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Effects of dietary *Astragalus mongholicus*, *Astragalus* polysaccharides and *Lactobacillus* on growth performance, immunity and antioxidant status in *Qingjiaoma* finishing broilers

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Abstract: Three hundred 21-day-old male *Qingjiaoma* broilers were randomly assigned to six groups to investigate the effect of dietary stems and leaves of *Astragalus mongholicus* (AMSLs), *Astragalus* polysaccharides (APSs), *Lactobacillus* (Lac) and their combinations on finishing broilers in a 42-day feeding experiment. Supplementary 1% AMSLs, 1 000 mg/kg APSs and 4.5×10^{10} CFU/kg Lac improved significantly growth performance. Dietary AMSLs, APSs and Lac increased the serum concentrations of immunoglobulins IgA, IgG and complements C3 and C4. Furthermore, AMSLs increased glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and total antioxidation capacity (T-AOC) and decreased the malondialdehyde (MDA) content in both serum and liver tissue. Compared with individual supplementation, the combination of Lac and AMSLs or APSs increased serum IgA, IgG, C3 and C4 concentrations. The combination of AMSLs and Lac increased serum GSH-Px activity and reduced the MDA content in the serum and liver tissue. These results suggested that AMSLs, APSs and Lac are beneficial feed additives, and the applications of combined Lac and AMSLs or APSs could synergistically improve immunity in broilers.

Keywords: medicinal plant; poultry; performance trait; immunity; probiotics

In recent years, the research and utilization of feed additives, such as probiotics and medicinal plants, have received extensive attention in the animal production industry. Medicinal plants and their extracts, such as *Astragalus*, *Ginseng* and *Lycium barbarum* polysaccharides, have been found to improve metabolism and enhance immunity. *Astragalus* species and their active components as feed additives are very promising (Che et al. 2019). The roots of *Astragalus membranaceus* (AMP) are widely used as a main component in many traditional Chinese herbal formulations to prevent and treat a variety

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of diseases owing to their immunomodulation, antioxidant and anti-inflammatory effects (Dong et al. 2022). Several studies confirmed that AMP supplementation in diets increased average daily gain (ADG), feed conversion ratio (FCR) and immunity in broilers (Wang et al. 2010). The stems and leaves of *Astragalus mongholicus* (AMSLs), by-products of *Astragalus mongholicus* (AM) processing, also contain the main active components of AM. A few studies have shown that AMSLs exert positive effects on the growth and physiological function of animals. AMSL supplementation was used to prevent diarrhoea and improve nutrient digestibility, growth and immunity in weaned pigs (Che et al. 2019). It was found that adding AMSLs to diets at an appropriate level improved growth performance, immunity and antioxidant status in quails (Guo et al. 2019). However, further research is needed to confirm the effect of AMSL supplementation on broilers. As aerial parts after harvesting the roots of *Astragalus* plants, AMSLs are usually discarded as waste, which results in a waste of resources and environmental problems. If AMSLs could be widely used as a feed additive, it would be very beneficial to animal production and the environment.

Astragalus polysaccharides (APSs), astragalosides and more than a hundred other chemical compounds have been found in AM (Tang and Huang 2022). APSs are a general term for all polysaccharides extracted from AM, and its pharmacological functions have been revealed. APSs are used as immunomodulators to treat diarrhoea, common colds, fatigue and anorexia (Li et al. 2020). APSs extracted from AMP promoted recovery of the intestinal barrier and alleviated intestinal inflammation in colitis rats (Zhao et al. 2014), and reduced cytotoxicity and promoted the proliferation of splenic lymphocytes in mice (Xu et al. 2019). APSs alleviated inflammation via the NF-κB/MAPK signalling pathway in porcine intestinal epithelial cells and in mice (Dong et al. 2020). However, belonging among the active components in AM, there is little knowledge about whether APSs have the same effects as AMP or AMSLs in broilers.

