IN VITRO ANTIOXIDANT ACTIVITY OF *ARTEMISIA ARGYI* POWDER AND THE EFFECT ON HEPATIC AND INTESTINAL ANTIOXIDANT INDICES IN BROILER CHICKENS*

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Abstract

This study was conducted to investigate the *in vitro* and *in vivo* antioxidant effect of *Artemisia argyi* powder (AAP). Two hundred forty mixed-sex one-day-old Arbor Acres broilers were randomly divided into five treatment groups, each consisting of six replicates (one replicate per cage) with eight broilers per replicate. Broilers were fed basal diets supplemented with 0, 2.5, 5, 10 and 20 g AAP per kg feed, respectively. The hepatic and intestinal samples were collected on d 21 and 42 for analysis of antioxidant indices and antioxidative enzyme gene expression. The *in vitro* results showed that the scavenging activity of *Artemisia argyi* against •OH and DPPH were 34.99±1.11% and 74.12±0.50%, respectively; the ferric reducing power was 2.58±0.03%. The *in vivo* results showed that dietary 20 g/kg of AAP significantly enhanced the hepatic total antioxidant capacity (T-AOC), catalase (CAT) activity, and glutathione peroxidase (GSH-Px) activity, also decreased the malondialdehyde (MDA) content; dietary 10 g/kg of AAP significantly increased the gene expression of superoxide dismutase (SOD) and CAT on d 42. For the duodenum, 10 g/kg of AAP increased SOD activity (P<0.05), and reduced MDA level (P<0.05) on d 21; the gene expression of CAT and SOD were increased in the 20 g/kg of AAP treatment compared with the control group on d 42. For the jejunum, on d 21, the T-AOC level was increased by inclusion of 10 g/kg of AAP, and CAT activity was enhanced significantly at 5, 10, and 20 g/kg of AAP group; dietary AAP significantly decreased MDA level at the concentration of 2.5, 5, 10 and 20 g/kg in contrast with control group on d 42; 5 and 20 g/kg of AAP increased the gene expression of SOD on d 21, and the gene expression of GSH-Px was increased (P<0.05) in 10 g/kg of AAP group on d 42. For the ileum, compared to the control group, 2.5 and 20 g/kg of AAP increased SOD activity (P<0.05); and dietary 10 and 20 g/kg of AAP significantly reduced MDA level; dietary 10 g/kg of AAP increased the gene expression of SOD, CAT and GSH-Px in broilers on d 42. In conclusion, dietary AAP could improve the antioxidant defenses of liver and small intestine, and the best concentration of the AAP improving hepatic and small intestinal antioxidant status was 20 g/kg and 10 g/kg, respectively.

Key words: *Artemisia argyi* powder; antioxidant, liver, small intestine, broiler

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In the animal body, the liver and intestine are the major organs involved in the digestion, absorption, and metabolism of dietary nutrients, and this process can lead to the accumulation of reactive oxygen species (ROS) and free radicals in the intestinal mucosa, liver, and peripheral sites of metabolism (Surai and Fisinin, 2015). In certain concentrations, ROS play important roles in regulating many cellular functions and acting as secondary messengers to activate specific transcription factors (Valko et al., 2007). However, excessive ROS could cause a breakdown of membrane phospholipids and initiate lipid oxidation, probably resulting in a functional alteration of internal organs and leading to widespread damage in broiler chickens (Lambeth, 2004; Liu et al., 2014). To prevent ROS-associated damage, the body has enzymatic and nonenzymatic antioxidant systems. The antioxidative enzymes mainly include catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), and the nonenzymic system includes water-soluble vitamin C, lipid-soluble vitamin E, selenium, copper, and zinc. However, these antioxidant systems are too vulnerable to protect these organs from oxidative damage, when these organs are in oxidative stress (Sun et al., 2009). Therefore, it is important to apply production strategies capable of enhancing the antioxidative function of broilers, and one of these strategies is dietary supplementation with exogenous antioxidants, such as vitamins, minerals, or natural plant extracts with antioxidative ingredients, including polysaccharides, flavonoids, and polyphenols (Delles et al., 2014; Hajati et al., 2014; Surai, 2014). Of these antioxidants, natural plant extracts are attracting interest and becoming major additives in poultry feed, due to their lower toxic effects and various biological activities (Salami et al., 2016).

