The effects of dietary supplementation of *Artemisia argyi* polysaccharide on immune and antioxidative functions in broilers

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**ABSTRACT**

In this study, the effects of *Artemisia argyi* polysaccharide (AAP) on broilers’ immune and antioxidative functions and the underlying regulatory mechanism were investigated. In this experiment, 288 one-day-old Arbor Acres plus broilers were randomly allocated into 6 treatments with 6 replicates where each treatment group contained 6 replicate groups and there were 8 broilers in each replicate group. The control group was fed the basal diet, and the experimental groups were fed the basal diet supplemented with 250, 500, 750 or 1000 mg/kg AAP in comparison with 50 mg/kg chlortetracycline for 42 d. The result demonstrated that the best amount of AAP addition was 1000 mg/kg for growth. It could promote the contents of cytokines, immunoglobulin as well as the activity of the antioxidant enzyme (GPx), and reduce the malondialdehyde (MDA) content in the serum of broilers on 42 d. The same dose of AAP could regulate the immune and antioxidant functions in the liver of broilers, and activate the TLR/NF-κB signal pathway in broilers. The growth-promoting effect of AAP was comparable to that of chlortetracycline.

**Highlights**

- *Artemisia argyi* polysaccharide could promote the growth performance of broilers
- *Artemisia argyi* polysaccharide could promote the immune regulation of broilers
- *Artemisia argyi* polysaccharide could promote the antioxidant function of broilers

**Introduction**

The challenges for the livestock and poultry farming industry have become increasingly prominent over the past few years. First of all, while countries have recently introduced stricter industry policies, public opinion on the sustainable development of the environment continues to rise. Secondly, free-range farmers are gradually withdrawing from the market. It has been inherited by large-scale livestock and poultry farms, enterprises and groups by mergers and expansions. Nowadays, consumers are paying more attention to the safety and quality of livestock products. To solve the problem of antibiotic residues in feed additives, it is urgent to find green and safe feed additives that can improve production performance.

Data on the existing literature have shown that herbal extracts can improve the growth performance of animals and enhance their immune function. One of the traditional Chinese medicines, *Artemisia argyi*, is a small perennial herbaceous shrub plant with a strong aroma, belonging to the family of Asteraceae. It is mainly distributed in the northern temperate regions, especially in Asia, Europe and North America (Bora and Sharma 2011). In China, there is a long history of research on *Artemisia argyi* with a lot of applications. In addition to being used as a medicinal herb, *Artemisia absinthium* is also an ingredient of dye. *Artemisia argyi* has many bioactive components such as polysaccharides (Zhang et al. 2018), flavonoids (Han et al. 2017), organic acids (Han et al. 2017) and essential oils (Abad et al. 2012). Modern science has also confirmed that *Artemisia argyi* is beneficial for animal health because of its antioxidant (Kim et al. 2015), anti-tumour (Seo et al. 2003), anti-inflammatory (Yun et al. 2016), anti-coagulant (Lv et al. 2018) and immunomodulatory (Zhang et al. 2018) effects.

Zhang et al. 2018 demonstrated that the alcoholic extract, the crude polysaccharide and the aqueous extract of *Artemisia argyi* had anti-inflammatory effects on broiler peripheral blood mononuclear cells (PBLs). *Artemisia argyi* polysaccharide (AAP) was able to increase the rate of nutrient metabolism and improve the morphology of intestinal mucosa in animals. However, a few studies have reported the effects of AAP on immune or antioxidant functions under conventional feeding conditions. The purpose of this study is to show the influence of AAP dietary supplementation on the immune and antioxidant functions of broilers under conventional feeding conditions.

**Materials and methods**

**Preparation of Artemisia argyi polysaccharide**

*Artemisia argyi* is often harvested from the territory of Hohhot, Inner Mongolia in May. The hot water decoction-ethanol precipitation method was used to prepare *Artemisia argyi*
polysaccharide (AAP). The supernatant was concentrated to 1/5 of the volume of the original supernatant by a rotary evaporator. The concentrated solution was mixed with anhydrous ethanol in a ratio of 1:4 for 48 h. The flocculent precipitate was freeze-dried and made into dry powder jelly (Artemisia argyi polysaccharide, AAP).

The average molecular weight of AAP was determined by the gel permeation chromatography (GPC) system to be 16 KDa. The monosaccharide composition was determined on an Agilent 1100 liquid chromatograph equipped with an Xttimate C18 column (4.6×200 mm, 5 µm radius). The monosaccharides contained in AAP mainly consisted of mannose (23.82%), ribose (1.41%), rhamnose (6.95%), glucuronic acid (1.49%), galacturonic acid (2.55%), glucose (23.89%), glucose-galactose (16.99%), xylose (7.58%), arabinose (12.03%) and fucose (3.29%).