Probiotics are live microorganisms that, when supplemented in adequate amounts, confer health and growth benefits to host animals. The roles of probiotics in digestion, modulation of the intestinal microbiome and barrier integrity, and meat quality of broilers have been reviewed (Neveling and Dicks 2021). There is increasing interest in probiotics as alternatives to antibiotics in animal feeds. Supplementary probiotics exerted positive effects on growth and feed conversion in broiler chickens (Amerah et al. 2013; Humam et al. 2019). *Lactobacillus* (Lac) is one of the most widely studied and used probiotics. A number of studies have shown that supplementing Lac could improve body weight (BW) gain, feed conversion, immune function and antioxidant status (Humam et al. 2019; Yulianto et al. 2020); alleviate intestinal impairments (Yang et al. 2020); resist *Listeria monocytogenes* infection (Deng et al. 2020); and prevent subclinical necrotizing enteritis, *Campylobacter jejuni* enteritis and other poultry diseases (Saint-Cyr et al. 2017). However, most of these studies focused on fast-growing broiler chickens, and the data on the effect of probiotics on slow- and medium-growing broilers are very limited to date. Therefore, the use of probiotics in poultry feed is still far from common practice (Neveling and Dicks 2021). This study first evaluated the effect of AMSLs, APSs and Lac on growth performance, antioxidant status, immune function and meat quality of *Qingjiaoma* broilers, a medium-growing broiler strain that is very popular locally due to the chewy texture of the meat, then investigated the potential benefits of the combination of Lac and AMSLs or APSs and discusses whether APSs contribute to the effects of AMSLs.

MATERIAL AND METHODS

Experimental diet preparation

The AMSLs were shattered and screened. The contents of APSs, flavonoids and triterpenoid saponins in the AMSL powder were 26.05, 1.23 and 0.91 mg/g dry matter (DM), respectively (measured value). The APSs, flavonoids and triterpenoid saponins were separately extracted from AMSL by water soluble, ethanol/water bath and ethanol/water bath/*n*-butanol extraction, and the contents in extracts were measured using colorimetry (Zhang et al. 2016). The APSs (APSs were prepared from AM using decoction extraction and ethanol precipitation methods; Realcan Pharmaceutical Co., Ltd, Yantai, China; purity was up to 98%) and the Lac (*Lactobacillus plantarum*; Luoxing Biology Studio, Shanghai, China; 3×10^9 CFU/g) used in the present study were commercial products.

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The dietary treatments were as follows: (1) control (basal diet containing 1% lucerne meal), (2) AMSL treatment (basal diet containing 1% AMSLs instead of 1% lucerne meal), (3) APS treatment (APSs 1 000 mg/kg in the basal diet), (4) Lac treatment (basal diet supplemented with Lac at 4.5×10^{10} CFU/kg), (5) AMSL + Lac treatment (basal diet with 1% AMSLs instead of 1% lucerne meal, supplemented with 4.5×10^{10} CFU/kg Lac), and (6) APS + Lac treatment (basal diet with both 1 000 mg/kg APSs and 4.5×10^{10} CFU/kg Lac). The basal diet (Table 1) was formulated according to the Broiler Feeding Standard in China (NY/T33-2004). The crude fibre, metabolic energy and crude protein contents in all diets were the same, and no antimicrobial agents were administered.

Table 1. Composition and nutrient levels of the basal diet (on air-dry basis)

Items	Composition
Ingredients (%)	
Corn	63.5
Soybean meal	24.0
Rapeseed meal	3.0
Wheat bran	1.0
Alfalfa meal	1.0
Soybean oil	5.0
Calcium diphasic phosphate	1.2
Calcium carbonate	0.6
NaCl	0.3
Premix[1]	0.4
Total	100.0
Calculated nutrient levels (%)	
ME (MJ/kg)	13.11
Crude protein	18.05
Calcium	0.74
Total P	0.61
Available P	0.40
Lysine	0.86
Methionine	0.28
Methionine + Cysteine	0.61

[1]The premix provided the following per kg of the diet: Fe (as ferrous sulphate) 50 mg, Cu (as copper sulphate) 9 mg, Zn (as zinc sulphate) 40 mg, Mn (as manganese sulphate) 66 mg, vitamin A 8 000 IU, vitamin D_3 1 000 IU, vitamin K_3 1 mg, vitamin B_1 2 mg, riboflavin 5.5 mg, nicotinic acid 50 mg, D-pantothenic acid 12 mg, vitamin B_{12} 0.6 mg, folic acid 0.55 mg, vitamin B_6 2.5 mg, biotin 0.15 mg, choline chloride 1 000 mg

Birds, housing and management

Three hundred 21-day-old male *Qingjiaoma* broilers obtained from a local commercial farm were used in this experiment. All chickens were reared in a single room on the poultry farm of Northwest Minzu University, Gansu, China. This room contained six floor pens, each measuring 300 × 350 cm. Each pen had solid plastic white walls and was divided by wire mesh into 10 compartments. Compartments were equipped with a round feeder pan (diameter = 30 cm) and one nipple drinker. Cork shavings were used as litter, and litter was replaced every three days.