Artemisia argyi, also known as Chinese mugwort, is a traditional herbal medicine used for treating diseases such as cancer, inflammation, hemostasis and hepatitis (Adams et al., 2006; Cai, 2001; Jeong et al., 2007). Phytochemical and pharmacological studies on this plant have revealed several bioactive chemicals such as polysaccharides, flavonoids, essential oils and triterpenoids, which have been shown to exhibit strong antioxidant and free-radical scavenging activity (Diaz et al., 2012; Huang et al., 2012; Lan et al., 2010; Zhang et al., 2013). Although the above studies have demonstrated the antioxidative activity of *Artemisia argyi*, the relevant studies were mostly limited to *in vitro* testing; scant literature is available about the antioxidative activity of *Artemisia argyi* in the animal body. Recently, our laboratory completed preliminary research evaluating the effect of *Artemisia argyi* powder (AAP) on broilers, which reported that AAP enhanced the total antioxidant capacity (T-AOC) and reduced the malondialdehyde (MDA) content of broilers (Cao et al., 2015 a). Based on that finding, we carried out further research to determine whether dietary AAP modulates antioxidative status in the small intestine and liver. Therefore, the present study was conducted to examine the T-AOC, the specific activity of antioxidant enzymes such as CAT, SOD, and GSH-Px, and the level of MDA in the small intestine and liver of broilers. Additionally, qRT-PCR analyses were carried out to examine whether the gene expression of the antioxidant enzymes was influenced by AAP.

Material and methods

Preparation of AAP

Artemisia argyi was collected from Hohhot (Inner Mongolia, China) in July; whole plants were sorted, cleaned with distilled water, and dried at ambient temperature (22–25°C) in the shade. The dried materials were then cut into 1–2 cm pieces, crushed by a grinder, and sieved through an 80-mesh sieve to obtain the powder (AAP), which was then stored at ambient temperature (22–25°C) pending its use.

Analysis of AAP

Before mixing with basal diet, the water extract of *Artemisia argyi* (WEAA) was prepared to determine the main components and antioxidant capacity *in vitro*. Total polysaccharide was determined by the phenol-sulfuric acid method using D-glucose as a standard (Saha and Brewer, 1994); the result was expressed as D-glucose equivalents (mg GE/g). Total flavonoid and phenol were measured according to the methods reported previously (Wan et al., 2016); the results were expressed as rutin equivalents (mg RE/g) for flavonoid and gallic acid equivalents for phenol (mg GAE/g), respectively. The antioxidant capacities were evaluated by scavenging activities against hydroxyl radical ($\bullet\text{OH}$) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), as well as ferric reducing antioxidant power (FRAP) test (Shen et al., 2017).

Experimental design, diets, and management

The experiment was conducted in the experimental farm of Inner Mongolia Agricultural University (Hohhot, China). All animal procedures were performed under the national standard Guideline for Ethical Review of Animal Welfare (GB/T 35892-2018).

A total of 240 mixed-sex 1-d-old Arbor Acres broilers were distributed according to a single factorial arrangement. All broilers were randomly divided into five treatment groups, each of which consisted of six replicates (one replicate per cage) with eight broilers per replicate. Treatments included one control group (broilers fed with a basal diet) and four AAP groups (broilers fed with a basal diet supplemented with 2.5, 5, 10, or 20 g AAP per kg feed). The maize- and soybean-based basal diet (Table 1), which was formulated to meet the nutritional requirements of broilers according to the National Research Council (1994) and Agricultural Industry Standards of P.R. China (Feed Standard of Chicken, NY/T 33-2004), was used throughout the 42-d trial, including the starter (d 1–21) and grower (d 22–42) periods. During the experimental period, broilers were housed in metal cages (0.8 m $\times$ 0.8 m $\times$ 0.5 m). Infrared heating devices were installed in the chicken house to maintain the temperature automatically. The temperature was set at 33°C for the first 3 d and then gradually reduced at a rate of 3°C per week to a final temperature of 21°C. The lighting regime was 23 h light/1 h dark for the first three days, followed by 18 h light/6 h dark for the rest of the trial period. Feed and water were provided *ad libitum* throughout the experiment. The vaccination program was made as follows:

Age of broilers	Vaccine name	Inoculation mode
3-day-old	Newcastle disease and infectious bronchitis combined live vaccine	Eye drops and nose drops
20-day-old		
13-day-old	Medium-virulence strains of infectious bursal live vaccine	Drinking water
24-day-old		
16-day-old	Avian influenza vaccine	Intramuscular injection

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

Ingredient	d 1–21	d 22–42
Corn	51.68	58.49
Soybean meal	41.00	34.30
Soybean oil	3.00	3.00
Dicalcium phosphate	1.90	1.80
Limestone	1.10	1.20
Salt	0.37	0.37
Lysine (98%)	0.05	0.03
Methionine	0.19	0.10
Premix ¹	0.71	0.71
Total	100	100
Nutrient levels ²		
Crude protein	21.84	19.95
ME (MJ/kg)	12.62	12.87
Calcium	1.00	1.00
Available phosphorus	0.48	0.46
Lysine	1.40	1.20
Methionine	0.56	0.44
L-cystine	0.40	0.37

¹Composition of premix (per kg of feed): vitamin A – 6141.5 IU; vitamin D₃ – 1789.2 IU; vitamin E – 7.99 mg; vitamin K – 1.82 mg; vitamin B₁ – 0.65 mg; vitamin B₂ – 3.93 mg; vitamin B₆ – 2.08 mg; vitamin B₁₂ – 0.01 mg; niacin – 18.06 mg; calcium pantothenate – 6.65 mg; folic acid – 0.59 mg; biotin – 0.07 mg; choline chloride – 332.28 mg; Fe – 60.91 mg; Cu – 6.01 mg; Zn – 65.75 mg; Mn – 62.3 mg; I – 0.9 mg; Se – 0.21 mg.

²Crude protein was measured value, while others were calculated values.

Sampling and measurements

Collection of liver and small intestine

On d 21 and 42, 12 broilers per treatment (2 broilers per replicate) were randomly selected and euthanized by cervical dislocation. Each broiler's liver was collected and placed in a cryogenic vial after being washed with ice-cold sterile saline solution to remove blood contamination. The small intestine was dissected, and a 7-cm intestinal segment was collected from the distal duodenum, mid-jejunum, and mid-ileum. The intestinal segments were opened longitudinally, and the contents were rinsed gently with sterile saline and then placed in cryogenic vials. All samples were stored at –80°C until analysis of antioxidative indexes and relative gene expression.

Preparation of the tissue homogenates

About 0.5 g of liver or small intestine was used to prepare the homogenate. The minced tissue was homogenized in an ice-cold 0.9% sodium chloride solution (w/v, 1:9) using a glass homogenate tube and then centrifuged at $1346\times g$ for 10 min at room temperature, and the supernatant was collected and stored at -80°C until further analysis.

Biochemical assay determination of liver and small intestine

The T-AOC, SOD, CAT, and GSH-Px activities and MDA content in both the liver and the small intestine were determined spectrophotometrically using a commercial kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China), according to the manufacturer's instructions. Briefly, T-AOC activity was measured using the ferric reducing/antioxidant power assay described by Benzie and Strain (1999), and the change in absorbance was detected at 520 nm; SOD activity was measured by the xanthine and xanthine oxidase/cytochrome *c* method (McCord and Fridovich, 1969), and the change in absorbance was detected at 550 nm; CAT activity was measured by the rate of H_2O_2 disappearance according to the method of Aebi (1984), and the change in absorbance was detected at 405 nm; GSH-Px activity was determined at an absorbance of 412 nm with the dithio-nitro benzene method described by Hafeman et al. (1974); and the concentration of MDA was assayed by measuring TBA-reactive substances with a spectrophotometer at 532 nm, as described by Placer et al. (1966). The activities of T-AOC, SOD, CAT, and GSH-Px in tissue homogenates were expressed as U per milligram of protein; the tissue homogenate MDA content was expressed as nanomoles per milligram of protein. The protein concentrations of the tissue homogenates were determined by using the bicinchoninic acid assay (Walker, 2009).