Birds, experimental design and diets

In this experiment, 288 one-day-old Arbor Acres plus broilers were randomly allocated into 6 treatments with 6 replicates each and 8 broilers in each replicate. The control group was fed the basal diet. The experimental groups were fed the basal diet supplemented with 250, 500, 750 or 1000 mg/kg AAP or 50 mg/kg chlortetracycline for 42 d. The basal diet used in this experiment was formulated with reference to the nutritional requirements of the Chinese agricultural industry standard for broiler feeding (NY/T 33-2004). The composition and nutritional levels are shown in Table 1. The broilers of test groups were kept in single-layer cages, and the six replicate groups of each treatment were arranged separately according to different cage locations to eliminate the influence of environmental factors. During the trial period, a combination of natural light and artificial lighting was used in the broiler house to meet the light requirements of broilers, and a natural ventilation system was used to keep the air in the house clean. Manure was cleaned manually once a day during the experiment, and the growth of broilers was observed and recorded in real-time. The test chickens had free access to feed and water. The vaccination procedure was conducted as follows: the broilers were vaccinated with Newcastle disease and infectious bronchitis combined vaccine on d 7 and 28, Newcastle disease, infectious bronchitis and avian influenza triple vaccine on d 10, infectious bursal disease vaccine on d 14 and 20.

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

| Ingredients             | 1–21 days of age | 22–42 days of age |
|-------------------------|------------------|-------------------|
| Corn                    | 52.50            | 58.80             |
| Soybean meal            | 40.00            | 33.80             |
| Soybean oil             | 3.00             | 3.00              |
| Dicalcium phosphate     | 1.90             | 1.80              |
| Limestone               | 1.08             | 1.22              |
| Salt                    | 0.37             | 0.37              |
| L-Lysine                | 0.05             | 0.03              |
| DL-Methionine           | 0.19             | 0.07              |
| Premix                  | 0.80             | 0.80              |
| Choline chloride        | 0.11             | 0.11              |
| Total                   | 100.0            | 100.0             |

| Nutrient levels<sup>a</sup> | 1–21 days of age | 22–42 days of age |
|----------------------------|------------------|-------------------|
| ME (MJ/kg)                 | 12.42            | 12.62             |
| CP                         | 21.77            | 19.65             |
| Calcium                   | 1.00             | 1.02              |
| Available phosphorus       | 0.44             | 0.42              |
| Lys                       | 1.34             | 1.15              |
| Met                       | 0.55             | 0.40              |
| Cys                       | 0.40             | 0.36              |

<sup>a</sup> premix provided the following per kilogram of diet: VA 9000 IU,VD3 3000 IU, VE 26 mg, VK1 1.20 mg, VB2 3.00 mg, VB6 8.00 mg, VB12 4.40 mg, VB3 0.012 mg, nicotinic acid 45 mg, folic acid 0.75 mg, biotin 0.20 mg, calcium pantothenate 15 mg, Fe 100 mg, Cu 10 mg, Zn 108 mg, Mn 120 mg, 11.5 mg and Se 0.35 mg.

<sup>b</sup> CP was a measured value, while others were all calculated values.

Measurement indicators and methods

Growth performance

The broilers were weighed on d 0, 21 and 42 of the trial for the determination of growth performance indicators. The average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G) and body weight (BW) were calculated for different periods of the experiment based on the data after weighing to evaluate the growth performance of the broilers in different treatment groups.

Sampling method

Six chickens (one from each replicate) were randomly selected from each treatment group for sampling on d 21 and 42 of the trial. The collected blood samples were left at an inclination of 45° for 1 h and centrifuged at 3500 × g for 15 min to obtain serum. Serum samples were then stored at −80°C until the analyses of the test-related indexes. The abdominal cavity of the slaughtered broiler chickens was quickly opened and the tissue samples of the liver were removed, mixed with physiological saline at a ratio of 1:9, ground and pulverized using an electric homogenizer. Then the samples were centrifuged at 3000 × g for 15 min to remove the supernatant and stored at −80°C until further analyses.

Immunological indicators

Serum and the tissue homogenate samples of the liver were prepared for the detection of immunological and antioxidant indicators: IL-1β, IL-2, IL-4, IL-6, IFN-γ, IgA, IgG, IgM contents, total superoxide dismutase (T-SOD, hydroxyl amine method), catalase (CAT, molybdate method), glutathione peroxidase (GPx, colorimetric method), malondialdehyde (MDA, TBA method) and total antioxidant capacity (T-AOC, ABTS rapid method). The enzyme-linked immunosorbent assay (ELISA) kits used in the test were purchased from Quanzhou Ruixin Biotechnology Company. The kits for the protein content of the tissue samples and the antioxidant indicators used in the experiment were purchased from the Nanjing Jiancheng Institute of Biological Engineering. The determination was performed according to the instructions for the kit.