Broilers were randomly allotted into six dietary groups, with 10 replications for each group, five birds per replication. Each group was allocated to one of the six dietary treatments described above. The average high and low ambient temperatures recorded during the experimental period were 23.6 °C and 21.4 °C, respectively. The photoperiod comprised natural lighting during the entire 42-day period. Feed and water were provided *ad libitum*.

All procedures involving animals were performed following the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China) and were approved by the Animal Care and Use Committee of Northwest Minzu University.

Growth performance

The BW of broilers was measured on day 0, 21 and 42 of the trial after fasting for 12 h, and the feed intake on a compartment basis was recorded. ADG, average daily feed intake (ADFI) and FCR were calculated. Mortality was recorded as it occurred.

Sample collection

After feed deprivation for 12 h at the end of the experiment, 20 broilers (two broilers per compartment) from each treatment were randomly selected, 5 ml of blood samples were taken via the wing vein per individual, and serum was collected to analyse

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immune and antioxidant parameters. After that, the broilers were killed and dissected immediately, and internal organs were weighed and expressed as relative organ weight to the live BW. Meat of the left breast and leg and liver tissues were sampled.

Meat analysis

The colour, pH and cooking loss 24 h post-mortem of the breast and thigh meat were evaluated. The meat colour was measured by the CIE (Commission International d'Eclairage) system [Hunter-L* (lightness), a* (redness), and b* (yellowness) values] with a chroma meter (CR-400; Minolta Co., Ltd, Tokyo, Japan). An average of three readings from the surface of the medial muscles that were free of colour defects, bruising and haemorrhages was used for colour evaluation. The pH was measured at three sites in the breast and thigh muscles by using a pH meter (HI8424C; HANNA Instrument, Ronchi, Italy). The pH probe was inserted at an angle of 45 degrees into the muscle and washed with sterile water between samples. Each sample was analyzed three times at various points, and the average values were used. For determination of the shear force, samples of skinless breast and thigh meat were first cooked to an internal temperature of 70 °C and then assessed with a C-LM3B shear apparatus (Northeast Agricultural University, Harbin, China). Muscle samples were weighed and measured using a computer water loss rate device (model RH-1000; RunHu Co., Guangzhou, China), in which a 2.25-cm diameter sample was pressed at a stable 35-kg force for 5 min and weighed. After trimming and weighing, the muscle sample was steam cooked for 30 min on a 1 500 W electric stove and cooled for 2 h; the percentage between the cooked weight and the raw weight was used to calculate cooking loss.

Determination of antioxidant status and immunity

Nine times cooled cold saline was added to 0.5 g of liver samples, which were ground in a glass homogenizer to prepare a 10% tissue homogenate. The homogenate was centrifuged at 2 000 rpm for 15 min, and the supernatant was preserved at –20 °C. The supernatant and serum samples were analyzed for glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

Serum immunoglobulins (IgA, IgG, and IgM) and complements (C3 and C4) were determined using chicken-specific ELISA kits following the manufacturer's protocol (Shanghai Jianglai Industrial Limited By Share Ltd, Shanghai, China).

Statistical analysis

All data were statistically analyzed by one-way ANOVA using SPSS software v19.0 (IBM Corp., Armonk, NY, USA). Duncan's multiple range test was used for multiple comparisons when significant differences were found. $P < 0.05$ was considered a significant difference.

RESULTS

Growth performance

The broilers were good in health without disease or death during the experiment. The growth performance of broilers was affected by supplementation with Lac, AMSLs or APSs in diets (Table 2). The ADG and FCR in the birds fed diets supplemented with AMSLs, APSs, Lac, AMSLs + Lac and APSs + Lac were significantly superior to those in the control group ($P < 0.05$), but there were no significant differences between the different supplementary diets ($P > 0.05$). The final body weight (FBW) of broilers fed supplementary diets was higher than that in the control group, and FBW was significantly better for the AMSLs + Lac group compared to all other groups ($P < 0.001$). No difference in the ADFI across treatments was observed ($P > 0.05$).

