma lucidum* on growth performance, antioxidant function and immune response of broilers

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ABSTRACT

This study was conducted to evaluate the effects of sporoderm-broken spores of *Ganoderma lucidum* (SSGL), a traditional Chinese medicinal herb, on growth performance, antioxidant ability, and immunity of broilers. Three hundred male broilers with similar body weights (40.0 ± 1.0 g) at 1 d of age were assigned randomly to 4 treatments. Each treatment contained 5 replicates of 15 birds per replicate. The dietary treatments were corn–soybean meal basal diet supplemented with SSGL at the concentrations of 0 (control), 100, 200 and 500 mg/kg diet. The results showed that diets supplemented with SSGL significantly increased ($P < 0.05$) the average daily gain and decreased ($P < 0.05$) the feed:gain (F:G) ratio of birds during the finisher period (22 to 44 d of age). Moreover, the total antioxidant capability, glutathione reductase and catalase activities in the liver and spleen were significantly higher ($P < 0.05$) in broilers fed diets with SSGL than in broilers fed the control diet. Additionally, dietary SSGL also increased ($P < 0.05$) the serum interleukin (IL)-2, immunoglobulin (Ig) A and IgG levels of broilers compared with the control diet. These results suggest that SSGL have ameliorative effects on growth performance, free radical-scavenging activity, antioxidant capability, and immune function of broilers.

1. Introduction

High stocking density, fast growth and environmental factors such as acute heat stress, health challenges, bacterial or viral exposure, and mycotoxin-contaminated diets may cause serious stress of broilers in modern intensive broiler production systems (Zhang et al., 2013; Ma et al., 2015). Excessive reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), can be stimulated by stressful conditions, breaking the balance between the oxidation and antioxidant defense systems (Papadopoulou et al., 2017). When initiated, oxidative stress causes lipid peroxidation (LPO) and oxidative damage to proteins, DNA and cells (Andrew et al., 2014). Furthermore, oxidative stress results in immune inhibition and inflammation (Yadav and Haldar, 2012), affecting the growth, feed conversion efficiency and health status of broilers (Jia et al., 2014). To reduce oxidative stress and increase growth performance, dietary antioxidants such as vitamin E and α-lipoic acid have been studied in poultry industries (Li et al., 2014; Ma et al., 2015; Urso et al., 2015).

Ganoderma lucidum, also known as Lingzhi, has been collected, cultivated and used as a traditional healthy function food and as a component of medicine in China and other eastern Asian countries for many centuries (Dong et al., 2012). In the last decades, the biological characteristics of *G. lucidum* were studied and well identified by many scientific researchers, and the results demonstrated that *G. lucidum* possesses strong bioactivities in immunomodulation, antioxidation, hepatoprotection, and anticancer and antitumor action, among others (Pan et al., 2013a; Dong and Han, 2015). The primary bioactive ingredients of *G. lucidum* include

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triterpenoids, polysaccharides, sterols, alkaloids, peptides, mineral elements and amino acids (Wang et al., 2013; Liu et al., 2015). Among them, triterpenoids and polysaccharides were proved to be the most effective compounds (Smina et al., 2011b; Zhang et al., 2016).

The spores of *G. lucidum* (SGL), ejected from the pileus of growing *Ganoderma* in the mature phase, are asexual reproductive bodies of approximately 6.5 to 8.0 μm × 9.6 to 12.6 μm enwrapped with the double-layered sporoderm (Fu et al., 2009). The spores also contain many bioactive ingredients, including triterpenoids, polysaccharides and steroids, similar to the *G. lucidum* (Guo et al., 2009). Additionally, the contents of some bioactive ingredients of SGL are higher than those of *G. lucidum*, e.g., the total triterpenoid content of the spores was 5- to 20-fold higher (Huie and Di, 2004). Therefore, many studies suggest that the bioactivities of SGL are much higher than those of *G. lucidum* (Min et al., 1998; Zhao et al., 2012). However, SGL were recognized and utilized only in the 20th century because of the extremely hard and resilient sporoderm, which inhibits the release of the inner bioactive components of the SGL (Chen et al., 2012). Zhou et al. (2012) observed that dietary SGL (2 g/kg) had no effect on oxidative stress and mitochondrial dysfunction in the hippocampus of rats. The activities of spores are closely related to the status of the sporoderm, and sporoderm-broken spores of *G. lucidum* (SSGL) are more effective in modulating the immune responses in rats than sporoderm-unbroken spores (Yue et al., 2008). In recent years, with successful collection of SGL on a large scale and a breakthrough in sporoderm-breaking technology, SGL were demonstrated to possess strong bioactivities, such as antioxidation, immunomodulation, and anticancer and antitumor actions in vitro (Kozarski et al., 2011; Heleno et al., 2012) in broilers (Liu et al., 2016a), rats (Hapuarachchi et al., 2016) and human promyelocytic leukemia cells (Gao et al., 2012). However, no study has evaluated the effects of dietary supplementation of SGL on productive performance, oxidative status and immune response of broilers under a high-stocking density environment.