Molecular biology indexes

The measurements were made according to the instructions of the kits (CodeNo: 9109, TaKaRa; Bao Bio, Dalian, China) and total RNA from the tissue samples of the liver was extracted. The integrity and purity of total RNA were assessed by 1% agarose gel electrophoresis, 260/280 nm absorbance ratio (ideal absorbance ratio between 1.8 and 2.0) and total RNA concentration. Afterwards, cDNA reverse transcription was
performed on total RNA extracted from the liver and intestinal samples according to the instructions of the Prime Script™ RT kit (CodeNo: RR047A, TaKaRa; Bao Bio, Dalian). The β-actin gene was used as an internal control to verify reverse transcription’s success and calibrate the cDNA template. The specific primers obtained from Shanghai Biotech, China are shown in Table 2. Real-time fluorescent quantitative PCR amplification assays were performed following the instruction for the SYBR Premix ExTaq™ II kit. The total volume was 20 μL, including 10 μL of SYBR Premix ExTaq™ II, 2 μL of cDNA template, 1 μL of upstream and downstream primers and 6 μL of DEPC-treated water. The PCR procedure was as follows: 95°C for 30 s; 95°C for 10 s, annealing temperature for 30 s and 72°C for 15 s, for 40 cycles. The procedure for the lysis curve was as follows: 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Each sample was tested in triplicate. The relative mRNA expression of the selected genes was calculated using the 2^ΔΔCT method (Livak and Schmittgen 2001).

**Statistical analysis**

The experimental data were collated by Microsoft Excel 2010, and one-way ANOVA was performed using the GLM model of the SAS 9.2 analysis software system and the means of different treatment groups were compared using Duncan's multiple comparisons and regression analysis was used to determine the dose-effect of AAP addition, with P < 0.05 as the difference or regression effect significant, 0.05 < P < 0.1 for difference or regression effect tending to be significant and P > 0.1 for difference or regression effect not significant.

**Results**

**Growth performance**

As shown in Table 3, the ADFI of the 750 mg/kg AAP addition group was significantly lower than that of the control group (P < 0.05), but had no significant difference compared with that of the chlortetracycline group during d 1–21. The ADG of the 1000 mg/kg AAP addition group was significantly higher than that of the control group (P < 0.05), but had no significant difference compared with that of the chlortetracycline group, and a linear and quadratic effect was observed with the increase in the amount of AAP during d 22–42. The feed-to-weight ratio of the AAP-added groups was significantly lower than that of the control group (P < 0.05) from d 22 to 42. On d 21 or d 42, the body weight was not significantly different from that of the control group and the chlortetracycline group (P > 0.1).

**Immunological index**

As shown in Table 4, for IL-1β and IL-4 levels in broiler serum on d 42, the AAP-added groups at 750 and 1000 mg/kg were significantly lower than that of the control group (P < 0.05), but had no significant difference compared with that of the control group, 0.05 < P < 0.1 for difference or regression effect tending to be significant and P > 0.1 for difference or regression effect not significant. Growth performance indicators were calculated on a repetitive basis and other indicators were calculated on a single broiler basis.

### Table 2. Primer sequences of immune and antioxidant-related genes.

| Genes     | Gene Bank no. | Primer sequences, 5'–3' | Length, bp | Ta (°C) |
|-----------|---------------|-------------------------|------------|---------|
| β-actin   | NM_205518     | F. GCCAACAGAGAGAGAGATGACAC<br>R. GAAAGCGATGAGCATGCTG | 118        | 60      |
| TLR2      | NM_001161650  | F. CATTCCATGAGGGCGAGGGAGTAG<br>R. GGTGCAGATCAAAGGACACTAGGA | 244        | 60      |
| TLR4      | NM_001030693  | F. TTCGAGAAGACTTTGAGTG<br>R. CAACCGAATAGCTGGTACGCTG | 131        | 60      |
| MyD88     | NM_001030963  | F. TTCAGAATGCTGAGATGCTA<br>R. TCCAAAGTCTGGAGATGCTA | 198        | 60      |
| Rac1      | NM_205017.1   | F. ACTCTATACACTACCCACGG<br>R. ACGCCAGACTTTGACGTTGGA | 186        | 60      |
| PI3K      | NM_001004410.1| F. GTCCAGGACGACTGATG<br>R. TGGTGCCCTACGCTGTG | 177        | 60      |
| NF-κB     | D13721        | F. CAGGCCATCAGCAGAACCC<br>R. CAGCCCAAGAAGCAACCTC | 151        | 60      |
| IL-1β     | NM_204524     | F. AGCTGTTGGTGCTGAGAATCT<br>R. ACTCTATACACTACCCACGG | 84         | 60      |
| IL-2      | AJ224516      | F. TGCTTTTAAACGCTCTTG<br>R. GATGCTTCAATACGCTGATG | 659        | 60      |
| IL-6      | HM179640      | F. AAATCCCTCTCGCCCAAATCT<br>R. GCCCATGCTTCCTCTCCATAAA | 106        | 60      |
| IFN-γ     | Y079221       | F. GATAGAACACTTTCAAGGAT<br>R. GTAAGAACACTTTCAAGGAT | 670        | 60      |
| CAT       | NM_001031215.1| F. GTGGCCGCTGGAGAGCTGCT<br>R. GTTCCATGAGGGGCTGCTG | 182        | 60      |
| SOD       | NM_205064.1   | F. TTGTCTGAGGAGATCTGAGCT<br>R. TGCTTTTAAACGCTCTTG | 98         | 60      |
| GPx       | NM_001163245.1| F. CAAAGTGGCGGCTAGTGGA<br>R. AGATCCCCAGGCTTACCATTTCC | 136        | 60      |