Relative organ weights

The relative weights of the kidney, heart, liver, pancreas, gallbladder, spleen and bursa of Fabricius did not differ between the dietary groups ($P > 0.05$) (Table 3). However, Lac, AMSLs + Lac or APSs

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Table 2. Effects of dietary AMSLs, APSs and Lac on growth performance of broilers after 42-day feeding

	Control	AMSLs	APSs	Lac	AMSLs + Lac	APSs + Lac	SEM	*P*-value
IBW (g)	504.1	510.8	509.2	505.5	496.6	506.4	30.813	0.951
FBW (g)	2 209.3[a]	2 363.0[b]	2 342.3[b]	2 349.3[b]	2 380.3[c]	2 377.3[b]	104.167	< 0.001
ADFI (g/day)	115.71	117.07	116.28	115.90	118.38	118.81	6.183	0.065
ADG (g/day)	40.6[a]	44.1[b]	43.6[b]	43.9[b]	44.9[b]	44.5[b]	3.233	0.035
FCR	2.85[a]	2.65[b]	2.66[b]	2.64[b]	2.63[b]	2.67[b]	0.170	0.048

ADFI = average daily feed intake; ADG = average daily gain; AMSLs = stems and leaves of *Astragalus mongholicus*; APSs = polysaccharides of *Astragalus mongholicus*; FBW = final body weight; FCR = feed conversion ratio; IBW = initial body weight; Lac = *Lactobacillus plantarum*; SEM = standard error of means

[a–c]Different superscripts within a row indicate significantly different means ($P < 0.05$)

Table 3. Effects of dietary AMSLs, APSs and Lac on relative organ weight in broilers after 42-day feeding (g/100 g of live body weight)

	Control	AMSLs	APSs	Lac	AMSLs + Lac	APSs + Lac	SEM	*P*-value
Dressed weight	85.23	83.15	85.15	84.45	83.69	82.89	3.873	0.781
Abdominal fat	1.54[a]	1.55[a]	1.58[a]	1.69[b]	1.65[b]	1.71[b]	0.297	0.048
Kidney	0.59	0.60	0.60	0.53	0.56	0.56	0.050	0.862
Heart	0.42	0.44	0.44	0.38	0.39	0.39	0.033	0.081
Liver	1.63	1.60	1.60	1.53	1.58	1.58	0.242	0.061
Pancreas	0.15	0.15	0.15	0.14	0.15	0.15	0.017	0.117
Gallbladder	0.13	0.11	0.11	0.12	0.13	0.10	0.013	0.142
Bursa of Fabricius	0.23	0.23	0.23	0.19	0.20	0.20	0.013	0.093
Spleen	0.15	0.13	0.13	0.12	0.14	0.14	0.013	0.104

AMSLs = stems and leaves of *Astragalus mongholicus*; APSs = polysaccharides of *Astragalus mongholicus*; Lac = *Lactobacillus plantarum*; SEM = standard error of means

[a,b]Different superscripts within a row indicate significantly different means ($P < 0.05$)

+ Lac supplementation increased the relative abdominal fat weight of the birds compared with the control ($P < 0.05$).

Meat quality

The effects of Lac, AMSLs and APSs on meat quality traits, including muscle colour, pH and shear stress, were investigated (Table 4). The results showed no significant effects of the different supplements on the pH values, L* or cooking loss in either breast or thigh muscle ($P > 0.05$). The shear stress of thigh muscle decreased after supplementation with Lac, AMSLs + Lac and APSs + Lac compared with the control ($P < 0.05$), although that of breast muscle did not differ between treatment groups. The b* of thigh and breast meat was significantly decreased by AMSL and AMSL + Lac supplementation ($P < 0.05$). The a* of breast and thigh muscles was significantly increased by AMSL + Lac supplementation ($P < 0.05$).