In this study, to improve the digestion and absorption of the bioactive ingredients in SGL, SSGL were chosen to evaluate the biological effects in broiler diets via measurements of growth performance, antioxidant ability and serum immunoglobulin contents in male Arbor Acres broilers under a high-stocking density environment.

2. Materials and methods

The protocol was reviewed and approved by the Animal Care and Use Committee of China Agricultural University (CARE NO. AW17109102-1-1). All procedures were performed strictly in accordance with the guidelines of recommendations in the Guide for Experimental Animals of the Ministry of Science and Technology (Beijing, China), and all efforts were made to minimize suffering.

2.1. Preparation of sporoderm-broken spores of G. lucidum

Riverside *Ganoderma Lucidum* Planting Co. Ltd. (Xiuyan Manchu Autonomous County, Liaoning Province, China) supplied the SGL. The spores were dried at 55 °C for 24 h and then were broken by a supercritical fluid extraction device according to Fu et al. (2009) and Li et al. (2011). Briefly, approximately 150 g of the spores was loaded into a steel cylinder equipped with mesh filters on both ends. Liquefied CO_2 was pumped into the vessel, and the pressure was raised to 35 MPa. The temperature was controlled at 25 °C during the processing. The pressure was released within 1 min at the end of the 4-h process. During the process of rapid depressurization, the resistance of the sporoderm created a pressure difference inside and outside the sporoderm, and the spores were broken when CO_2 burst out of the sporoderm. Sporoderm-broken spores of *G. lucidum* were collected and stored at −20 °C before adding into the diets.

The contents of triterpenoids, polysaccharides, and α-tocopherol in the SSGL sample were determined before feeding trial. The triterpenoids content was determined to be 4.55% by spectrophotography using oleanolic acid as a standard according to Yang and Zhu (2010). The polysaccharides content was determined to be 7.98% by ultraviolet spectrophotometer according to the phenol-sulfuric acid method described by Yang et al. (2018). The αdtocopherol content was determined to be 36.9 IU/kg according to HPLC method described by Zhang and Zhang (1997).

2.2. Birds, diets and management

A total of 300 one-d-old male Arbor Acre broilers were purchased from a local commercial company (Beijing Huadu Yukou Poultry Co., Ltd, Beijing, China). The experiment lasted for 44 d with 2 feeding periods. The starter period was from 1 to 21 d of age, and the finisher period was from 22 to 44 d of age.

All broilers were raised in wire-floored pens (100 cm × 100 cm × 60 cm; 15 broilers per pen) in an environmentally controlled room. The lighting program in the room was 24 h of constant light (L) on d 1, providing sufficient time for learning to drink water and feed diet, and then 23L:1 h dark (D; between 22:00 and 23:00) up to d 7, then for 20L:4D (between 22:00 and 02:00) until d 44. The temperature of the room was maintained at 35 to 37 °C for the first 2 d, 33 to 35 °C for d 3 to 7 and then gradually decreased by 2 °C per week until d 35 and maintained at 24 °C thereafter. The relative humidity was maintained at 65% to 70%. Ventilation was controlled by negative pressure air fans. Water and diet were provided *ad libitum*. All birds were inoculated with Newcastle disease vaccine and infectious bronchitis vaccine on d 7 and 21 and with infectious bursa disease vaccine on d 14.

The composition of the basic diet is presented in Table 1. All nutrients met or exceeded the NRC (1994) recommendations. The 4 dietary treatments were the basic diet supplemented with 0 (control), 100, 200, and 500 mg SSGL/kg diet.