Note: TLR2 (Toll-like receptor 2), TLR4 (Toll-like receptor 4), MyD88 (myeloid differentiation factor 88), Rac1 (Ras-related C3 botulinum toxin substrate 1), PI3K (phosphatidylinositol 3-kinase), NF-κB (nuclear transcription factor-κB), IL-1β (interleukin-1β), IL-2 (interleukin-2), IL-6 (interleukin-6), IFN-γ (interferon-γ), CAT (catalase), SOD (superoxide dismutase), GPx (glutathione peroxidase).

F = forward primer; R = reverse primer.
For IL-2 levels in serum on d 42, the 750 mg/kg AAP-added group was significantly higher than the control group (P < 0.05), but not significantly different compared with the CTC group. The IL-2 level indicated a linear or quadratic effect with increasing AAP additive level. On d 42, the IL-2 level of 1000 mg/kg AAP group was significantly different from that of the chlorotetracycline group, and showed a linear or quadratic effect with the increase of the AAP additive level. On d 21, the IL-2 level of 750 mg/kg AAP group was higher than that of the control group (P = 0.05), but it was not significantly different from that of the chlorotetracycline group, and showed a linear or quadratic effect with an increased AAP additive level.

As shown in Table 6, on d 21, the liver IL-1β content of 500, 750 and 1000 mg/kg AAP additive groups was significantly lower than that of the control group (P < 0.05). On d 42, the IL-4 level of 1000 mg/kg AAP group was significantly higher than that of the control group (P < 0.05). On d 42, the IL-4 level of 500 and 1000 mg/kg AAP groups was significantly higher than that of the control group (P < 0.05), but 500 and 1000 mg/kg of AAP groups were not significantly different from that of the chlorotetracycline group, and showed a quadratic increase with the increase in AAP additive level.

As shown in Table 5, on d 21, AAP supplementation had no significant effect on immunoglobulin IgA, IgG and IgM in broiler serum (P > 0.1). On d 42, IgG and IgM serum levels in the 1000 mg/kg AAP group were higher than those of the control group (P < 0.05), but it was not significantly different compared with the chlorotetracycline group and showed a linear or quadratic effect with an increased AAP additive level.
content showed a linear or quadratic effect by increasing the AAP additive amount. In addition, on d 42, the IFN-γ content of 500, 750, and 1000 mg/kg AAP group was significantly higher than that of the control group and the chlortetracycline group (P<0.01), and showed a linear effect with the increase of the AAP additive amount.

As shown in Table 7, on d 21 and 42, the liver IgA content of 500, 750, and 1000 mg/kg AAP addition groups was significantly higher than that of the control and chlortetracycline groups (P<0.01), and showed a linear or quadratic effect with the increase of AAP additive level (P<0.05). On d 21, the IgM content of 750 mg/kg AAP group was higher than that of the control group (P<0.05). On d 42, the IgM content of the 1000 mg/kg AAP group was significantly higher than that of the control and chlortetracycline groups (P<0.05), and showed a linear increase effect with the increase of AAP additive level (P<0.05).

**TLR/NF-κB signalling pathway-related gene expression**

As shown in Table 8, on d 42, the gene expression of TLR2 in the AAP-added groups was lower than that in the control group (P<0.01).
Table 8. Effects of AAP on the expression of TLR/ NF-κB signalling pathway-related genes in the liver of broilers.

