Effects of *Achyranthes bidentata* polysaccharides on performance, immunity, antioxidant capacity, and meat quality in Pekin ducks

X. Ao*† and I. H. Kim*,[1]

*Department of Animal Resource and Science, Dankook University, Cheonan, Chungnam 330-714, South Korea; and †Swine institute, Tie Qi Li Shi Group. Co., Mianyang, Sichuan, 621006, P. R. China

ABSTRACT This study was conducted to evaluate the effects of *Achyranthes bidentata* polysaccharide (**ABP**) on growth performance, antioxidant capacity, immune function, relative organ weight, ileal microflora, and meat quality in Pekin ducks. A total of 1,200 female 1-day-old Pekin ducklings (51.2 ± 0.2 g) were blocked based on body weight (**BW**) and randomly allocated into 3 treatments with 10 replicates of 40 birds each. The experiment lasted for 6 wk, and dietary treatments included corn–soybean meal–based diet supplemented with 0, 0.02, and 0.04% ABP. The supplementation of ABP increased ($P < 0.05$) body weight gain (**BWG**) and final BW linearly during day 22 to 42 and day 1 to 42, respectively, but decreased ($P < 0.05$) feed-to-gain ratio (**F/G**) linearly during day 22 to 42 and day 1 to 42. The inclusion of ABP increased ($P < 0.05$) serum superoxide dismutase, glutathione peroxidase, total antioxidative capacity, catalase, complement3, complement4, immunoglobin A, immunoglobin G, interleukin-2, interferon-γ, and tumor necrosis factor-α linearly. The relative weight of breast meat was increased ($P < 0.05$) linearly, but the relative weight of abdominal fat was decreased ($P < 0.05$) linearly with the increasing dietary ABP supplementation. The supplementation of ABP increased ($P < 0.05$) ileal *Lactobacilli* counts linearly, whereas decreased ($P < 0.05$) *Escherichia coli* counts linearly. Taken together, the inclusion of ABP promoted BWG and final BW during day 22 to 42 and the entire experiment, decreased F/G during day 22 to 42 and day 1 to 42, and partially improved antioxidant activities, immunity, and gut microflora in Pekin ducks.

Key words: antioxidant capacity, *Achyranthes bidentata* polysaccharides, ducks, immunity, performance

2020 Poultry Science 99:4884–4891
https://doi.org/10.1016/j.psj.2020.06.026

INTRODUCTION

Polysaccharides are polymeric carbohydrates composed of long chains of monosaccharide units joined by α-glycosidic and β-glycosidic linkages with a general formula of $(C_6H_{10}O_5)n$, which can be divided into 3 main subclasses (animal polysaccharides, microbial polysaccharides, and plant polysaccharides). The plant polysaccharides are derived from plant roots, leaves, skins, seeds, and flowers. It is well known that the plant polysaccharides from Chinese herb own a wide variety of biological activities, such as antioxidant, antiinflammatory, antiviral, antitumor, hypoglycemic, and immunity-stimulating properties (Li et al., 2015; Xie et al., 2016; Chen and Huang, 2018; Yin et al., 2019).

Achyranthes bidentata is a perennial member of the *Achyranthes* genus in the *Amaranthaceae* family, which is widely used in traditional medicines in China, Korea, and Japan. It is officially listed in the Chinese Pharmacopoeia and used as a tonic. The root of *A. bidentata* contains various bioactive components, including alkaloids, saponin, steroid, triterpenoids, phytoecdysteroid, 20-hydroxyecdysone, and inokosterone (Zhang and Lin, 2012; Liu et al., 2018; Al-Mijan et al., 2018). The *A. bidentata* polysaccharides (**ABP**), gray white powder, are extracted from the root of Chinese medicinal herb *A. bidentata*. They are composed of (2→1)-linked-β-D-fructofuranosyl, (2→6)-linked-β-D-Fruf and (2→1,6)-linked-β-D-Fruf residues and terminated with fructose and glucose residue (Guo et al., 2008; Zhang et al., 2019). Thus, ABP have relatively small molecular mass. Pharmacological and clinical studies have shown that ABP can exert antioxidant, antiallergic,

Received December 3, 2019.
Accepted June 13, 2020.

[1]Corresponding author: inhokim@dankook.ac.kr

immunomodulatory, arthritis alleviation, antiinflammatory, hepatoprotective, and anticancer effects (Kim and Park, 2010; Bang et al., 2012; Jang et al., 2012). Previous studies have indicated that ABP have significant immune-stimulating activity, which are achieved through the generation of reactive oxygen species, the secretion of cytokines, cell proliferation, and the phagocytic activity of macrophages (Li and Li, 1997; Yin et al., 2019; Zhang et al., 2019). Hence, it has the potential to be used as an immune modulator in animal nutrition. Several researches have been conducted to evaluate its effects on growth performance, antioxidant activities, gut health, and immune function in broilers (Qiu et al., 2007; Liu et al., 2018), weaning pigs (Guo et al., 2008; Kang et al., 2010; Chen et al., 2011, 2014), and rats (Zhang and Lin, 2012), which indicated positive effects, especially on immunity. Our recent study also demonstrated that ABP increased growth performance and total tract digestibility, regulated cecal microflora, and decreased excreta ammonia gas emission and abdominal fat weight in broilers (Park and Kim, 2020). To the best of our knowledge, no information about the effect of ABP on ducks was available. Thus, we hypothesize that ABP may exert antioxidant capacity, stimulate immune system, and hence improve growth performance in Pekin ducks. Therefore, the aim of this study was to evaluate the impact of ABP on growth performance, immunity, antioxidant capacity, relative organ weight, ileal microflora, and meat quality in Pekin ducks.