2.3. Serum and tissue preparation

On d 44 of the experiment, one bird from each pen (5 birds per treatment) with a body weight (BW) close to the average was selected and slaughtered. Birds were slaughtered by bleeding the left jugular vein. Feed was withdrawn 12 h before slaughter. Blood was drawn from the wing vein with vacuum blood collection tubes and then centrifuged at 3,000 × *g* for 10 min to obtain serum. The serum was stored at −20 °C immediately. Portions of liver and spleen tissues were harvested, coded, snap-frozen in liquid nitrogen, and then maintained at −80 °C for analyses.

2.4. Measurements

2.4.1. Growth performance

The BW and feed intake were recorded based on pens on d 21 and 44 after a 12-h feed withdrawal. The average daily feed intake (ADFI), average daily gain (ADG) and feed:gain (F:G) ratio were calculated subsequently.

2.4.2. Oxidative stress indices in the liver and spleen

Liver or spleen tissues (approximately 1 g) were cut into small pieces and homogenized in ice-cold physiological saline with a ratio 1:9 (wt/vol) using an Ultra-Turrax (T8, IKA-labortechnik

Table 1
Ingredient and nutrient composition of the basal diet (as-fed basis, % unless noted).

Item	Starter period (1 to 21 d of age)	Finisher period (22 to 44 d of age)
Ingredients		
Corn	57.95	60.79
Soybean meal	14.20	13.30
Peanut meal	20.00	18.00
Ground limestone	1.30	1.00
Dicalcium phosphate	1.80	1.50
Salt	0.30	0.30
Soybean oil	3.00	4.00
Lysine	0.46	0.34
DL-methionine	0.37	0.20
Threonine	0.19	0.14
Choline chloride	0.10	0.10
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.30	0.30
Total	100.00	100.00
Nutrition components		
Crude protein	21.48	20.03
Lysine	1.15	1.01
Methionine	0.63	0.45
Metabolizable energy, MJ/kg	12.52	12.93
Calcium	1.01	0.82
Total phosphorus	0.67	0.61

[1] Provided 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 mg; pyridoxine, 4.40 mg; vitamin B_{12}, 0.02 mg; calcium pantothenate, 25.6 mg; nicotinic acid, 65.8 mg; folic acid, 0.96 mg; biotin, 0.20 mg.
[2] Provided per kilogram of diet: Fe, 100 mg; Cu, 8 mg; Zn, 78.0 mg; Mn, 105 mg; I, 0.5 mg; Se, 0.3 mg.

Staufen, Germany) and following the procedure described by Jia et al. (2014) and Ma et al. (2015). The homogenates were centrifuged at 1,000 × *g* for 15 min at 4 °C, and the supernatants were collected for assays of hydrogen peroxide (H_2O_2), hydroxyl radical-scavenging activity (HRSA), malondialdehyde (MDA), lipid peroxidation (LPO), total antioxidant capability (T-AOC), catalase (CAT), total superoxide dismutase (T-SOD), glutathione reductase (GR), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), and total protein (TP). The assays were conducted using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) according to the kit instructions.

2.4.3. Serum concentrations of selected immunoglobulins (Ig) and interleukin (IL)-2

The levels of IgA, IgM, IgG and IL-2 in serum were measured using ELSA methods, and the measurements were conducted following the manufacturer's instructions of the commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

2.5. Statistical analyses

Pen was used as the experimental unit, and the homogeneity of variances and normality of the data were tested first. Then one-way analysis of variance was performed using the GLM procedure with the SAS statistical software package (Version 9.0; SAS Institute, Inc., Cary, NC, USA). Polynomial orthogonal contrasts were used to determine linear and quadratic responses to increasing dietary SSGL levels (0, 100, 200 and 500 g SSGL/kg diet). Tukey's multiple range tests was used for multiple comparisons when a significant difference was detected. All statements of significance were based on the <0.05 level of probability.

3. Results

3.1. Growth performance

The growth performance measured as ADFI, ADG, and F:G ratio (Table 2) showed no differences among the dietary treatments ($P > 0.05$) during the starter period (1 to 21 d of age). However, the ADFI and ADG of broilers in groups that received SSGL were on average 1.71% and 5.18% higher ($P < 0.05$) than those in the control group during the finisher period (22 to 42 d of age), respectively. Supplementation of SSGL significantly decreased the F:G ratio of broilers ($P < 0.05$) compared with that of the control group. No significant differences were observed for ADFI, ADG and F:G ratio of broilers among SSGL-treated groups (diets that received 100, 200 and 500 mg SSGL/kg feed; $P > 0.05$).