| Item       | AAP supplemental level mg/kg | CTC mg/kg | SEM | P-Value |
|------------|-----------------------------|-----------|-----|---------|
| TLR2       | 0  | 250 | 500 | 750 | 1000 | 50 | SEM | ANOVA | Linear | Quadratic |
| 21d        | 1.00 | 0.74 | 0.87 | 0.61 | 0.75 | 0.64 | 0.24 | 0.25 | 0.06 | 0.10 |
| 42d        | 1.00 | 0.67 | 0.87 | 0.23 | 0.19 | 0.36 | 0.14 | <0.01 | <0.01 | <0.01 |
| TLR4       | 21d | 1.00 | 1.30 | 1.06 | 1.16 | 1.81 | 1.31 | 0.43 | 0.05 | 0.22 | 0.27 |
| 42d        | 1.00 | 1.16 | 1.02 | 1.46 | 1.15 | 1.18 | 0.31 | 0.25 | 0.53 | 0.69 |
| MyD88      | 21d | 1.00 | 0.88 | 0.94 | 0.86 | 0.69 | 0.87 | 0.29 | 0.67 | 0.08 | 0.19 |
| 42d        | 1.00 | 0.87 | 0.86 | 0.86 | 0.71 | 0.78 | 0.34 | 0.86 | 0.26 | 0.54 |
| TLR4       | 21d | 1.00 | 0.98 | 1.13 | 1.03 | 0.93 | 1.71 | 0.28 | <0.01 | 0.77 | 0.51 |
| 42d        | 1.00 | 1.05 | 0.81 | 0.76 | 0.86 | 1.07 | 0.33 | 0.62 | 0.23 | 0.44 |
| TLR2       | 21d | 1.00 | 0.98 | 1.12 | 1.17 | 1.12 | 1.33 | 0.34 | 0.64 | 0.37 | 0.63 |
| 42d        | 1.00 | 0.98 | 1.12 | 1.17 | 1.12 | 1.33 | 0.34 | 0.64 | 0.37 | 0.63 |
| TLR4       | 21d | 1.00 | 1.24 | 1.23 | 1.42 | 1.51 | 1.28 | 0.37 | 0.47 | 0.04 | 0.13 |
| 42d        | 1.00 | 1.17 | 1.61 | 1.57 | 1.70 | 1.68 | 0.58 | 0.25 | 0.01 | 0.04 |
| IL-1β      | 21d | 1.00 | 0.84 | 0.79 | 0.86 | 0.97 | 1.31 | 0.21 | 0.03 | 0.90 | 0.21 |
| 42d        | 1.00 | 0.87 | 0.44 | 0.46 | 0.46 | 0.64 | 0.22 | <0.01 | <0.01 | <0.01 |
| IL-2       | 21d | 1.00 | 0.93 | 0.90 | 0.89 | 0.86 | 0.99 | 0.33 | 0.98 | 0.43 | 0.72 |
| 42d        | 1.00 | 1.00 | 1.01 | 1.16 | 1.19 | 1.35 | 1.35 | 0.14 | 0.01 | <0.01 | <0.01 |
| IL-6       | 21d | 1.00 | 0.79 | 0.76 | 0.84 | 0.82 | 0.67 | 0.35 | 0.82 | 0.52 | 0.61 |
| 42d        | 1.00 | 0.65 | 0.50 | 0.43 | 0.42 | 0.42 | 0.41 | <0.01 | 0.53 | 0.53 | 0.19 |

Note: a, b and c Means within the same line that do not share a common superscript are significantly different (P<0.05).
TLR: Toll-like receptor; MyD88: myeloid differentiation factor 88; Rac1: Ras-related C3 botulinum toxin substrate 1; PI3K: phosphatidylinositol 3-kinase; NF-κB: nuclear transcription factor-κB; IL: interleukin; IFN-γ: interferon-gamma; AAP: Artemisia argyi polysaccharide; CTC: chlortetracycline, SEM: standard error of the mean.

0.05) and showed a linear or quadratic decrease effect with the increase of AAP dose. On d 21, the liver TLR4 gene expression of 1000 mg/kg AAP addition groups was significantly higher than that of the control (P = 0.05), and the difference was not significant compared with the chlortetracycline group. On d 21, the gene expression of IL-1β in the chlortetracycline group was significantly higher than that in all other groups (P < 0.05). When AAP was added at 500 mg/kg, the relative gene expression of IL-1β was significantly higher than that in the control group (P < 0.05), but the difference was not significant compared with the chlortetracycline group. On d 42, the relative gene expression of IL-2 in the 1000 mg/kg AAP group was significantly higher than that in the control group (P < 0.05), but the difference was not significant compared with that in the chlortetracycline group, and showed a linear or quadratic lowering effect with an increasing AAP dose. On d 42, the relative gene expression of IL-6 in the AAP-added groups was significantly lower than that in the control group (P < 0.05).

Antioxidant capacity

The effects of Artemisia argyi polysaccharide dietary supplementation on serum antioxidant indexes in broilers are shown in Table 9. On d 21, there was no significant difference in the content or activity of T-SOD, MDA, CAT, T-AOC and GPx between AAP groups and the control or chlortetracycline group (P > 0.1). On d 42, the GPx activity of 1000 mg/kg AAP groups was significantly higher than that of the control group (P < 0.05), and was not significantly different from that of the chlortetracycline group, and showed a linear or quadratic effect with the increase of AAP additive. The MDA content of AAP groups was significantly lower than that of the control group (P < 0.05), and was not significantly different from that of the chlortetracycline group, and showed a linear or quadratic decrease with the increase of AAP additive.

The effects of AAP on the antioxidant indexes of broiler liver are presented in Table 10. On d 21, the T-SOD activity of 500, 750 and 1000 mg/kg AAP groups was significantly higher than that of the control group (P < 0.05), but not significantly different from that of the CTC group, and showed a linear or quadratic increase effect with the increase of AAP additive amount. The CAT activity of 750 and 1000 mg/kg AAP groups was significantly higher than that of the control group (P < 0.05), but the difference was not significant compared with that of the CTC group and showed a linear or quadratic effect with the increase in the amount of AAP additive. When AAP was added at 1000 mg/kg, the T-AOC was significantly higher than that of the control group (P < 0.05), but the difference was not significant compared with that of the CTC group, and showed a linear effect with the increase of AAP additive.
the T-AOC was higher than that of the control and chlortetracycline groups on d 42 (P = 0.05).