MATERIALS AND METHODS

Experimental Design and Duck Husbandry

The experiment received prior approval from the Animal Protocol Review Committee of Dankook University (Cheonan, Choongnam, South Korea). The ABP used in our study was obtained from a commercial company (Synergen Inc., Bucheon, Korea) and produced according to our previous study (Park and Kim, 2020) with small modifications. *A. bidentata* roots from China were washed 3 times with clean water and then grinded with a mill (IKAM20; IKA, Staufen, Germany). The dried sample was extracted with distilled water at 100°C and was then refluxed for 6 h to obtain an initial extract. The residues were extracted with distilled water (1: 5) at 80°C for 2 h. The extract solution was filtered under low temperature by a high-velocity centrifugal machine. The useful parts were collected by column and eluted with ethanol (95%). After cooling to room temperature (25°C) and filtering (Whatman No. 2; Whatman Ltd., Kent, UK), the samples were vacuum-dried at a temperature below 40°C. The extracts were completely dried in a freeze-drier. The content of polysaccharide was 68.69%, which was analyzed by high performance liquid chromatography (Agilent 1100 series, Palo Alto, CA).

A total of 1,200 female Pekin ducklings (No. 4 strain) at 1 D of age with an average initial body weight (**BW**) of 51.2 ± 0.2 g were blocked based on BW in this 42-D experiment and placed in stainless steel battery brooders. The cages were equipped with feeder, nipple drinker, and raised plastic floors. All ducks were housed in an environmentally controlled facility. This experiment consisted of 3 treatments with 10 replications (cages) per treatment and 40 ducks per cage in a randomized complete block design. The dietary treatments were 1) CON, basal diet; 2) ABP2, CON + 0.02% ABP; and 3) ABP4, CON + 0.04% ABP. *Achyranthes bidentata* polysaccharide was included at the expense of corn. A 2-phase feeding program was used: a starter diet from day 1 to 21 and a grower diet from day 22 to 42. All diets were formulated to meet or exceed the NRC (1994) requirements of ducks (Table 1). Diets were fed in pellet form, and ducks were provided with water and feed *ad libitum* throughout the experiment. The environmental temperature and humidity were kept at 29°C and 60%, respectively, during 1 to 14 D. Afterward, the temperature was kept at 24°C.

Feed samples were analyzed for dry matter (Method 934.01), crude protein (Method 990.03), total ash (Method 942.05), calcium, and phosphorus (Method 985.01) according to the standard procedures of the AOAC (2002). The amino acids of all diets were determined, following acid hydrolysis with 6 N HCl at 110°C for 24 h, using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Cambridge, England). Before acid hydrolysis, methionine and cystine were oxidized with formic acid (Liu et al., 2019).

Table 1. Diet composition (as-fed basis).

Items	Starter[1]	Grower[1]
Ingredients, %		
Corn	59.20	64.22
Soybean meal (CP 46%)	31.36	24.69
Wheat bran	0.50	0.40
Soybean oil	2.03	2.83
Corn gluten meal	2.00	4.00
Dicalcium phosphate	1.39	1.27
Limestone	1.10	0.97
Bentonite	0.90	-
Sodium chloride	0.20	0.25
Choline chloride (60%)	0.10	0.10
DL-Methionine (99%)	0.15	0.11
L-Lys·HCl (78%)	0.07	0.16
Vitamin premix[2]	0.70	0.70
Trace mineral premix[3]	0.30	0.30
Total	100.00	100.00
Analyzed composition		
ME, kcal/kg[4]	3,000	3,200
Crude protein, %	22.25	18.28
Lysine, %	1.00	0.80
Methionine, %	0.50	0.45
Methionine + Cystine, %	0.81	0.74
Threonine, %	0.97	0.81
Calcium, %	0.70	0.60
Available phosphorus, %	0.40	0.35

[1]Starter diets, provided during day 1 to 21; grower diets, provided during day 22 to 42.

[2]Provided per kg of diet: choline chloride, 1,000 mg; vitamin A, 10,000 IU; vitamin D_3, 3,000 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; thiamin, 2 mg; riboflavin, 8 mg; pyridoxine hydrochloride, 4 mg; cyanocobalamin, 0.02 mg; calcium-D-pantothenate, 20 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

[3]Provided per kg of diet: Cu, 10 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Se, 0.3 mg; I, 0.2 mg.

[4]Calculated values.

Sampling and Measurements

Body weight and feed intake (**FI**) were recorded on day 1, 21, and 42 of the experiment on a per replicate basis. Body weight gain (**BWG**), FI, and feed-to-gain ratio (**F/G**) were calculated accordingly. Mortality was recorded as it occurred, and the weights of dead birds were used to adjust F/G.