During the entire experimental period (1 to 44 d of age), birds fed diet with 100 mg/kg SSGL had a significant higher ADG ($P < 0.05$) and lower F:G ratio ($P < 0.05$), and birds fed diet with 200 mg/kg SSGL only had a significant lower F:G ratio ($P < 0.05$) as compared with the control group. No significant differences were observed for ADFI among all the treatments ($P > 0.05$), ADG and F:G ratio among SSGL-treated groups ($P > 0.05$) during the whole experimental period (1 to 44 d of age).

3.2. Oxidant status in the liver and spleen of broilers

The H_2O_2 content in the liver of broilers under SSGL-treatments was on average 11.45% lower ($P < 0.01$) than that of broilers in the control group (Table 3). However, the hepatic HRSA of broilers that received SSGL was higher ($P < 0.01$) than that of broilers in the control group, and dietary SSGL (100, 200 and 500 mg/kg) increased the HRSA by 8.26%, 6.61% and 35.12%, respectively, compared with that of the control. Dietary supplementation of SSGL also decreased ($P < 0.05$) MDA and LPO contents in the liver of broilers. This decrease was linear with the increase in level of SSGL in diets ($P < 0.01$), and the contents of MDA and LPO under SSGL treatment at the level of 500 mg/kg were lower than those at 100 mg/kg. Similar to the trends in the liver, the HRSA in the spleen of broilers increased ($P < 0.05$), and the MDA and LPO content decreased ($P < 0.01$) with the increasing levels of dietary SSGL. Low levels of dietary SSGL (100 and 200 mg/kg) had no effect ($P > 0.05$) on the MDA content, but dietary treatment with 500 mg SSGL/kg feed decreased ($P < 0.05$) MDA content by 6.42% compared with that of the control diet (Table 3). No significant difference was detected in splenic H_2O_2 content among all dietary treatments.

3.3. Antioxidant status in the liver and spleen of broilers

No significant differences were observed for the hepatic activities of T-SOD and GSH-Px among all treatments ($P > 0.05$; Table 4). However, the activities of T-AOC and GR in the liver of broilers improved linearly ($P < 0.01$) with the increase in the dietary SSGL supplementation. Additionally, the hepatic T-AOC and GR of broilers fed 500 mg SSGL/kg feed were higher than those fed 100 mg SSGL/kg feed. Compared with the control group, dietary 100, 200 and 500 mg SSGL/kg feed significantly increased ($P < 0.05$) the hepatic activity of CAT by 18.20%, 16.00% and 17.47%, respectively. The hepatic GSH level of broilers fed diets containing 100, 200 and 500 mg SSGL/kg feed increased ($P < 0.05$) by 16.25%, 19.70% and 19.21% as compared with that of the broilers fed control diet.

Similar to the trend in the liver, the T-SOD and GSH-Px in the spleen were not significantly affected ($P > 0.05$) by the dietary SSGL. However, the activities of T-AOC and CAT measured in the spleen of broilers fed diets supplemented with SSGL were on average 39.02% and 3.71% higher ($P < 0.05$) than those in broilers in the control

Table 2
Effect of SSGL on growth performance of broilers.[1]

Item	SSGL, mg/kg				SEM	*P*-value		
	0	100	200	500		Multiple range test	Linear	Quadratic
1 to 21 d of age								
ADFI, g/d	53.0	52.6	52.2	52.8	0.37	0.58	0.22	0.95
ADG, g/d	33.5	34.0	33.3	33.0	0.53	0.58	0.81	0.46
F:G ratio	1.58	1.55	1.55	1.60	0.37	0.34	0.77	0.35
22 to 44 d of age								
ADFI, g/d	135.7	138.0	138.0	138.1	0.86	0.18	0.079	0.24
ADG, g/d	70.4^{b}	74.0^{a}	74.0^{a}	74.2^{a}	0.70	0.004	0.003	0.028
F:G ratio	1.93^{a}	1.87^{b}	1.87^{b}	1.86^{b}	0.01	0.006	0.003	0.031
1 to 44 d of age								
ADFI, g/d	98.1	99.2	99.0	99.3	0.57	0.45	0.19	0.53
ADG, g/d	53.6^{b}	55.8^{a}	55.5^{ab}	55.4^{ab}	0.46	0.017	0.026	0.027
F:G ratio	1.83^{a}	1.78^{b}	1.78^{b}	1.79^{ab}	0.01	0.01	0.23	0.016

SSGL = sporoderm-broken spores of *Ganoderma lucidum*; ADFI = average daily feed intake; ADG = average daily gain; F:G ratio = feed:gain ratio, equal to ADFI/ADG.
[a, b]Means with different superscripts within the same row are different ($P < 0.05$).
[1] Each value represents the mean of 5 replicate values.