Immune and antioxidant properties

The effects of diets on serum Ig concentrations in the broilers are shown in Table 5. Compared with the control, the serum contents of IgA, IgM, IgG, C3 and C4 were increased significantly when AMSLs or APSs were supplemented in the diet ($P < 0.05$). The serum contents of IgA, IgG, C3 and C4, but not IgM, were significantly increased by Lac supplementation in the diet ($P < 0.05$). These Ig concentrations were affected more by dietary AMSL or APS supplementation than by Lac supplementation. Combinations of Lac and AMSLs or APSs exerted synergistic effects in increasing

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Table 4. Effects of dietary AMSLs, APSs and *Lac* on meat quality of broilers after 42-day feeding

	Control	AMSLs	APSs	Lac	AMSLs + Lac	APSs + Lac	SEM	*P*-value
Breast muscle								
L* (lightness)	47.01	45.73	47.37	45.77	46.64	46.81	2.227	0.055
a* (redness)	6.66^{a}	7.09^{a,b}	6.92^{a,b}	7.04^{a,b}	7.48^{b}	6.89^{a,b}	0.650	0.048
b* (yellowness)	17.67^{a}	16.19^{b}	17.90^{a}	17.67^{a}	16.4^{b}	17.45^{a}	1.198	0.035
Shear stress (kgf)	6.13	6.01	6.20	6.17	6.16	6.19	0.457	0.784
pH	6.13	6.10	6.17	6.02	6.23	6.14	0.175	0.101
Cooking loss (%)	32.09	32.46	29.28	32.87	30.73	31.55	1.553	0.815
Thigh muscle								
L* (lightness)	52.73	52.82	52.84	53.37	53.67	53.90	2.415	0.787
a* (redness)	7.63^{a}	7.87^{a}	7.58^{a}	7.77^{a}	8.36^{b}	7.66^{a}	0.572	0.049
b* (yellowness)	17.39^{a}	16.29^{b}	17.41^{a}	17.33^{a}	16.73^{b}	17.67^{a}	0.698	0.019
Shear stress (kgf)	6.67^{a}	6.53^{a,b}	6.73^{a}	6.21^{b}	6.33^{b}	6.25^{b}	0.533	0.036
pH	6.33	6.20	6.43	6.38	6.39	6.51	0.183	0.351
Cooking loss (%)	30.99	31.89	29.13	32.04	31.39	30.81	1.648	0.827

AMSLs = stems and leaves of *Astragalus mongholicus*; APSs = polysaccharides of *Astragalus mongholicus*; Lac =*Lactobacillus plantarum*; SEM = standard error of means

^{a,b}Different superscripts within a row indicate significantly different means ($P < 0.05$)

Table 5. Effects of dietary AMSLs, APSs and Lac on serum immunoglobulin and complement levels of broilers after 42-day feeding

	Control	AMSLs	APSs	Lac	AMSLs + Lac	APSs + Lac	SEM	*P*-value
IgA (μg/ml)	78.23^{a}	104.64^{b}	99.73^{b}	92.99^{b}	133.65^{c}	116.73^{d}	3.767	0.024
IgM (μg/ml)	223.85^{a}	301.11^{b}	335.80^{c}	230.11^{a}	286.53^{b}	381.29^{d}	9.432	0.008
IgG (μg/ml)	643.21^{a}	986.07^{b}	948.89^{b}	875.98^{c}	1 139.56^{d}	1 145.15^{d}	17.350	0.005
C3 (μg/ml)	59.16^{a}	159.68^{c}	168.39^{c}	143.46^{b}	204.21^{e}	197.88^{d}	3.992	< 0.001
C4 (μg/ml)	43.98^{a}	67.88^{c}	70.45^{c}	55.33^{b}	84.23^{e}	78.10^{d}	1.727	0.017

AMSLs = stems and leaves of *Astragalus mongholicus*; APSs = polysaccharides of *Astragalus mongholicus*; C3 = complement 3; C4 = complement 4; Lac = *Lactobacillus plantarum*; SEM = standard error of means

$^{a–e}$Different superscripts within a row indicate significantly different means ($P < 0.05$)

the contents of IgM, IgG, C3 and C4 compared with AMSL, APS or Lac supplementation alone.

The effects of diets on antioxidant property in the broilers are shown in Table 6. The activities of GSH-Px and the T-AOC and MDA contents in both serum and liver tissue and SOD activity in liver tissue were not affected by Lac, APS or APS + Lac supplementation in diets ($P > 0.05$). However, compared with the control, supplementation with AMSLs, APSs, AMSLs + Lac, APSs + Lac increased the SOD activity in serum and supplementation with AMSLs and AMSLs + Lac increased the SOD activity in liver tissue ($P < 0.05$). Supplementation with AMSLs or AMSLs + Lac significantly enhanced the activities of GSH-Px, increased the content of T-AOC, and reduced the content of MDA in both serum and liver tissue compared with the control ($P < 0.05$). Moreover, the combination of AMSLs and Lac increased GSH-Px activity in serum and T-AOC in the liver tissue and reduced the MDA content in serum and in the liver tissue compared with AMSL or Lac supplementation alone ($P < 0.05$).