At the end of the experiment, 10 birds from each replicate were randomly selected from each cage, and blood samples were collected from the jugular vein into a sterile syringe and stored at −4°C. Blood samples were then centrifuged at 3,000 × *g* for 15 min, and serum was separated. The levels of superoxide dismutase (**SOD**), catalase (**CAT**), glutathione peroxidase (**GSH-PX**), total antioxidative capacity (**T-AOC**), malondialdehyde (**MDA**), immunoglobin A (**IgA**), immunoglobin M (**IgM**), immunoglobin G (**IgG**), complement3 (**C3**) and complement4 (**C4**), interleukin-2 (**IL-2**), interleukin-6 (**IL-6**), tumor necrosis factor-α (**TNF-α**), and interferon-γ in the serum were measured using ELISA method (Jiancheng Biotechnology Institute, Nanjing, China) following the kit instructions (Liu et al., 2020; Yan et al., 2020). The serum T-AOC was measured by the method of ferric reducing-antioxidant power assay. The activity of GSH-PX was determined by colorimetric method of 5,5′-dithiobis-*p*-nitrobenzoic acid. The activity of SOD was detected by the xanthine oxidase method. The activity of CAT was measured by ammonium molybdate colorimetry. The MDA level was determined as an indicator of lipid peroxidation via 2-thiobarbituric acid color reaction.

After blood collection, the same birds were weighed individually and then sacrificed by cervical dislocation and exsanguinated. The carcass weight (without neck and feet), breast meat, liver, gizzard, pancreas, thymus, bursa of fabricius, spleen, and abdominal fat were removed by trained personnel and weighed after flushing with saline. Organ size was expressed as a percentage of BW. The pH of the breast meat was measured by a calibrated, glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL). The breast meat lightness (L*), redness (a*), and yellowness (b*) values were determined (Minolta CR410 Chromameter; Konica Minolta Sensing Inc., Osaka, Japan). The water-holding capacity was measured in accordance with the methods described by Kauffman et al. (1986). Drip loss was measured with approximately 2 g of heat sample according to the plastic bag method described by Honikel (1998). Cook loss was determined as described previously by Sullivan et al. (2007). The 2-thiobarbituric acid reactive substances (**TBARS**) were measured by the method described by Witte et al. (1970). The TBARS values were expressed in terms of milligrams of MDA per kilogram of muscle. Trichloroacetic acid solution (20% wt/vol) was utilized for the extraction. The chromium concentration was determined by spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan).

On day 42, the samples of small intestine tissues (approximately 2 cm from ileum) were collected for determination of microflora after weighing. Samples of fresh digesta (2 g) from the ileum were collected aseptically in preweighed 20-mL sterilized plastic tubes. One gram of the composite ileal digesta sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in ileal digesta samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates and *lactobacilli* spp. medium III agar plates to isolate the *Escherichia coli* and *Lactobacilli*, respectively. The *lactobacilli* medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. MacConkey agar plates were incubated for 24 h at 37°C. *E. coli* and *lactobacilli* colonies were counted immediately after removal from the incubator (Ao and Kim, 2019).

Statistical Analysis

All data were analyzed using Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). Orthogonal polynomials were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental ABP. Variability in the data is expressed as the SEMs, and a probability level of $P < 0.05$ were considered to be statistically significant.

RESULTS

Growth Performance

During day 1 to 21, dietary treatments did not affect ($P > 0.05$) BWG, FI, or F/G (Table 2). During day 22 to 42, the inclusion of ABP increased ($P < 0.05$) BWG linearly but decreased ($P < 0.05$) F/G without any effect on FI ($P > 0.05$). During the whole experiment, BWG and final BW was increased ($P < 0.05$) linearly in ABP2 and ABP4 treatments compared with CON, whereas F/G was reduced ($P < 0.05$) linearly without any effect on FI ($P > 0.05$).

Antioxidant Activities and Immune Function

The supplementation of ABP improved ($P < 0.05$) SOD, GSH-PX, T-AOC, and CAT in the serum linearly, whereas did not affect ($P > 0.05$) serum MDA (Table 3).

The inclusion of ABP increased ($P < 0.05$) serum C3, C4, IgA, IgG, IL-2, INF-γ, and TNF-α linearly (Table 4). No differences were observed ($P > 0.05$) in serum IgM or IL-6 among treatments.

Relative Organ Weight and Meat Quality

The administration of ABP increased ($P < 0.05$) relative weight of breast meat but decreased ($P < 0.05$) abdominal fat linearly (Table 5). There were no differences ($P > 0.05$) in relative weight of carcass, liver,

Table 2. Effects of *Achyranthes bidentata* polysaccharide on growth performance in ducks.[1]

Item[2]	CON[3]	ABP2[3]	ABP4[3]	SEM	*P*-value	
					Linear	Quadratic
Initial BW, g	51.2	51.2	51.2	0.20	0.73	0.87
Final BW, g	3,194	3,235	3,301	19	0.03	0.81
Day 1–21						
BWG, g	1,334	1,347	1,349	13	0.26	0.67
FI, g	2,574	2,582	2,590	15	0.22	0.79
F/G	1.93	1.92	1.92	0.02	0.72	0.24
Day 22–42						
BWG, g	1,809	1,837	1,901	18	0.02	0.88
FI, g	4,722	4,743	4,768	22	0.19	0.74
F/G	2.61	2.58	2.51	0.02	0.03	0.82
Day 1–42						
BWG, g	3,143	3,184	3,250	16	0.03	0.69
FI, g	7,296	7,325	7,358	20	0.07	0.76
F/G	2.32	2.30	2.26	0.01	0.04	0.81

[1]Means represent 10 replicates with 40 birds per cage (n = 10/group).
[2]BWG, body weight gain; FI, feed intake; F/G, feed-to-gain ratio.
[3]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

pancreas, gizzard, bursa of Fabricius, thymus, or spleen among treatments.