Table 3
Effect of SSGL on oxidant status in the liver and spleen of broilers.[1]

Item	SSGL, mg/kg				SEM	*P*-value		
	0	100	200	500		Multiple range test	Linear	Quadratic
Liver								
H_2O_2, mmol/g protein	10.7^{a}	9.49^{b}	9.60^{b}	9.44^{b}	0.21	0.003	0.002	0.026
HRSA, U/g protein	2.42^{b}	2.62^{b}	2.58^{b}	3.27^{a}	0.08	<0.001	<0.001	0.007
MDA, nmol/g protein	2.57^{a}	2.14^{b}	2.07^{bc}	2.03^{c}	0.02	<0.001	<0.001	0.002
LPO, μmol/g protein	0.92^{a}	0.86^{b}	0.83^{bc}	0.81^{c}	0.01	<0.001	<0.001	0.27
Spleen								
H_2O_2, mmol/g protein	14.4	14.6	14.2	14.4	0.27	0.75	0.65	0.40
HRSA, U/g protein	1.90^{b}	2.30^{a}	2.48^{a}	2.50^{a}	0.05	<0.001	<0.001	0.074
MDA, nmol/g protein	1.87^{a}	1.84^{a}	1.79^{ab}	1.75^{b}	0.03	0.037	0.004	0.61
LPO, μmol/g protein	4.20^{a}	3.46^{b}	3.34^{bc}	3.28^{c}	0.04	<0.001	<0.001	<0.001

SSGL = sporoderm-broken spores of *Ganoderma lucidum*; H_2O_2 = hydrogen peroxide; MDA = malondiadehyde; LPO = lipid peroxidation; HRSA = hydroxyl radical-scavenging activity.
[a, b, c]Means with different superscripts within the same row are different ($P < 0.05$).
[1] Each value represents the mean of 5 replicate values.

Table 4
Effect of SSGL on antioxidant status in the liver and spleen of broilers.[1]

Item	SSGL, mg/kg				SEM	*P*-value		
	0	100	200	500		Multiple range test	Linear	Quadratic
Liver								
T-AOC, U/mg protein	1.99^{c}	2.18^{b}	2.22^{b}	2.44^{a}	0.03	<0.001	<0.001	0.76
CAT, U/mg protein	8.13^{b}	9.61^{a}	9.43^{a}	9.55^{a}	0.08	<0.001	<0.001	<0.001
T-SOD, U/mg protein	17.0	16.6	16.1	16.7	0.50	0.70	0.60	0.35
GR, U/g protein	4.10^{c}	6.04^{b}	6.74^{ab}	7.05^{a}	0.22	<0.001	<0.001	0.066
GSH-Px, U/mg protein	43.3	43.0	43.5	43.0	0.62	0.92	0.91	0.85
GSH, mg/g protein	2.03^{b}	2.36^{a}	2.43^{a}	2.42^{a}	0.05	<0.001	<0.001	0.008
Spleen								
T-AOC, U/mg protein	2.46^{b}	3.43^{a}	3.38^{a}	3.45^{a}	0.06	<0.001	<0.001	<0.001
CAT, U/mg protein	5.30^{b}	5.47^{ab}	5.52^{a}	5.50^{a}	0.05	0.017	0.006	0.008
T-SOD, U/mg protein	21.2	20.2	20.6	22.1	0.78	0.35	0.62	0.50
GR, U/g protein	6.34^{b}	8.25^{a}	9.23^{a}	9.30^{a}	0.30	<0.001	<0.001	0.177
GSH-Px, U/mg protein	47.7	48.4	47.6	47.4	0.35	0.28	0.28	0.27
GSH, mg/g protein	3.75^{c}	3.87^{c}	4.32^{b}	4.63^{a}	0.05	<0.001	<0.001	0.099

SSGL = sporoderm-broken spores of *Ganoderma lucidum*; T-AOC = total antioxidant capability; CAT = catalase; T-SOD = total superoxide dismutase; GR = glutathione reductase; GSH-Px = glutathione peroxidase; GSH = reduced glutathione.
[a, b, c]Means with different superscripts within the same row are different ($P < 0.05$).
[1] Each value represents the mean of 5 replicate values.

group, but the differences among treatments that received different levels of SSGL (100, 200 and 500 mg/kg) were not significant ($P > 0.05$). Compared with the control, the GR activity and GSH level in the spleen increased linearly ($P < 0.01$) with the increase in SSGL supplementation.