Quantitative real-time PCR

The mRNA levels of the antioxidative enzymes (SOD, CAT, and GSH-Px) in the liver and small intestine were determined by the SYBR Green II chimeric fluorescence method (Zhang et al., 2014). Total RNA was extracted from tissue samples using the RNAiso Plus kit (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's protocol. The RNA integrity was checked using agarose gel electrophoresis with ethidium bromide staining. The RNA concentration and purity were determined using an automatic microplate reader (Synergy H4, BioTek, Tokyo, Japan) at an OD 260/280 reading (ratio 1.8–2.1). After determining the RNA concentration, 1 μg of total RNA was reverse-transcribed into complementary DNA (cDNA) using the PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa Biotechnology). Real-time PCR was performed with an ABI StepOnePlus Real-Time PCR system (Applied Biosystems, Grand Island, NY) using the SYBR Premix Ex Taq II Reagent Kit (TaKaRa Biotechnology), according to the manufacturer's instructions with a modification (Zhang et al., 2018). Briefly, the volume of the reaction system is 20 μL , including 10 μL of SYBR Premix Ex Taq II, 0.4 μL of PCR forward primer (10 μM), 0.4 μL of PCR reverse primer (10 μM), 2 μL of cDNA, and 7.2 μL of RNase-free H_2O . The PCR reaction procedure was as follows: a pre-run at 95°C for

30 s and 40 cycles of denaturation at 95°C for 5 s, followed by an annealing step for 30 s (different annealing temperatures for different target genes). The melting curve program was as follows: one cycle of denaturation at 95°C for 10 s, followed by an increase in temperature from 70 to 95°C at a rate of 0.5°C/s. The primer sequences of the target and reference genes (SOD, CAT, GSH-Px, and β -Actin), obtained from GenBank, are shown in Table 2. The relative levels of mRNA expression were calculated using the $2^{-\Delta\Delta CT}$ method after normalization against the reference gene β -Actin (Livak and Schmittgen, 2001).

Table 2. Genes and their primer sequences

Gene name ¹	Gene number	Primer sequence ²	Fragment size
β -Actin	NM_205518	F-GCCAACAGAGAGAAGATGACAC R-GTAACACCATCACCAGAGTCCA	140 bp
SOD	NM_205064.1	F-TTGCTCTGATGGAGATCATGGCTTC R-TGCTTGCCTTCAGGATTAAAGTGA	98 bp
CAT	NM_001031215.1	F-GTTGGCGGTAGCAGTCTGGTCT R-GTGGTCAAGGCATCTGGCTTCTG	182 bp
GSH-Px	NM_001163245.1	F-CAAAGTTGCGGTCAGTGGA R-AGAGTCCCAGGCCTTTACTACTTTC	136 bp

¹CAT=catalase; SOD=superoxide dismutase; GSH-Px=glutathione peroxidase.

²F=forward primer; R=reverse primer.

Statistical analysis

All data were analyzed using SAS 9.0 software (SAS, 2003) to determine the statistical significance of the treatment groups. Results were expressed as treatment means and SEM. Compatibility of characteristics distribution with normal distribution was examined with Shapiro-Wilk's test. Orthogonal comparison was used to evaluate the linear and quadratic effects of AAP on the various antioxidative responses, and the significant levels were expressed with P-value. In addition, Duncan's multiple comparisons test was used to identify the significant difference between treatment groups, and the significant level was differentiated with different letters (a, b). A probability level of $P < 0.05$ was chosen as the limit for statistical significance.

Results

Main content and antioxidant activities (*in vitro*) of AAP

The extraction rate of WEAA (Table 3) was 73.9 ± 0.20 mg per g of *Artemisia argyi* powder. The contents of total polysaccharide, flavonoid and phenol in WEAA were 93.8 ± 0.31 mg GE per g, 109.7 ± 0.92 mg RE per g and 51.0 ± 0.13 mg GAE per g, respectively. The scavenging activity of WEAA against $\bullet$ OH and DPPH were $34.99 \pm 1.11\%$ and $74.12 \pm 0.50\%$, respectively; the ferric reducing power was $2.58 \pm 0.03\%$.