Dietary treatments did not affect ($P > 0.05$) pH_{45min}, pH_{24h}, lightness (L*), redness (a*), yellowness (b*), water-holding capacity, cook loss, TBARS, or drip loss (Table 6).

Ileal Microflora

The inclusion of ABP increased ($P < 0.05$) ileal microbial shedding of *Lactobacilli* linearly, whereas decreased ($P < 0.05$) *E. coli* linearly (Table 7).

DISCUSSIONS

Growth Performance

Previous studies have demonstrated that various herb extracts have the potential to enhance growth performance in poultry (Alçiçek et al., 2003; Park et al., 2014; Diaz-Sanchez et al., 2015). This is the first study about the effect of ABP on Pekin ducks. The findings of present study showed that 0.02 to 0.04% ABP improved BWG and feed efficiency in the grower period and the whole experiment, which was consistent with the results of our recent study (Park and Kim, 2020). The supplementation of ABP (0.025–0.1%) increased BWG during day 1 to 7, day 22 to 25, and day 1 to 35 as well as feed efficiency during day 1 to 7, day 8 to 21, and day 1 to 35 linearly in broilers (Park and Kim, 2020). The improvement in BWG and F/G may be because of the increased nutrient digestibility. Park and Kim (2020) found that the digestibility of dry matter and nitrogen was increased linearly with the increasing dietary ABP supplementation in broilers. Besides, several studies suggest that herb extracts may stimulate the bile secretion, increase the activities of digestive enzymes (Brenesa and Roura, 2010), and ameliorate gut intestinal morphology (Khalaji et al., 2011; Liu et al., 2018). Moreover, Liu et al. (2018) and Ou et al. (2019) reported that the inclusion of ABP (0.04–0.05%) could improve final BW and average daily gain in broilers. Similar results were observed in weaning pigs (Chen et al., 2009; Kang et al., 2010). Chen et al. (2011) observed that weaning pigs fed 0.05% ABP had greater average daily gain and feed efficiency. The beneficial effects of ABP may be more pronounced in broilers and weaning pigs under stress. Liu et al. (2018) indicated

Table 3. Effects of *Achyranthes bidentata* polysaccharide on antioxidant activities in ducks.[1]

Item[2]	CON[3]	ABP2[3]	ABP4[3]	SEM	*P*-value	
					Linear	Quadratic
SOD, U/mL	128	147	162	5.5	0.03	0.80
GSH-PX, U/mL	257	286	294	5.2	0.02	0.85
MDA, nmol/mL	5.49	4.97	4.82	0.3	0.08	0.90
T-AOC, U/mL	16.7	19.3	19.6	1.2	0.04	0.33
CAT, U/mL	119	148	161	4.9	0.03	0.67

[1]Means represent 10 replicates with 10 birds per cage (n = 100/group).
[2]SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidative capacity; CAT, catalase.
[3]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

Table 4. Effects of *Achyranthes bidentata* polysaccharide on immune function in ducks.[1]

Item[2]	CON[3]	ABP2[3]	ABP4[3]	SEM	*P*-value	
					Linear	Quadratic
C3, g/L	0.15	0.17	0.19	0.01	0.03	0.21
C4, g/L	0.06	0.08	0.09	0.01	0.04	0.16
IgA, μg/mL	24.8	26.7	28.4	1.4	0.04	0.75
IgM, μg/mL	44.8	45.6	45.8	2.0	0.28	0.31
IgG, μg/mL	82.3	88.1	92.1	2.5	0.03	0.89
IL-2, ng/mL	132	144	149	2.7	0.04	0.67
IL-6, ng/mL	23.4	24.1	23.9	0.4	0.58	0.16
IFN-γ, ng/mL	21.1	23.5	24.6	0.7	0.04	0.79
TNF-α, pg/mL	22.3	25.1	26.9	0.6	0.04	0.80

[1]Means represent 10 replicates with 10 birds per cage (n = 100/group).
[2]C3, complement3; C4, complement4; IgA, immunoglobin A; IgM, immunoglobin M; IgG, immunoglobin G; IL-2, interleukin 2; IL-6, interleukin 6; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
[3]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

that the supplementation of ABP (0.05%) could improve growth performance and intestinal morphology in broilers challenged with *E. coli* K88. The inclusion of ABP (0.05%) alleviated the negative effects of lipopolysaccharide challenges in weaning pigs (Chen et al., 2014). Similar results were also observed in weaning pigs challenged with *E. coli* (Guo et al., 2008) and rats under oxidative stress induced by exhaustive exercise (Zhang and Lin, 2012). However, several studies failed to observe growth-promoting effects in broilers fed 0.02% ABP (Chen, 2002) and weaning pigs fed 0.08% ABP (Xie et al., 2018). The inconsistent results may be attributed to the different supplementation dosages, sources, diet composition, age, and species. However, more studies are needed to determine the effects of ABP on growth performance in ducks to verify the growth-stimulating effects.