3.4. Serum immune parameters of broilers

A significant linear response of serum IL-2 ($P < 0.01$) to the SSGL concentrations in the diets was detected (Table 5). Among all treatments, the levels of serum IgA and IgG were the highest in

Table 5
Effect of SSGL on serum immune parameters of broilers.[1]

Item	SSGL, mg/kg				SEM	*P*-value		
	0	100	200	500		Multiple range test	Linear	Quadratic
IL-2, ng/L	36.6^{c}	37.9^{bc}	39.4^{b}	41.2^{a}	0.39	<0.001	<0.001	0.48
IgA, g/L	0.281^{c}	0.284^{bc}	0.298^{a}	0.294^{ab}	0.003	0.002	0.001	0.37
IgG, g/L	0.266^{b}	0.268^{ab}	0.277^{a}	0.276^{a}	0.003	0.037	0.007	0.59
IgM, g/L	0.224	0.226	0.229	0.227	0.002	0.62	0.34	0.45

SSGL = sporoderm-broken spores of *Ganoderma lucidum*; IL-2 = interleukin-2; Ig = immunoglobulin.
$^{a, b, c}$Means with different superscripts within the same row are different ($P < 0.05$).
[1] Each value represents the mean of 5 replicate values.

broilers that received 200 SSGL/kg diet. Compared with the control, supplementing SSGL to diets at 100, 200 and 500 mg/kg increased serum IgA by 1.07% ($P > 0.05$), 6.05% ($P < 0.05$) and 4.63% ($P < 0.05$), respectively. The level of serum IgG had a similar trend to that of IgA, and with the increase in supplement of SSGL to diets, the level of IgG increased by 0.75% ($P > 0.05$), 4.14% ($P < 0.05$) and 3.76% ($P < 0.05$) with 100, 200 and 500 mg SSGL/kg feed, respectively. No effect of dietary SSGL on serum IgM of broilers was detected ($P > 0.05$).

4. Discussion

4.1. Growth performance

In the present study, we did not observe a significant effect of dietary SSGL on growth performance measured as ADFI, ADG, and F:G ratio during the starter period (1 to 21 d of age), but a significant increase in ADG and decrease in F:G ratio of the broilers fed dietary SSGL were detected during the finisher period (22 to 44 d of age), suggesting that the effect of dietary supplementation of SSGL on the growth performance of broilers is associated with the duration of feeding on SSGL and the age of the broilers. Broilers are more stressed in their late stage than in their earlier one. In the current study, the stocking density was higher during the finisher period than that during the starter period (0.60 to 11.3 kg BW/m^2, average 5.95 kg/m^2 during d 1 to 21 vs. 11.3 to 37.4 kg BW/m^2, average 24.4 kg BW/m^2 during d 22 to 44); therefore, the stress caused by the competition for space and feeding increased. Moreover, rapidly growing broilers during the finisher period produce an increase in endogenous calories (Aluwong et al., 2017) and oxidative free radicals (Werner et al., 2011) because of the pronounced age or BW-related changes in metabolic rate, body composition and endocrine function (Buyse et al., 2004). The SGL are characterized as an antioxidant and immunomodulator (Zhou et al., 2012; Zhang et al., 2016) and also contain some other plant-type functional additives such as those from *Astragalus polysaccharide*, *Capsicum frutescens*, and *Nigella sativa* L., which possess bioactivities in antioxidation and immunomodulation and stimulate growth performance of broilers (Ghasemi et al., 2014; Shang et al., 2014; Wang et al., 2015b). Moreover, the polysaccharides from *G. lucidum* increase serum SOD and GSH-Px and decrease serum MDA levels in mice with chronic pancreatitis, resulting in a significant increase in BW (Li et al., 2016). Wang et al. (2015a) also observed that SGL attenuated the level of oxidative stress in diabetic rats and accelerated the recovery of BW lost during the stress. In our previous study, the addition of 200 mg SSGL/kg to aflatoxin B1 (AFB_1)-contaminated diet (25.0 and 22.5 μg AFB_1/kg in starter and finisher diets respectively) could counteracted its negative effects on ADFI, ADG and F:G ratio of broilers during 0 to 44 d (Liu et al., 2016a). These findings are in support of the present results. Broilers are continuously confronted by a multitude of stressors that can last for a few hours (e.g., catching, crating and transport) or for nearly the entire rearing period (e.g., heat stress and immune challenges). Consequently, intrinsic and extrinsic adverse forces or stressors constantly challenge their internal homeostasis (Lin et al., 2004). The pro-oxidant/antioxidant balance maintains the redox homeostasis, and an imbalance in favor of the pro-oxidant system will result in oxidative stress (Sies, 1991). The maintenance of the redox balance is important for the health of broilers (Iqbal et al., 2002). Thus, the improvement of ADG and F:G ratio observed in this study during the finisher period (22 to 44 d of age) could be partially due to the antioxidative characteristics of SSGL. The antioxidant activities of SGL and SSGL and their extracts have also been demonstrated in vitro (Heleno et al., 2012), in mice (Bao et al., 2001), in rats (Zhou et al., 2012) and in broilers (Liu et al., 2016a).