DISCUSSION

The effects of AMP/AMSL and APS supplementation on the immune function, growth performance and the antioxidant status of animals have been reported (Wang et al. 2010; Guo et al. 2019; Li

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Table 6. Effects of dietary AMSLs, APSs and Lac on antioxidant capacity of broilers after 42-day feeding

	Control	AMSLs	APSs	Lac	AMSLs + Lac	APSs + Lac	SEM	*P*-value
Serum								
GSH-Px (IU/ml)	186.95^{a}	233.75^{b}	184.56^{a}	191.44^{a}	251.26^{c}	183.09^{a}	13.892	0.009
SOD (IU/ml)	11.48^{a}	14.11^{b}	13.87^{b}	12.21^{a}	14.23^{b}	14.03^{b}	0.361	0.033
T-AOC (IU/ml)	21.84^{a}	30.36^{b}	19.36^{a}	20.42^{a}	32.35^{b}	22.04^{a}	3.180	< 0.001
MDA (nmol/ml)	10.13^{c}	8.06^{b}	8.90^{c}	9.89^{c}	6.37^{a}	9.34^{c}	0.738	0.037
Liver tissue								
GSH-Px (IU/mg)	19.46^{a}	24.75^{b}	19.75^{a}	18.21^{a}	24.46^{b}	18.32^{a}	1.643	0.021
SOD (IU/mg)	20.55^{a}	29.35^{b}	22.14^{a}	21.68^{a}	34.05^{b}	21.74^{a}	1.553	0.006
T-AOC (IU/mg)	19.84^{a}	30.66^{b}	22.66^{a}	19.65^{a}	37.16^{c}	21.16^{a}	2.497	0.004
MDA (nmol/mg)	2.13^{c}	1.37^{b}	1.91^{c}	2.06^{c}	1.16^{a}	1.95^{c}	0.305	0.045

AMSLs = stems and leaves of *Astragalus mongholicus*; APSs = polysaccharides of *Astragalus mongholicus*; GSH-Px = glutathione peroxidase; Lac = *Lactobacillus plantarum*; MDA = malondialdehyde; SEM = standard error of means; SOD = superoxide dismutase; T-AOC = total antioxidation capacity

$^{a–c}$Different superscripts within a row indicate significantly different means ($P < 0.05$)

et al. 2020). AMSLs supplemented at a concentration of 1–5% or AMP at 1% in the diet increased the daily gain and FCR in quails, but there was no positive correlation between the AMSL concentration and growth performance due to the high crude fibre content in AMSLs (Guo et al. 2019). Diets containing 5% and 7.5% AMSLs increased the ADFI, ADG and FCR, but 2.5% AMSLs had no effect in weaned piglets (Che et al. 2019). It was found that APS supplementation at 350 mg/kg or 200 mg/kg did not affect the growth performance of broilers (Chen et al. 2003; Wang et al. 2010). In contrast, Wang et al. (2006) observed that APSs exerted positive effects on growth and feed efficiency when added at concentrations of 245 mg/kg and 370 mg/kg in broiler diets. Other studies showed that 500–2 000 mg/kg APSs in diets improved BW gain and FCR in broilers (Yin et al. 2009; Wu 2018). It appears that the effects of APSs on the growth of animals depend on the concentration of APS supplementation. In the present study, the crude fibre content was standardized in all diets. The ADG in broilers fed diets supplied with 1% AMSLs or 1 000 mg/kg APSs was significantly increased, although the ADFI was not improved. The content of APSs in AMSLs in this study was 26.05 mg/g DM, which was similar to the value of 27.04 mg/g DM reported by Zhang et al. (2016), and the concentration of APSs in the diet supplemented with 1% AMSLs was approximately 260 mg/kg. The effects of APSs supplied at a concentration of 1 000 mg/kg on the growth performance in broilers were similar to those of 1% AMSLs, suggesting that the influence of AMSLs on the growth performance of broilers was at least partly due to APSs. Undeniably, multiple bioactive substances in AMSLs with antioxidative (Yu et al. 2009), immunomodulatory (Qiu and Cheng 2019), anti-inflammatory (Dong et al. 2019) and antimicrobial activities (Guo et al. 2019) might also be involved in the beneficial effects on the broilers.