Antioxidant Activities and Immune Function

Previous studies have indicated that ABP might improve antioxidant capacity in rats (Xue et al., 2009; Zhang and Lin, 2012). In the present study, ABP exerted antioxidative activities by improving serum SOD, GSH-PX, T-AOC, and CAT. Similarly, Xie (2018) also found that ABP increased serum SOD, GSH-PX, and T-AOC in weaning pigs. This may be because of the generation of reactive oxygen species stimulated by ABP (Li and Li, 1997).

It is reported that the improved antioxidant capacity may enhance their immunity in poultry (Kamboh et al., 2015). A recent review demonstrated that plant polysaccharides activate macrophages by recognizing and binding to specific receptors on the surfaces of macrophages, which initiates the immune response and exerts an immunomodulatory effect (Yin et al., 2019). The serum C3, C4, IgA, IgG, IL-2, and INF-γ levels have been measured as indicators of humoral immunity. Complement3and C4 are involved in the body's immune defense system, which may bind to plant polysaccharides and thus reduce the amounts of nitric oxide (Yin et al., 2019). Interferon-γ serves as an important regulator in the activation of lymphocytes and monocytes (Ao and Kim, 2019). The serum IL-2, as an important cytokine, plays a key role in the cell-mediated immune response which can promote the proliferation of activated natural killer cells, B lymphocytes, T lymphocytes, and antibody production (Li et al., 2011). Our study confirmed that ABP could exert immune-stimulating activities by improving serum C3, C4, IgA, IgG, IL-2, and INF-γ.

Table 5. Effects of *Achyranthes bidentata* polysaccharide on relative organ weight in ducks.[1]

Item	CON[2]	ABP2[2]	ABP4[2]	SEM	*P*-value	
					Linear	Quadratic
Carcass weight, %	71.2	71.1	71.2	0.40	0.42	0.26
Breast meat, %	18.1	19.5	19.6	0.18	0.04	0.64
Abdominal fat, %	2.70	2.46	2.35	0.05	0.03	0.81
Liver, %	2.74	2.72	2.76	0.06	0.57	0.16
Gizzard, %	2.17	2.14	2.15	0.05	0.18	0.36
Pancreas, %	0.37	0.36	0.36	0.02	0.24	0.40
Thymus, %	3.47	3.48	3.50	0.05	0.12	0.56
Bursa of Fabricius, %	0.15	0.16	0.16	0.02	0.11	0.40
Spleen, %	0.17	0.16	0.19	0.01	0.41	0.26

[1]Means represent 10 replicates with 10 birds per cage (n = 100/group).
[2]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

Table 6. Effects of *Achyranthes bidentata* polysaccharide on meat quality in ducks.[1]

Item[2]	CON[3]	ABP2[3]	ABP4[3]	SEM	*P*-value	
					Linear	Quadratic
$pH_{45\ min}$	6.62	6.61	6.63	0.02	0.60	0.25
$pH_{24\ h}$	5.61	5.65	5.78	0.03	0.06	0.84
WHC, %	47.6	49.1	49.6	1.30	0.14	0.75
Cook loss, %	29.8	29.3	29.5	0.55	0.69	0.28
TBARS, mg MDA/kg	1.59	1.57	1.56	0.05	0.11	0.67
Meat color						
Lightness (L*)	53.2	54.1	54.0	0.71	0.63	0.26
Redness (a*)	13.1	13.8	13.4	0.40	0.60	0.25
Yellowness (b*)	10.3	10.5	10.2	0.31	0.69	0.21
Drip loss, %						
day 1	2.58	2.56	2.54	0.11	0.21	0.63
day 3	4.51	4.48	4.47	0.10	0.18	0.58
day 5	7.15	7.09	7.05	0.13	0.17	0.72

[1]Means represent 10 replicates with 10 birds per cage (n = 100/group).
[2]WHC, water holding capacity; TBARS, 2-thiobarbituric acid reactive substances.
[3]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

Luo (2008) reported that ABP increased serum IgA in broilers, which was also observed in weaning pigs (Xie, 2018). Qiu et al. (2007) and Ou et al. (2019) demonstrated that ABP possessed immune-enhancing properties by increasing lymphocyte proliferation in broilers. Moreover, the immune-stimulating effects were also verified in weaning pigs (Kang et al., 2010; Chen et al., 2011; Xie, 2018). However, we found the pro-inflammatory cytokine TNF-α was increased by ABP, which was inconsistent with Guo et al. (2008) in weaning pigs and Liu et al. (2018). The underlying immunomodulatory mechanism of ABP may be achieved through the generation of reactive oxygen species, the secretion of cytokines, cell proliferation, and the phagocytic activity of macrophages (Yin et al., 2019), which was demonstrated by the improved serum antioxidant capacity and immune response in our study. The nuclear factor-kappa B and mitogen-activated protein kinase signaling may be the important cell signaling pathways to regulate the production of cytokines (Yin et al., 2019), which was demonstrated by Liu et al. (2018) in broilers fed diets with ABP under the challenge of *E. coli* K88 and Wang et al. (2017) in weaning pigs receiving lipopolysaccharides stress.