The hepatic antioxidant status

As shown in Table 4, on d 42, the hepatic T-AOC was improved linearly ($P=0.01$) or quadratically ($P=0.04$) with the increase of AAP supplementation, and the greatest level was observed in the 20 g/kg AAP group which was significantly increased compared with the control group ($P<0.05$). Adding 20 g/kg AAP in diet increased the hepatic CAT activity of broilers on d 21 ($P<0.05$), and the CAT activity was elevated linearly ($P=0.02$ on d 21 and $P=0.01$ on d 42) or quadratically ($P=0.04$ on d 42) by the inclusion of AAP. In addition, on d 21, diets supplemented with 5, 10, 20 g/kg of AAP significantly enhanced the hepatic GSH-Px activity compared with the control diet, and the GSH-Px activity showed a quadratic dose-dependent effect ($P=0.02$). In contrast with control group, dietary 20 g/kg AAP significantly reduced the hepatic MDA level on d 42, and the MDA level showed the linear or quadratic dose-dependent effect ($P=0.04$).

Table 3. Main chemical components and antioxidant activity of water extract of *Artemisia argyi* (WEAA)[†]

Items	Results
Extraction rate of WEAA ¹	73.9±0.20
Total polysaccharide (mg GE/g)	93.8±0.31
Total flavonoid (mg RE/g)	109.7±0.92
Total phenol (mg GAE/g)	51.0±0.13
Ferric reducing power (%)	2.58±0.03
•OH scavenging power ² (%)	34.99±1.11
DPPH clearance rate ³ (%)	74.12±0.50

[†]Each value is expressed as mean±SD ($n=3$).

¹Extraction rate = Weight of AAE dry powder / weight of *Artemisia argyi* plant powder (mg/g).

²•OH, Hydroxyl radical.

³DPPH, 2, 2-diphenyl-1-picrylhydrazyl.

Table 4. Effect of AAP on hepatic antioxidant of broilers

Items ¹	AAP supplemental level (g/kg)					SEM ²	P-value ³		
	0	2.5	5	10	20		P	Lin-P	Quad-P
1	2	3	4	5	6	7	8	9	10
T-AOC									
21 d	1.26	1.45	1.86	1.58	1.69	0.18	0.38	0.37	0.41
42 d	1.22 b	1.54 ab	1.47 ab	1.55 ab	1.89 a	0.15	0.13	0.01	0.04
SOD									
21 d	169.72	241.47	295.64	220.95	229.85	24.79	0.23	0.97	0.73
42 d	226.94	227.14	215.06	240.08	275.89	23.45	0.79	0.21	0.43
CAT									
21 d	18.58 b	22.20 ab	26.38 ab	26.82 ab	38.57 a	5.12	0.23	0.02	0.06
42 d	34.03	31.12	31.14	39.19	41.95	3.04	0.08	0.01	0.04

Table 4 – contd.

1	2	3	4	5	6	7	8	9	10
GSH-Px									
21 d	13.20 b	17.17 b	47.90 a	38.12 a	35.23 a	5.89	<0.01	0.15	0.02
42 d	24.78	26.63	22.23	34.07	28.17	2.95	0.08	0.23	0.27
MDA									
21 d	0.72	0.62	0.57	0.40	0.45	0.11	0.60	0.15	0.23
42 d	1.97 a	1.69 ab	1.48 ab	1.42 ab	1.22 b	0.19	0.18	0.02	0.04

a, b– within the same row, different letters indicate significant differences between groups ($P<0.05$).
¹AAP = *Artemisia argyi* powder; T-AOC (U/mgprot) = total antioxidant capacity; SOD (U/mgprot) = super dismutase; CAT (U/mgprot) = catalase; GSH-Px (U/mgprot) = glutathione peroxidase; MDA (nmol/mgprot) = malondialdehyde.
²SEM = standard error of the mean.
³P represents the differences between five treatment groups analyzed by one-way ANOVA; Lin-P and Quad-P represent the dose-dependent effect of AAP tested by orthogonal comparisons.

The hepatic gene expressions of antioxidative enzymes are shown in Figure 1. Compared with control group, diet supplemented with 10 g/kg of AAP significantly increased the gene expression of SOD and CAT on d 42.

Figure 1. Effect of dietary AAP on the relative gene expression of CAT, SOD and GSH-Px in the liver of broilers. Results are presented as mean±SD ($n=6$). Each bar carrying different letters (a, b) was significantly different ($P<0.05$)

The small intestinal antioxidant status

Duodenum

With the increase in dietary AAP, SOD activity was enhanced quadratically in the duodenum ($P=0.03$) on d 21 (Table 5). In addition, compared to the control group, 10 g/kg of AAP increased the duodenal SOD activity on d 21 ($P<0.05$); the gene expression was increased both on d 21 and on d 42 (Figure 2 A). Diet supplemented with 10 g/kg of AAP reduced the duodenal MDA level of broilers on d 21 ($P<0.05$);

the dose-dependent effect analysis showed that diet with AAP quadratically reduced the MDA content in the duodenum ($P=0.03$). Although the increased CAT activity was not observed, the gene expression was increased in the 20 g/kg of AAP treatment compared with the control group on d 42 ($P<0.05$, Figure 2 A).