A number of studies have shown that BW gain and feed conversion were improved by supplementing Lac or a mixture of Lac strains in broiler diets (Kalavathy et al. 2003; Humam et al. 2019; Deng et al. 2020; Yulianto et al. 2020). However, others reported that there were no positive effects on the performance of broilers when the *L. plantarum* was supplied in feed (Wang et al. 2017; Song et al. 2022a), and dietary supplementation with *L. plantarum* did not improve the growth performance of broilers challenged with *E. coli* O78 (Ding et al. 2019). Although the controversial results from different studies could be due to differences in the viability and concentrations of bacteria used and health status of broilers, bird age may also play a role. In fact, it was observed that the beneficial effect of *L. plantarum* B1 on broilers occurred during the finisher period (Peng et al. 2016). In contrast, some studies found that dietary Lac improved ADG and FCR during the 1- to 21-day period but had no effect during the 22 to 42-day period (Wu et al. 2019a). In this study, 21-day-old *Qingjiaoma* broilers were used, and it was demonstrated that Lac

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supplemented in diets increased the growth performance and decreased the FCR after 21 days of age. The ADFI was not influenced by Lac supplementation, which was in accordance with a previous study (Shokryazdan et al. 2017).

Regarding the meat quality, the addition of AMSLs did not affect the cooking loss, pH, shear force, L* and a* values in breast and thigh muscle, while the b* value was decreased, suggesting that dietary AMSLs had a positive effect on the meat quality of broilers, although the effect was weak. Furthermore, supplementary APSs had no effect on meat quality. The effect of probiotics in improving the meat quality of broilers is controversial (Neveling and Dicks 2021). Some studies have noted that probiotics could improve meat characteristics, such as tenderness, colour, sensory properties and microbial safety (Bai et al. 2017; Khan et al. 2018), whereas another study reported that probiotics did not affect the meat quality in broilers (Kim et al. 2016). In this study, supplementary Lac decreased the shear stress of thigh muscle, but the other meat quality indices were not changed. Furthermore, the abdominal fat weight was increased after supplementation with Lac, which conflicts with the result reported by Kalavathy et al. (2003), who found that supplying Lac in diets reduced abdominal fat deposition in broilers. Although the mechanisms underlying these disparate results are complex, the variation in meat colour might be related to the probiotic strains used.

This study also found that both AMSLs and APSs enhanced the immune response of broilers by significantly increasing the levels of IgA, IgM, IgG, C3 and C4 in serum. The immunoglobulin is a kind of protein with antibacterial and antiviral functions that reinforces cell phagocytosis and dissolves or kills pathogenic microorganisms synergistically with C3 and C4 (Fassbinder-Orth et al. 2016). IgG, IgM and IgA are three major Igs in avian species, and their content in serum can reflect the immune status of animals. The theory is that the higher the content, the stronger the immunity. Increased secretion of C3 and C4, two important components of the complement system, indicates intensive immunity and enhanced disease resistance (Delanghe et al. 2014). The results herein were consistent with those of previous studies in which supplying AMSLs or APSs in the diet enhanced immunity in quails by promoting the development of the thymus and bursa of Fabricius and by increasing the levels of Igs and complements (Wu 2018; Guo et al. 2019). Other studies on chickens also reported that AMSL or APS supplementation enhanced the immune function by increasing antibody titres and the levels of IFN-γ and IL-2, and improved immunity against H5N1 avian influenza virus (Xi et al. 2014; Abdullahi et al. 2016). Furthermore, the intestinal mucosal immune function and immune organ index of the thymus were strengthened by APSs (Shan et al. 2019). The developments of the spleen, thymus and bursa of Fabricius directly impacted the immune function (Cheng et al. 2017). However, neither AMSL nor APS supplementation affected the relative weight of these immune-related organs and other organs of the broilers in the present study. The differences in bird age might explain these disparate results. One-day-old birds were used in the aforementioned studies (Xi et al. 2014; Abdullahi et al. 2016; Guo et al. 2019), and 21-day-old broilers were employed in this study.