Antioxidant gene mRNA expression levels

The effects of AAP dietary inclusion on the expression of antioxidant enzyme genes in the liver of broilers are demonstrated in Table 11. On d 21, the expression of antioxidant enzyme genes in the liver of AAP groups had no significant change compared with the control and chlortetracycline groups. On d 42, the expression of CAT genes in 500, 750 and 1000 mg/kg AAP groups increased significantly compared to the control group (P < 0.05), and showed a linear or quadratic effect with increasing AAP supplemental level.

Table 9. Effects of AAP on serum antioxidant indexes of broilers.

| Item                  | AAP supplemental level mg/kg | CTC mg/kg | SEM | ANOVA | Linear | Quadratic |
|-----------------------|-------------------------------|-----------|-----|-------|--------|-----------|
| GPx U/mL              | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| T-SOD U/mL            | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| MDA nmol/mL           | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| CAT U/mL              | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| T-AOC mmol/mL         | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |

Note: a, b and c Means within the same line that do not share a common superscript are significantly different (P < 0.05).

Table 10. Effects of AAP on liver antioxidant indexes of broilers.

| Item                  | AAP supplemental level mg/kg | CTC mg/kg | SEM | ANOVA | Linear | Quadratic |
|-----------------------|-------------------------------|-----------|-----|-------|--------|-----------|
| GPx U/mg prot.        | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| T-SOD U/mg prot.      | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| MDA mmol/mg prot.     | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| CAT U/mg prot.        | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| T-AOC mmol/mg prot.   | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |

Note: a, b Means within the same line that do not share a common superscript are significantly different (P < 0.05).

Table 11. Effects of AAP on the expression of antioxidant-related genes in the liver of broilers.

| Item | AAP supplemental level mg/kg | CTC mg/kg | SEM | ANOVA | Linear | Quadratic |
|------|-------------------------------|-----------|-----|-------|--------|-----------|
| CAT  | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| SOD  | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |
| GPx7 | 0 250 500 750 1000             | 0 250 500 |     |       |        |           |

Note: a, b Means within the same line that do not share a common superscript are significantly different (P < 0.05).

AAP: Artemisia argyi polysaccharide, CTC: chlortetracycline, SEM: standard error of the mean.
dose, but the difference was not significant compared to the CTC group.

**Discussion**

In recent years, the use of polysaccharides in feed as prebiotics and growth promoters has received much attention in poultry farming. Functional polysaccharides are indigestible due to the presence of β-1,4 bonds in the polysaccharide structure (Guo et al. 2020). Functional polysaccharides for feeding and medicinal purposes could regulate intestinal flora and improve health, thereby enhancing the growth performance of broiler chicks (Wu 2018; Yadav and Jha 2019; Liu et al. 2020). A previous study showed that the addition of algal polysaccharides to the diet could promote the growth performance of broilers, which was similar to our results (Guo et al. 2020). ADFI (d 1–21) was decreased in the 750 mg/kg of AAP. ADG (d 22–42) was increased in the 1000 mg/kg of AAP and F/G was improved in all AAP groups within d 22–42, but only in the 500 and 750 mg/kg of AAP within d 1–42. It is suggested that AAP has a more pronounced effect on ADG later in the experiment. The optimal amount addition of 750 mg/kg of AAP in the diet significantly improved the growth performance of broilers and was not significantly different from the chlorotetracycline group. Li et al. (2017) also observed that the addition of 0.5% of *Enteromorpha prolifera* polysaccharides to the diet could improve the ADG and FCR of broilers by enhancing immune function. Li et al. (2019a) found that broilers fed a diet containing 30 mg/kg chitosan had higher FCR than the control group. Similarly, Huang et al. (2005) and Li et al. (2007) found that the addition of 150 or 100 mg/kg of chitosan increased the average daily weight gain and feed conversion ratio of broilers. However, Biggs and Parsons (2008) and Shang et al. (2015) observed that feeding diets containing natural polysaccharides had no significant effect on the growth performance of broilers. Differences in response to plant polysaccharides from one trial to another may be related to the degree of polymerization of the polysaccharide, purity, amount added, time and conditions of the trial and the source of the polysaccharide. All the above evidence suggests that *Artemisia argyi* polysaccharide is a potential prebiotic candidate that can be considered a viable alternative to antibiotic growth promoters.

Immunoglobulin is the main antibody involved in humoral immunity, and its content in serum is one of the important indicators to judge the humoral immune function of animals. In this study, we found that the contents of IgG and IgM in the serum and liver of broilers in the high dose of *Artemisia argyi* polysaccharide group were significantly increased, in the serum the difference was not significant compared with that in the chlorotetracycline group. The contents of IgG and IgM in the liver the difference were significant compared with that in the chlorotetracycline group. This indicated that *Artemisia argyi* polysaccharide had a promoting effect on the humoral immunity of broilers. In a study with mice, it was found that the plant polysaccharide could significantly enhance the activity of B cells, make the body secrete higher levels of tumour-specific IgG antibodies and improve the body’s humoral immunity (Jia 2016). Wan et al. (2013) reported that the addition of plant polysaccharides in the diet could significantly increase the IgG content in the serum of broilers, and the IgA and IgM content in the serum of the high-dose addition groups (1.0 and 1.5 g/kg) were also significantly increased.