Relative Organ Weight and Meat Quality

In the current study, ABP decreased the relative weight of abdominal fat but increased breast meat, which was in agreement with our recent study in broilers (Park and Kim, 2020). The reason for the decreased relative weight of abdominal fat may be because of the lipolytic effect of ABP (Krishnakumari and Priya, 2006; Latha et al., 2011). It is proposed that the relative weight of immune organs may be increased because of the immune-stimulating effect of ABP. However, we did not observe any effect of ABP on bursa of Fabricius, thymus, or spleen in our study. Similarly, Park and Kim (2020) showed that ABP did not affect the relative weight of bursa of Fabricius, thymus, or spleen. Notwithstanding, the results were not always consistent. The relative weight of bursa of Fabricius was increased in broilers fed diets with ABP (Ou et al., 2019).

It is speculated that ABP may exert antioxidative activities and thus decrease water loss, which may improve meat quality. Unfortunately, we failed to observe any effect of ABP on meat quality in the current study. In agreement with our results, Park and Kim (2020) demonstrated that ABP did not affect meat quality in broilers.

Ileal Microflora

The stabilization of ileal microflora is critical to gut health and function (Song et al., 2014). Flint et al. (2008) indicated that polysaccharides could be utilized by the gut microbiota and result in beneficial effects on gut bacteria balance. In the present study, the ABP

Table 7. Effects of *Achyranthes bidentata* polysaccharide on ileal microflora in ducks.[1]

Item	CON[2]	ABP2[2]	ABP4[2]	SEM	*P*-value	
					Linear	Quadratic
Escherichia coli, log^{10} cfu/g	5.09	4.73	4.36	0.07	0.03	0.67
Lactobacilli, log^{10} cfu/g	7.17	7.42	8.01	0.06	0.03	0.70

[1]Means represent 10 replicates with 10 birds per cage (n = 100/group).
[2]CON, basal diet; ABP2, basal diet containing 0.02% ABP; ABP4, basal diet containing 0.04% ABP.

supplementation exerted a positive effect on ileal bacterial populations, which was similar to the findings of Park and Kim (2020). They observed the inclusion of ABP increased cecal microbial shedding of *Lactobacilli*, whereas decreased *E. coli* in broilers. Similarly, previous study indicated that plant extract could increase the ileal and cecal *Lactobacilli* counts, but reduce *E. coli* counts in broilers (Vidanarachchi et al., 2006). Liu et al. (2018) found that ABP decreased cecal *E. coli* but increased *Lactobacilli* counts in broilers, indicating that ABP might enhance the growth of specific beneficial bacteria strains in the intestinal tract and inhibit certain bacterial pathogens. This is supported by the finding of Xie et al. (2018) in weaning pigs, which showed that ABP promoted the growth of *Lactobacilli*. The increased ileal *Lactobacilli* might increase the production of short-chain fatty acid, which could not only inhibit the pathogen such as *E. coli* but also serve as important substrate for energy metabolism of intestinal cells (Liu et al., 2018). The beneficial effects of ABP on gut health may mirror the improvement in growth performance and immune function in our study. The beneficial bacteria (*Lactobacillus*) may indirectly enhance gut health and thus immunity (Paszkiewicz et al., 2012).

CONCLUSIONS

The inclusion of ABP (0.02–0.04%) caused a positive effect on feed efficiency, antioxidant activities, immune function, and ileal microflora in Pekin ducks during day 22 to 42 and day 1 to 42 and hence improved growth performance, which indicated that ABP can be used as a potential additive for ducks.

ACKNOWLEDGMENTS

Conflict of Interest Statement: The authors declare that they have no financial and personal relationships with other people or organizations that can inappropriately influence their work, and 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 position presented in, or the review of, the manuscript entitled.

REFERENCES

Al-Mijan, M. S., H. Park, Y. M. Lee, and B. O. Lim. 2018. Evaluation of the antioxidant and anti-inflammatory potential of fermented *Achyranthes japonica* Nakai extract. Nat. Prod. Chem. Res. 6:337–343.

Alçiçek, A., M. Bozkurt, and M. Çabuk. 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. S. Afr. J. Anim. Sci. 33:89–94.

AOAC International. 2002. Official Methods of Analysis of AOAC International. 17th ed. AOAC Int., Gaithersburg, MD.

Ao, X., and I. H. Kim. 2019. Effects of astaxanthin produced by *Phaffia rhodozyma* on growth performance, antioxidant activities and meat quality in Pekin ducks. Poult. Sci. 98:4954–4960.

Bang, S. Y., J. H. Kim, H. Y. Kim, Y. J. Lee, S. Y. Park, S. J. Lee, and Y. Kim. 2012. *Achyranthes japonica* exhibits anti-inflammatory effect via NF-κB suppression and HO-1 induction in macrophages. J. Ethnopharmacol. 144:109–117.

Brenes, A., and E. Roura. 2010. Essential oils in poultry nutrition: main effects and modes of action. Anim. Feed Sci. Tech. 158:1–4.