It was observed that probiotics supplemented in diets did not alter the blood immunity level of broilers (Amerah et al. 2013). In contrast, numerous studies have documented that dietary Lac improves the serum content of Igs in broilers (Shen et al. 2014; Wu et al. 2019a). Our results showed that Lac supplementation increased the serum contents of IgA, IgG, C3 and C4, suggesting that Lac improved humoral immunity by promoting the synthesis of Igs in broilers. Recently, it was reported that *L. plantarum* had the potential to protect intestinal morphology, integrity and barrier function (Yang et al. 2020) and reduced the morphological changes in the intestine and oxidative stress due to its high antioxidant activity in broilers challenged with deoxynivalenol (de Souza et al. 2020).

The antioxidant system in animals eliminates free radicals that attack biological membranes to form lipid peroxides and plays an important role in preventing diseases. GSH-Px, SOD and other antioxidant enzymes are important components of the antioxidant system and clear reactive oxygen species (ROS) produced in the body and reduce MDA, an end product of lipid peroxidation contributing to tissue damage and thus leading to the development of diseases (Shen et al. 2014; Bai et al. 2017). In this study, the activities of SOD and GSH-PX and T-AOC levels in the liver tissue and serum were increased, and the MDA concentration was decreased when AMSLs were added to the diet, indicating

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an improved antioxidant status. Previous studies showed that AMSL/AMP supplementation improved the serum antioxidant status by increasing GSH-PX activity and the T-AOC (Guo et al. 2019). Juvenile broilers fed diets supplemented with APSs exhibited increased GSH-PX and SOD activities and decreased MDA levels after 42 days (Wu 2018). However, Wang et al. (2010) reported that APSs enhanced the T-AOC and GSH-PX activity only in broilers aged 1–21 days and not in those aged 21–42 days. In this study, supplementation of APSs had no influence on the antioxidant status of broilers over the age of 21 days. It was also indicated that the antioxidant activity of APSs was weaker than that of AMP and that flavonoids or saponins from AMP might play a greater role in scavenging free radicals than APSs (Yu et al. 2009).

L. plantarum supplementation in broiler diets improved the antioxidant capacity by increasing the activities of GSH-Px in serum and liver tissue and decreasing the content of MDA in serum (Shen et al. 2014; Wu et al. 2019b). Dietary supplementation with *L. plantarum* enhanced the antioxidant capacity of broilers to a certain extent (Song et al. 2022b). In contrast, in this study, supplementary Lac in the diets had no effect on the antioxidant capacity of broilers, which was consistent with the result reported by Yu et al. (2022). Whether these variations are related to age or the type of broiler needs to be further studied.

In view of the biological function of AMSLs and APSs, we were especially interested in the effects of a combination of Lac and AMSLs or APSs on the growth and health of broilers. To our knowledge, the effects of such combinations in broilers have rarely been reported. Li et al. (2009) reported that probiotics (lactobacilli and *Bacillus cereus*) combined with APS supplementation in feed had synergistic effects on the immunity in chicks. The present study found that Lac combined with AMSLs or APSs in diets increased ADG and relative abdominal fat weight, decreased FCR and the shear stress of thigh muscle, but there were no significant differences compared with AMSLs, APSs or Lac supplementation alone, suggesting that their combinations had no synergistic effect in improving the growth performance and meat quality of broilers. Nevertheless, compared with AMSLs, APSs or Lac supplementation alone, the combination of Lac and AMSLs or APSs significantly increased IgA, IgG, C3 and C4 concentrations in the serum of broilers, and the combination of APSs and Lac increased IgM, although there was no difference in IgM between the AMSLs and the combined AMSLs and Lac treatments. These results suggested that their combination could improve the immunity of broilers more effectively. The combination of AMSLs and Lac in the diet increased serum GSH-Px activity and liver T-AOC levels and reduced the MDA content in serum and liver tissue compared with AMSLs and Lac supplementation alone.

CONCLUSION

In conclusion, dietary supplementation with AMSLs, APSs or Lac improved the growth performance and immunity without adverse impacts on meat quality attributes, and AMSLs enhanced antioxidant capacity in broilers. The applications of combined AMSLs or APSs and Lac synergistically improve immunity in broilers. These results suggested that AMSLs, APSs and their combinations with Lac were safe and effective as feed additives in the poultry industry.

Conflict of interest

The authors declare no conflict of interest.

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