Choi et al. (2010) found that rockweed polysaccharides reduced IL-6 levels in the serum of rats with aspirin-induced acute gastric ulcer model and attenuated gastric mucosal injury. Brito et al. (2014) showed that sulfated polysaccharides inhibited the secretion of pro-inflammatory factors TNF-α and IL-1β and attenuated myeloperoxidase (MPO) activity in a rat model of enteritis, thereby reducing intestinal inflammatory cell infiltration and improving intestinal tissue damage. The results of the present study showed that the addition of *Artemisia argyi* polysaccharide in diets increased the levels of cytokines interleukin (IL) and interferon-γ (IFN-γ) in broiler serum, but inhibited the secretion of pro-inflammatory factors IL-1β and IL-6 in the liver, and promoted the secretion of anti-inflammatory factors IL-2 and IL-4 in the liver. In addition, IFN-γ is an important indicator cytokine for initiating cell-mediated immune responses and has broad biological activity against viruses and tumour cells (Park et al. 2008). Qiao et al. (2013) demonstrated that the addition of polysaccharides in diets increased the production of IFN-γ in the serum of weaned piglets. In addition, it has also been reported that the addition of dandelion root extract (the main bioactive components are polysaccharides and flavonoids) and *Astragalus* polysaccharides in diets increased the serum IFN-γ levels in pigs (Yuan et al. 2006; Zhao et al. 2019). It can be concluded that the addition of herbal extracts to diets can improve the immune function of livestock and poultry.

With the development of the poultry industry, broilers are constantly exposed to a variety of external stressors, leading to excessive production of reactive oxygen species (ROS) and disturbing the redox balance in the broiler, resulting in oxidative stress (Lee et al. 2018). Oxidative stress is one of the major adverse factors affecting the performance of broilers and leads to many metabolic disorders. Antioxidant enzymes, such as SOD and CAT, are known for their oxygen radical scavenging properties and, therefore, provide the first line of cellular defence against oxidative damage (Yin et al. 2013; Farag et al. 2019). As an end product of lipid peroxidation, MDA is used as a biomarker to measure the level of oxidative stress (Yang et al. 2008).

Natural polysaccharides are of great interest to researchers in animal nutrition because of their antioxidant biological activity, and it is suggested that the antioxidant effect of polysaccharides in poultry may be attributed to the presence of hemiacetal hydroxyl structures in polysaccharides that scavenge free radicals. It has been found that the addition of plant polysaccharides to the drinking water of broilers could improve their antioxidant capacity (Park et al. 2014). Long et al. (2019) found that the addition of herbal polysaccharides in the diet increased SOD and GPX activities and decreased MDA concentrations in the serum and liver of broilers at 42 days of age. The intestinal mucosa acts as a barrier to the external environment. In addition, it is susceptible to oxidative damage due to the large load on the intestine and high oxidative metabolic rate, which leads to a large release of ROS (Birendra and Rajesh 2019). Thus, the intestine is a key source of reactive oxygen species and is particularly sensitive to
stress compared to other tissues (Mahmood et al. 2018). The results of this study showed that the addition of Artemisia argyi polysaccharide in diets increased the activity of intestinal mucosal antioxidant enzymes (GPx, SOD, CAT) and successfully inhibited lipid peroxidation and reduced MDA content. The results are in agreement with the existing literature on natural polysaccharides. For example, the addition of 350 mg/kg chitosan to broiler diets reduces intestinal mucosal oxidative damage by increasing SOD and GPx enzyme activities and decreasing MDA content (Li et al. 2019a). Aqueous extracts of Artemisia argyi rich in natural polysaccharides, fed to broilers at 500–2000 mg/kg levels, improved total antioxidant capacity, SOD, and GPx activity by reducing MDA levels in small intestinal tissues (Zhao et al. 2016). Hu et al. (2015) investigated the antioxidant properties of Artemisia argyi polysaccharide in vitro. The results of the present study showed that the addition of Artemisia argyi polysaccharide in diets increased GPx, SOD and CAT activity in the liver.

Thus, natural polysaccharides can act as a defense mechanism for the antioxidant status of the broiler and can prevent the uncontrolled formation of ROS or direct scavenging of free radicals. It could be speculated that the increased antioxidant capacity observed in the present study is mainly attributed to the antioxidant properties of Artemisia argyi polysaccharide. This shows that Artemisia argyi polysaccharide can be used as an ideal natural antioxidant in poultry farming. Furthermore, in the present study, the improved antioxidant capacity may be closely associated with improved growth performance.