Table 5. Effect of AAP on small intestinal antioxidant of broilers

Items		AAP supplemental level (g/kg)					SEM	P-value		
		0	2.5	5	10	20		P	Lin-P	Quad-P
1	2	3	4	5	6	7	8	9	10	11
Duodenum	T-AOC									
	21 d	1.09	1.03	0.95	0.96	1.01	0.07	0.66	0.64	0.34
	42 d	0.88	0.82	0.96	0.93	0.90	0.06	0.63	0.60	0.63
	SOD									
	21 d	217.45 b	245.63 ab	258.66 ab	304.48 a	256.26 ab	19.61	0.12	0.36	0.03
	42 d	225.97	298.94	259.85	266.64	254.24	18.21	0.19	0.69	0.73
	CAT									
	21 d	1.27	1.84	2.06	1.55	1.51	0.27	0.69	0.87	0.71
	42 d	0.84	0.91	0.91	0.78	1.07	0.13	0.88	0.46	0.65
	GSH-Px									
	21 d	6.00	7.05	10.04	7.47	7.86	1.00	0.09	0.67	0.41
	42 d	6.32	5.26	6.02	5.74	6.14	0.46	0.62	0.84	0.73
	MDA									
	21 d	1.05 a	0.91 ab	0.73 ab	0.65 b	0.69 ab	0.10	0.15	0.06	0.03
	42 d	1.64	1.41	1.66	1.22	1.11	0.21	0.40	0.08	0.22
Jejunum	T-AOC									
	21 d	2.35 b	2.84 ab	3.76 ab	4.40 a	2.51 b	0.64	0.38	0.96	0.03
	42 d	0.67	0.66	0.73	0.79	0.81	0.06	0.14	0.05	0.08
	SOD									
	21 d	193.11 ab	178.26 b	193.14 ab	192.67 ab	214.94 a	9.87	0.17	0.43	0.33
	42 d	38.04 c	49.88 ab	54.27 a	41.03 bc	41.45 bc	3.50	0.01	0.04	0.08
	CAT									
	21 d	2.19 b	2.37 b	3.69 a	3.80 a	4.72 a	0.50	<0.01	<0.01	<0.01
	42 d	1.89	1.85	2.14	2.08	2.30	0.39	0.96	0.47	0.77
	GSH-Px									
	21 d	15.16	10.30	18.44	19.27	11.36	2.98	0.35	0.86	0.34
	42 d	6.58	5.29	5.13	7.21	8.87	1.14	0.24	0.04	0.11
	MDA									
	21 d	1.53	1.53	1.18	1.10	0.99	0.17	0.10	0.01	0.03
	42 d	0.60 a	0.41 b	0.40 b	0.31 b	0.41 b	0.06	0.06	0.21	0.02

Table 5 – contd.

1	2	3	4	5	6	7	8	9	10	11
Ileum	T-AOC									
	21 d	2.92	2.27	3.32	2.96	2.25	0.33	0.11	0.43	0.30
	42 d	0.92	1.16	1.03	1.19	1.03	0.12	0.59	0.81	0.46
	SOD									
	21 d	70.55	73.81	78.49	85.10	79.81	6.98	0.76	0.35	0.38
	42 d	173.65 c	219.94 ab	190.39 c	196.09 bc	226.33 a	10.56	0.01	0.03	0.09
	CAT									
	21 d	1.49	1.51	2.04	2.09	2.26	0.22	0.12	0.02	0.04
	42 d	2.63	2.47	2.20	2.07	2.63	0.26	0.73	0.86	0.34
	GSH-Px									
	21 d	11.73	17.36	17.70	17.26	17.24	2.37	0.57	0.43	0.41
	42 d	14.15	18.95	18.11	15.97	14.45	1.40	0.22	0.34	0.29
	MDA									
	21 d	1.40	1.31	1.35	1.28	1.36	0.18	0.99	0.97	0.94
	42 d	0.79 a	0.74 a	0.58 ab	0.46 b	0.44 b	0.09	0.04	0.01	0.01