4.2. Oxidant status in the liver and spleen of broilers

Increased production of ROS, including H_2O_2 and the ·OH, can lead to attack on biological molecules (Loschen and Azzi, 1975). The primary component of ROS is H_2O_2 released from the mitochondria. The ·OH is also an extremely reactive free radical that reacts rapidly with almost every type of molecule including sugars, amino acids, phospholipids, DNA bases and organic acids (Lee and Lee, 2006). Above-physiological levels of ROS cause or increase lipid peroxidation in cells and subsequently damage the cell membrane function by decreasing the membrane fluidity and modifying the activity of membrane-bound enzymes and receptors (Misro et al., 2004; Arulselvan and Subramanian, 2007). Usually, MDA is formed at the end of lipid peroxidation and therefore is used broadly as an indicator of lipid peroxidation (Ozen et al., 2009). The polysaccharides (Kozarski et al., 2011) and phenolic extracts (Heleno et al., 2012) of SGL can scavenge diphenylpicryl phenylhydrazine (DPPH) radicals, increase reducing power and inhibit lipid peroxidation in vitro. Jin et al. (2013) observed that mice treated with SGL for 7 d, and subsequently challenged with $CdCl_2$ had a reduced level of hepatic MDA. In type 2 diabetic rats, oral SGL significantly increased serum activities of GSH-Px and SOD, and decreased serum level of MDA at 4 weeks of age (Wang et al., 2015a). In this study, we found that supplementation of SSGL in the diet not only significantly decreased the hepatic H_2O_2 content, but also increased the HRSA in both liver and spleen of the broilers. In our previous study, Liu et al. (2016a) found that dietary supplementation of 200 mg SSGL/kg could relieve the oxidative stress by AFB_1-contaminated diet, and resulted in lower H_2O_2, MDA and LPO content in the liver and spleen of broilers.

Hydroxyl radical-scavenging activity (HRSA) has been applied to investigate the abilities of antioxidants to scavenge hydroxyl and superoxide radicals (Sánchez-Moreno, 2002). Cherian et al. (2009) reported that *G. lucidum* had free radical scavenging and mitochondrial antioxidant activities. Consistent with such activity, the content of MDA and level of LPO in the liver and spleen of the broilers that received diets with SSGL were reduced compared with those of broilers that received the control diet. Therefore,

supplementation of SSGL in the diets, as occurred in vitro and with rodent species, improved the capability to scavenge ROS and reduce the lipid oxidation in the tissues of broilers.