Chen, H. L. 2002. Studies on the Extraction, Immunomodulating Activities of Chinese Herbal Polysaccharides and Approach to the Mechanism. Ph.D. Dissertation. Chinese Academy of Agriculture Science, Beijing, China.

Chen, Q., Z. Liu, and J. H. He. 2009. *Achyranthes bidentata* polysaccharide enhances immune response in weaned piglets. Immunopharmacol. Immunotoxicol. 31:253–260.

Chen, Q. H., Z. Y. Liu, H. H. He, Y. R. Zhao, and X. S. Wu. 2011. *Achyranthes bidentata* polysaccharide enhances growth performance and health status in weaned piglets. Food Agr. Immunol. 22:17–29.

Chen, Q. H., Z. H. Ding, W. Qiu, and J. H. He. 2014. *Achyranthes bidentata* polysaccharide decreases inflammatory cytokine secretion in weaned piglets after LPS challenge. J. Anim. Vet. Adv. 13:454–459.

Chen, L., and G. Huang. 2018. Antitumor activity of polysaccharides: an overview. Curr. Drug Targets 19:89–96.

Diaz-Sanchez, S., D. D'Souza, B. Debrabrata, and H. Irene. 2015. Botanical alternatives to antibiotics for use in organic poultry production. Poult. Sci. 94:1419–1430.

Flint, H. J., E. A. Bayer, M. T. Rincon, R. Lamed, and B. A. White. 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat. Rev. Microbiol. 6:121–131.

Guo, G. L., Y. L. Liu, W. Fan, J. Han, Y. Q. Hou, Y. L. Yin, H. L. Zhu, B. Y. Ding, J. X. Shi, J. Lu, H. R. Wang, J. Chao, and Y. H. Qu. 2008. Effects of *Achyranthes bidentata* polysaccharide on growth performance, immunological, adrenal, and somatotropic responses of weaned pigs challenged with *Escherichia coli* lipopolysaccharide. Asian-aust. J. Anim. Sci. 21:1189–1195.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristic of meat. Meat Sci. 49:447–457.

Jang, G. Y., H. Y. Kim, S. H. Lee, Y. Kang, I. G. Hwang, K. S. Woo, T. S. Kang, J. S. Lee, and H. S. Jeong. 2012. Effects of heat treatment and extraction method on antioxidant activity of several medicinal plants. J. Korean Soc. Food Sci. Nutr. 41:914–920.

Kamboh, A. A., M. A. Arain, M. J. Mughal, A. Zaman, Z. M. Arain, and A. H. Soomro. 2015. Flavonoids: health promoting phytochemicals for animal production-A review. J. Anim. Health Prod. 3:6–13.

Kang, P., H. L. Xiao, Y. Q. Hou, B. Y. Ding, Y. L. Liu, H. L. Zhu, Q. Z. Hu, Y. Hu, and Y. L. Yin. 2010. Effects of *Astragalus* polysaccharides, *Achyranthes bidentata* polysaccharides, and *Acantbepanax senticosus* saponin on the performance and immunity in weaned pigs. Asian-aust. J. Anim. Sci. 23:750–756.

Kauffman, R. G., G. Eikelenboom, P. G. van der Wal, B. Engel, and M. Zaar. 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18:307–322.

Kim, C. S., and Y. K. Park. 2010. The therapeutic effect of *Achyranthis radix* on the collagen-induced arthritis in mice. Korea J. Herbol. 22:155–167.

Khalaji, S., M. Zaghari, K. H. Hatami, S. Hedari-Dastjerdi, L. Lotfi, and H. Nazarian. 2011. Black cumin seeds, Artemisia leaves (Artemisia sieberi), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. Poult. Sci. 90:2500–2510.

Krishnakumari, S., and K. Priya. 2006. Hypolipidemic efficacy of *Achyranthes aspera* on lipid profile in sesame oil fed rats. Anc. Sci. Life 25:49–56.

Latha, B. P., T. Vijaya, R. M. Reddy, M. Ismail, and S. D. Rao. 2011. Therapeutic efficacy of Achyranthes aspera saponin extract in high fat diet induced hyperlipidaemia in male wistar rats. Afr. J. Biotechnol. 10:17038–17042.

Li, Z. K., and D. D. Li. 1997. The immunomodulatory effect of *Achyranthes bidentata* polysaccharides. Acta Pharm. Sin. 12:881–887.

Li, L. L., F. G. Yin, B. Zhang, H. Z. Peng, F. N. Li, N. S. Zhu, D. X. Hou, Y. L. Yin, J. J. Luo, Z. R. Tang, and G. Liu. 2011. Dietary supplementation with *Atractylodes Macrophala Koidz* polysaccharides ameliorate metabolic status and improve immune function in early-weaned pigs. Livest. Sci. 142:33–41.

Li, H. L., P. Y. Zhao, Y. Lei, M. M. Hossain, and I. H. Kim. 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens. Livest. Sci. 181:1–6.

Liu, Z., X. Wang, S. Ou, M. Arowolo, D. X. Hou, and J. He. 2018. Effects of *Achyranthes bidentata* polysaccharides on intestinal morphology, immune response, and gut microbiome in yellow broiler chickens challenged with *Escherichia coli* K88. Polymers 10:1233–1247.