Polysaccharides isolated from natural plants show biological activities such as immunomodulatory, antiviral, anti-inflammatory, antitumor and antioxidant properties. Toll-like receptors (TLRs) are a group of pathogen recognition receptors expressed in antigen-presenting cells that play an important role in pathogen recognition and initiation of the innate immune response. The involvement of this receptor also triggers the innate immune response and the production of various cytokines (Zhang et al. 2016). Polysaccharide binding to TLR2 and TLR4 activates a variety of signalling pathways, including the PI3K pathway and NF-κB pathway, and mediates a range of intracellular actions.

Artemisia argyi as a natural herbal medicinal plant, rich in polysaccharides, also has many biological activities. Previous studies reported various health-promoting effects of Artemisia argyi extracts in anti-inflammatory, immunomodulatory and scavenging of free radicals. However, the molecular regulatory mechanisms for the effects of Artemisia argyi polysaccharide on the antioxidant and immune function of broilers have not been systematically evaluated. Therefore, this study was conducted to further investigate the possible molecular regulatory mechanisms of AAP supplementation in the diet on the immune and antioxidant functions of broiler chicks.

Immune regulation is an extremely complex process, and moderate immune stimulation helps the animal organism to recover; however, the too strong or persistent inflammatory response can cause some damage to normal cells and tissues. As indicated in the present study, IL-1β content and the relative mRNA expression of IL-1β in the liver mucosa of broilers in the Artemisia argyi polysaccharide group were significantly lower compared with the control group. The reasons for this phenomenon may be the following two aspects: on the one hand, broilers in the control group were affected by persistent high-density environmental and vaccine injection stress to start producing an immune response, which resulted in the production of more cytokines, and too many cytokines are harmful to the organism. On the other hand, the addition of Artemisia argyi polysaccharide could reduce the inflammatory response, resulting in a decrease in the content and expression of inflammatory factors.

Numerous studies have pointed out the immunomodulatory effects of plant polysaccharides, which are also considered to enhance the immune response of the body by modulating the NF-κB signalling pathway. Since plant polysaccharides could down-regulate the mRNA expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) through mechanisms such as inhibition of nuclear transcription factor-activating protein-1 (AP-1) activation, they could inhibit the release of inflammatory mediators nitric oxide and prostaglandin E2 (PGE2) and exert anti-inflammatory effects (Sanjeeewa et al. 2017). It was found that polysaccharides not only reduced the secretion of pro-inflammatory factors IL-6, TNF-α and IL-1β, but also increased the relative mRNA expression of anti-inflammatory factors IL-10 and transforming growth factor-β (TGF-β), thus restoring the balance of cytokines and playing an anti-inflammatory role (Li et al. 2019c). The mRNA expression of IL-2 and IFN-γ in the jejunum was increased by the addition of Astragalus polysaccharide to the diet (Li et al. 2019b). We know that NF-κB plays an active role in the immune regulation of broilers. Under normal physiological conditions, NF-κB binds to IκBα in the cytoplasm, whereas environmental stress leads to altered cellular signalling that induces degradation of IκBα, which, in turn, activates NF-κB and enhances its expression in the nucleus, thereby regulating the expression of downstream inflammatory cytokines (Goel et al. 2021). Cytokines are associated with helper T cells, with Th1 cells secreting mainly IL-2 and IFN-γ, and Th2 cells secreting mainly IL-1β, IL-4, IL-6 and IL-10. The important role of Th1/Th2 balance is maintaining the cellular and humoral immune response. Combined with the above, this study concluded that AAP in diets regulated NF-κB signalling pathway, maintained the Th1/Th2 balance and improved the immunomodulatory function of broilers.

In this study, it was confirmed that the antioxidant capacity of Artemisia argyi polysaccharide from molecular biology, by measuring the relative gene expression of the antioxidant indicators CAT, SOD and GPx7 in the liver. SOD and GPx7 scavenge reactive oxygen species to achieve antioxidant effects. Superoxide is first degraded to hydrogen peroxide by SOD and then converted to water by a series of enzymes such as GPx7 (Vnukov et al. 2017). In this experiment, the addition of Artemisia argyi polysaccharide in diets increased the gene expression of CAT in tissues of broilers, indicating that Artemisia argyi polysaccharide could improve antioxidative enzyme activity. Hu et al. (2015) reported that Artemisia argyi polysaccharide has the ability to scavenge free radicals. Combined with the results of a large body of literature, we infer that Artemisia argyi polysaccharide can enable the organism to improve antioxidative function.
Summary

Under the present experimental conditions, the addition of AAP to broiler diets could significantly improve the growth performance, regulate immune function and enhance the antioxidative capacity of broilers, and the best effect was achieved by adding 1000 mg/kg, and its growth-promoting effect was comparable to that of chlorotetracycline.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethic Approval

The care and use of laboratory animals reported in this study were approved by the Animal Care and Use Committee and the Ministry of Agriculture and the Inner Mongolia Agricultural University. The care and use of laboratory animals reported in this study were approved by the Inner Mongolia Agricultural University. This work was supported by the college of animal science of Inner Mongolia Agricultural University.

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