a, b – within the same row, different letters indicate significant differences between groups ($P<0.05$).
¹AAP = *Artemisia argyi* powder; T-AOC (U/mgprot) = total antioxidant capacity; SOD (U/mgprot) = super dismutase; CAT = catalase; GSH-Px(U/mgprot) = glutathione peroxidase; MDA (nmol/mgprot) = malondialdehyde.
²SEM = standard error of the mean.
³P represents the differences between five treatment groups analyzed by one-way ANOVA; Lin-P (linear) and Quad-P (quadratic) represent the dose-dependent effect of AAP tested by orthogonal comparisons.

Figure 2. Effect of dietary AAP on the relative gene expression of CAT, SOD and GSH-Px in the small intestine of broilers. (A) The gene expression in duodenum; (B) The gene expression in jejunum; (C) The gene expression in ileum. Results are presented as mean±SD ($n=6$). Each bar carrying different letters (a, b) was significantly different ($P<0.05$)

Jejunum

As shown in Table 5, on d 21, compared with the control group, the diet with AAP quadratically increased the T-AOC of broilers ($P=0.03$), and the greatest level was observed in the 10 g/kg AAP group ($P<0.05$). The SOD activity was linearly increased ($P=0.04$) with the increase of AAP on d 42, but the significant differences were not found as compared to the control group. Surprisingly, the gene expression of SOD was increased by 5 and 20 g/kg of AAP on d 21 ($P<0.05$; Figure 2 B), but not on d 42. In terms of the CAT activity, compared with the control group, the significant differences were observed in 5, 10, and 20 g/kg of AAP groups. The inclusion of AAP linearly or quadratically elevated CAT activity on d 21 ($P<0.01$). On d 42, GSH-Px activity in the jejunum was linearly increased ($P=0.04$) by the inclusion of AAP; the gene expression of GSH-Px was increased ($P<0.05$; Figure 2 B) in 10 g/kg of AAP treatment group, but there was no significant difference for GSH-Px activity between the treatment groups. MDA level was decreased linearly ($P=0.01$ on d 21) or quadratically ($P=0.03$ on d 21; $P=0.02$ on d 42) with the increase of AAP supplementation. Dietary AAP significantly decreased MDA level at the concentration of 2.5, 5, 10 and 20 g/kg on d 42.

Ileum

With the increase in dietary AAP, the SOD activity in ileum was enhanced linearly ($P=0.03$) on d 42 (Table 5). In addition, compared to the control group, 2.5 and 20 g/kg of AAP increased the ileal SOD activity ($P<0.05$). The inclusion of AAP linearly ($P=0.02$) or quadratically ($P=0.04$) elevated CAT activity on d 21. Diets with AAP reduced MDA content linearly ($P=0.01$) or quadratically ($P=0.01$) on d 42, and dietary 10 and 20 g/kg of AAP significantly reduced MDA level compared with the control diet. As for the gene expression (Figure 2 C), diet with 10 g/kg of AAP increased the gene expression of SOD, CAT and GSH-Px in broilers on d 42 ($P<0.05$). In addition, GSH-Px gene expression was also increased by inclusion of 5 and 20 g/kg AAP.

Discussion

In living animals, the balance between antioxidants and pro-oxidants (redox homeostasis) is an important physiological process, and dietary antioxidants can help maintain this balance in the animal body (Surai and Fisinin, 2015). The analysis of bioactive components in the current study have identified the content of polysaccharides, flavonoids, phenols existing in AAP; the antioxidative effects (*in vitro*) also were proved, which is similar to the results of previous reports (Cai et al., 2004; Lan et al., 2010; Lv et al., 2013). As the main component in AAP was total flavonoid (109.7 ± 0.92 mg rutin equivalents), it is reasonable to speculate that AAP may have the same antioxidant effect as rutin, and can be used as feed additive of chickens (Awad et al., 2019; Hassan et al., 2019). Cao et al. (2015 a) found that dietary AAP could increase the serum T-AOC and reduce the serum MDA content of broilers,

which suggested that AAP also exerts its antioxidative effect in animal body. In addition, the previous study demonstrated that 2.5 g/kg of AAP is the best concentration to increase average daily weight gain of broilers during the grower period, and 5 g/kg of AAP is the optimal dose to improve feed utilization (Cao et al., 2015 b). Thus, the improved growth performance induced by AAP may partly relate to its antioxidant effect on broilers. As the AAP can be absorbed and digested with the diet by the small intestine and liver, we may speculate that AAP also can improve the antioxidant of small intestine and liver.