4.3. Antioxidant status in the liver and spleen of broilers

The levels of free radical molecules and LPO are controlled by the antioxidant defense system, consisting of enzymatic components such as SOD, CAT, and GR and nonenzymatic components such as GSH and vitamin E (Loch-Caruso et al., 2005; Aaron et al., 2015). Therefore, when oxidative stress develops as a pathologic event, the defense system promotes an upregulation of the enzymatic gene/protein expression and the production of nonenzymatic antioxidants (McEligot et al., 2005). The polysaccharides, polysaccharide-peptide complex, triterpenes and phenolic components of *G. lucidum* exhibit antioxidant properties (Mehta, 2014; Kan et al., 2015). *G. lucidum* glucans perform in food as scavengers of free radicals and restrain lipid peroxidation, concurrently stimulating interferone production in human blood cells (Giavasis, 2014). Triterpenes of *G. lucidum* exhibit antioxidative characteristics in vitro and can decrease oxidative stress by scavenging free radicals generated in cells (Bhardwaj et al., 2016). Triterpenes also increase the actions of antioxidant enzymes and diminish radiation-induced oxidative DNA injury in mice splenocytes (Smina et al., 2011a). Indeed, supplementation of SSGL in the diets significantly increased the T-AOC, CAT, GR and GSH in the liver and spleen of the broilers in this study, suggesting that the antioxidant defense system was stimulated in broilers after receiving SSGL. The stimulation was related to the concentration of SSGL in the diet. Similar results are also reported also in the hippocampus of rats (Zhou et al., 2012), the liver of mice and the blood of patients with menopausal symptoms (Chung and Tong, 2005). Because of component complexity in SGL and SSGL, it is difficult to attribute these protective effects to specific ingredients or their combination. The contents of triterpenoids, polysaccharides, and α-tocopherol in the SSGL sample of this study were 4.55%, 7.98%, 36.9 IU/kg respectively, so the addition of 100 mg SSGL/kg in diets meant the dosage of 3 active ingredients were 9.1 mg/kg, 16.0 mg/kg and 0.007 IU/kg, respectively. The relative amount of α-tocopherol from SSGL supplementation to total diet content (>20 IU VE/kg, noted under Table 1) was so low, so its effect could be negligible in this study. Although the mechanism by which SSGL promote the antioxidant defense system is not very clear currently, the polysaccharides might play an important role in the promotion because of the linear response of the hepatic activities of GSH-Px and SOD to the levels of *G. lucidum* polysaccharides in diets (Zhao et al., 2012).

4.4. Serum immune parameters of broilers

Pharmacologically active compounds from *G. lucidum* augment the proliferation and maturation of T and B lymphocytes, dendritic cells, natural killer (NK) cells and splenic mononuclear cells (Zhu et al., 2007; Ma et al., 2008). *G. lucidum* polysaccharides (β-D-glucans) lead to changes in the activities of macrophages, T-helper cells, NK cells and other effector cells using lymphocyte surfaces through specific receptors or serum specific proteins (Wang et al., 1997; Lin and Zhang, 2004; Ishimoto et al., 2017). Polysaccharides of *G. lucidum* also increase the contents of DNA and RNA and change the cell ultrastructure in murine splenocytes (Jedinak et al., 2011). The IL-2 is secreted primarily by Th1 cells and plays a central role in the activation of T cell-mediated immune response (Shen et al., 2012). The polysaccharides from *G. lucidum* induced the expression of IL-2, IL-17, IL-10 and interferon-γ (IFN-γ) in in vitro culture of human peripheral blood mononuclear cells (Habijanic et al., 2015). Pan et al. (2013b) reported that *G. lucidum* polysaccharides significantly reduced the levels of serum IL-6 and tumor necrosis factor α (TNF-α), and increased the levels of serum IL-2, IL-4 and IL-10. Ma et al. (2008) also observed that the polysaccharides from SGL could induce the expression of IL-12, TNF-α and cell proliferation in mouse splenic mononuclear cells. In this study, IL-2 level in SSGL-treated groups also increased. Additionally, a strong linear relationship was found between IL-2 level in serum and SSGL concentration in the diets.

Zhang et al. (2002) reported that a bioactive *G. lucidum* immunomodulating substance isolated from the fruiting body stimulates the proliferation, activation and differentiation of B lymphocytes. Serum immunoglobulins, secreted by B cells, are the major effective molecules and might moderately reflect the actual humoral immunity (Bao et al., 2001). In our previous study, the addition of SSGL significantly increased the concentrations of serum IgA and IgG of broilers fed AFB_1-contaminated diets, although it did not change those fed uncontaminated diets (Liu et al., 2016a). The concentrations of serum IgA and IgG in the present study increased significantly in the broilers fed the diets containing 200 and 500 mg SSGL/kg feed. These results are consistent with those previously observed in mice (Liu et al., 2016b), in pigs (Li et al., 2015) and in broilers (Liu et al., 2016a).

5. Conclusions

The results from this study clearly demonstrated that SSGL is effective in improving growth performance, increasing ROS scavenging activity and strengthening the antioxidant defense system and immune system of broilers. Thus, supplementation of SSGL in the poultry diet may be of great benefit to the poultry industry. However, the beneficial effects could be associated with the feeding stage and the dietary level of SSGL, which require future tests. Because of component complexity in SSGL, our present study has limitations to explain the mechanism, and further studies using single component are still needed.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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