Liu, J. B., H. L. Yan, Y. D. Hu, and H. F. Zhang. 2019. Effects of dietary energy and protein content and lipid source on growth performance and carcass traits in Pekin ducks. Poult. Sci. 98:4829–4837.

Liu, J. B., H. L. Yan, Y. Zhang, Y. D. Hu, and H. F. Zhang. 2020. Effects of stale maize on growth performance, immunity, intestinal morphology and antioxidant capacity in broilers. Asian-australas. J. Anim. Sci. 33:605–614.

Luo, L. Y. 2008. Studies on the Technology of Extraction and the Immunomodulation Effect of the *Achyranthes Bidentata* Polysaccharides in Chicken. MS. D. Dissertation. Sichuan Agricultural University, Ya'an, China.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Ou, S. Q., Z. Y. Liu, D. Guo, and J. H. He. 2019. Effects of sanguinarine and *Achyranthes bidentata* polysaccharides on growth performance and immune performance of yellow-feather broilers. Chin. J. Anim. Nutr. 31:360–368.

Park, J. H., S. N. Kang, G. M. Chu, and S. K. Jin. 2014. Growth performance, blood cell profiles, and meat quality properties of broilers fed with *Saposhnikovia divaricata, Lonicera japonica*, and *Chelidonium majus* extracts. Livest. Sci. 165:87–94.

Park, J. H., and I. H. Kim. 2020. Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. Poult. Sci. 99:463–470.

Paszkiewicz, M., A. Budzynska, B. Rozalska, and B. Sadowska. 2012. Immunomodulacyjna rola polifenoli roślinnych. Postępy Hig. Med. Dosw. 66:637–646.

Qiu, Y., Y. L. Hu, B. A. Cui, H. Y. Zhang, X. F. Kong, D. Y. Wang, and Y. G. Wang. 2007. Immunopotentiating effects of four Chinese herbal polysaccharides administered at vaccination in chickens. Poult. Sci. 12:2530–2535.

Sullivan, Z. M., M. S. Honeyman, L. R. Gibson, and K. J. Prusa. 2007. Effects of triticale-based diets on finishing pig performance and pork quality in deep-bedded hoop barns. Meat Sci. 76:428–437.

Song, J., K. Xiao, Y. L. Ke, L. F. Jiao, C. H. Hu, Q. Y. Diao, B. Shi, and X. T. Zou. 2014. Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci. 93:581–588.

Vidanarachchi, J. K., L. L. Mikkelsen, I. M. Sims, P. A. Iji, and M. Choct. 2006. Selected Plant Extracts Modulate the Gut Microflora in Broilers. Pages 145–148 in Proc. 18th Aust. Poult. Sci. Sym, Sydney, Australia.

Wang, X., M. W. Li, J. Ma, and Q. H. Chen. 2017. *Achyranthes bidentata* polysaccharides: regulation on immunological stress in piglet intestinal epithelial cells and its action mechanism. Chin. J. Anim. Sci. 29:4116–4122.

Witte, V. C., G. F. Krause, and M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbuturic acid values of pork and beef during storage. J. Food Sci. 35:582–585.

Xie, J. H., M. L. Jin, A. Morris, X. Gordon, Q. Zha, H. Q. Chen, Y. Yi, J. E. Li, Z. J. Wang, J. Gao, S. P. Nie, P. Shang, and M. Y. Xie. 2016. Advances on bioactive polysaccharides from medicinal plants. Crit. Rev. Food Sci. Nutr. 56:60–84.

Xie, H. H., Y. Zou, L. L. Liu, Y. S. Yang, and J. H. He. 2018. Effects of botanical polysaccharides on growth performance and intestinal environment of weaned piglets. Chin. J. Anim. Nutr. 30:2662–2671.

Xie, H. B. 2018. Effects and Mechanism of Botanical Polysaccharides on Intestinal Physiological Characteristics and Immune Function of Weaned Piglets. Ph.D. Dissertation. Hunan Agricultural University, Changsha, China.

Xue, S., X. Chen, J. Liu, and L. Jin. 2009. Protective effect of sulfated *Achyranthes bidentata* polysaccharides on streptozotocin-induced oxidative stress. Carbohyd. Polym. 75:415–419.

Yan, H. L., S. C. Cao, Y. D. Hu, H. F. Zhang, and J. B. Liu. 2020. Effects of methylsulfonylmethane on growth performance, immunity, antioxidant capacity and meat quality in Pekin ducks. Poult. Sci. 99:1069–1074.

Yin, M., Y. Zhang, and H. Li. 2019. Advances in research on immunoregulation of macrophages by plant polysaccharides. Front. Immunol. 10:145–150.

Zhang, Z., and J. Lin. 2012. The Influence of polysaccharides from the roots of achyranthes bidentata on biochemical parameters related to oxidative stress induced by exhaustive exercise of rats. J. Anim. Vet. Adv. 24:4693–4696.

Zhang, D., C. Wang, X. Hou, and C. Yan. 2019. Structural characterization and osteoprotective effects of a polysaccharide purified from *Achyranthes bidentata*. Int. J. Biol. Macromol. 139:1063–1067.