A recent study demonstrated that the oxidation-reduction process started in the intestine (Surai and Fisinin, 2015). The dietary nutrients absorbed into the small intestine contain plenty of antioxidants but also a range of potential pro-oxidants, and consumption of pro-oxidants may cause oxidative damage to the intestine. It is therefore important to enhance antioxidative capacity in the small intestine. In the present study, dietary AAP enhanced SOD activity in the duodenum alone, whereas jejunal SOD, CAT, and GSH-Px activities were all improved by AAP, and increased SOD and CAT activities were also observed in the ileum, similar to a previous study by Zhao et al. (2016), who reported that dietary *Artemisia argyi* aqueous extract enhanced intestinal antioxidative enzyme activities. In addition, increased relative gene expressions were also observed in the small intestine, but this may partially explain the increased antioxidative enzyme activities, because the variation in relative gene expression was not totally in line with the variation in the enzymatic activities. This was probably because the enzymatic activity was affected by various factors, not just gene expression (Halliwell and Gutteridge, 2015). MDA level in the small intestine was reduced by dietary AAP, which suggested that dietary *Artemisia argyi* could protect the small intestine from damage caused by lipid peroxidation and help maintain redox homeostasis. This benefit might be due to the improved enzymatic activities, or might be attributed to the high antioxidant content of *Artemisia argyi* (Cai et al., 2004; Diaz et al., 2012). Although we observed increased enzyme activity and a compromised MDA level in the small intestine of broilers fed with AAP diets, the increased T-AOC level was observed in the jejunum only, which suggested that AAP had strong effect on antioxidative capacity in the jejunum. Taking all these results together, it is reasonable to assume that dietary *Artemisia argyi* could exert a beneficial effect on antioxidative capacity by enhancing enzymatic activity and directly scavenging the hydroxide radical and superoxide anion radical in small intestine, and then could potentially help maintain redox homeostasis.

The liver is the primary detoxifying organ in animal body, and has a high metabolic rate of nutrients from dietary feed. The potential antioxidants (polysaccharides, flavonoids, phenols) in AAP will reach the liver via the portal vein after absorption along the gastrointestinal tract. Therefore, the potential beneficial effect of antioxidants would take place primarily in liver. In the present study, no improvement of hepatic SOD activity was observed, even though the corresponding mRNA was increased on d 42. This may be because the superoxide anion radical in the liver was directly removed by the potential antioxidants in AAP, not by enhancing SOD activity (Huang et al., 2012). We observed that CAT activity was enhanced throughout the entire experiment, whereas GSH-Px activity was promoted only on d 21. This

suggests that AAP may possess the ability to eliminate H_2O_2 by enhancing CAT or GSH-Px activity, and the enhancing effect on CAT is stronger. Such observations can be explained by the fact that CAT is primarily concentrated in the liver (Halliwell and Gutteridge, 2015). In addition, the increased CAT gene expression on d 42 partially supports this finding, but the increasing tendency of GSH-Px gene expression does not make sense. The increased antioxidative enzyme activity observed in our study would lead to a decrease in the formation of lipid peroxidation products such as MDA, and the compromised MDA level observed on d 42 could be good evidence. We also found that the hepatic T-AOC of broilers was improved by AAP supplementation on d 42, which indicated that AAP showed a certain degree of antioxidative ability, and this was at least partly due to the enhanced activity of CAT and GSH-Px, or the compromised MDA level.

Conclusion

In summary, the present study provided some evidence that AAP supplementation improved antioxidative status in the liver and small intestine of broilers by elevating antioxidative enzyme activity and reducing lipid peroxidative products. Furthermore, AAP increased antioxidative enzyme activity, at least in part, by up-regulating the gene expression of antioxidative enzymes in the liver and small intestine. In addition, the best concentration of AAP improving hepatic and small intestinal antioxidant status was 20 g/kg and 10 g/kg, respectively.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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