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Original research article

# Effects of dietary supplementation of *Artemisia argyi* aqueous extract on antioxidant indexes of small intestine in broilers

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#### ABSTRACT

The present study was conducted to investigate the effect of Artemisia argyi aqueous extract (AAE) on antioxidant indexes in the small intestine. A total of 192 Arbor Acre broiler chickens (one-day-old) were randomly divided into 4 treatments with 6 replicates of 8 chickens. These 4 diets were formulated by adding 0, 500, 1,000 and 2,000 mg/kg AAE to the basal diet. The results showed as follows: 1) compared with the control, the total antioxidant capacity (T-AOC) in ileum for the 2,000 mg/kg treatment group was significantly increased at 21 days of age (P < 0.05); the T-AOC levels in jejunum and ileum were significantly increased in broilers supplemented with 500 mg/kg AAE at 42 days of age (P < 0.05), and the T-AOC levels in jejunum and ileum were significantly improved in 1,000 mg/kg treatment group (P < 0.01). 2) At 21 days of age, supplementation of 500 mg/kg AAE significantly increased the catalase (CAT) activity of small intestine, and the glutathione peroxidase (GSH-Px) activity of jejunum was improved (P < 0.01), meanwhile, the GSH-Px activity of duodenum and the total superoxide dismutase (T-SOD) activity of duodenum and jejunum were significantly higher than those of the control group (P < 0.05); supplementation of 1,000 mg/kg AAE significantly increased the CAT activity of duodenum and ileum and the GSH-Px activity of duodenum and jejunum (P < 0.05), and the ileum GSH-Px activity was significantly increased (P < 0.01); supplementation of 2,000 mg/kg AAE significantly increased the CAT activity of duodenum and ileum (P < 0.05). At 42 days of age, supplementation of 500 mg/kg AAE significantly increased the GSH-Px activity of ileum and the T-SOD activity of duodenum (P < 0.05), meanwhile, the T-SOD activity of jejunum was significantly increased (P < 0.01); supplementation of 1,000 mg/kg AAE significantly increased the CAT activity of jejunum and the T-SOD activity of ileum (P < 0.01), and the GSH-Px activity of jejunum was significantly increased (P < 0.05); supplementation of 2,000 mg/kg AAE significantly increased the T-SOD activity of ileum (P < 0.05), but significantly decreased the CAT activity of ileum and the GSH-Px activity of jejunum (P < 0.05). 3) The malondialdehyde (MDA) levels of 3 AAE supplementation groups were significantly decreased at 21 and 42 days of age (P < 0.05). The results suggested that dietary supplementation with AAE could improve the antioxidative capacity of small intestine in broilers.

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# 1. Introduction

The small intestine is the main organ and place in the digestion and absorption of broiler. Nutrients and water enter the blood and lymph circulation through the intestinal epithelial cells. Therefore, the integrity of the intestinal epithelial cell structure and function is very important to the chicken. However, with the aging of the body, the immunologic function declined and antioxidant capacity gradually weakened from 21 to 42 days in the growth of broiler, resulting in the body's new metabolic dysfunction, free radicals

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generated excess (Shen and Wang, 2000). When free radicals generated too much or radical scavenging ability declined, accumulation of oxygen free radicals will attack on biofilm polyunsaturated fatty acids, leading to lipid peroxidation, producing metabolites malondialdehyde – malondialdehyde (MDA). However, the MDA can cause proteins, nucleic acids and other vital macromolecules cross-linked polymer, and damage the cell membrane fluidity and permeability, thereby enabling the normal function of membrane damage (Guo et al., 2007).

Artemisia argyi, also known as medical grass, Artemisia argyi grass, perennial herbaceous plants of Compositae artemisia, widely distributed in most areas of our country. Artemisia argyi likes warm and wet conditions, born in the wild land margin, having strong cold and drought resistance. Artemisia argyi grows best in fertile and rich humus place (Zhang et al., 2006). Artemisia argyi contains many kinds of bioactive composition such as volatile oil, flavonoids, eudesmane and triterpene (Zhou et al., 2000), also contains polyunsaturated fatty acid, total phenols matter, vitamin C and essential amino acids (Kim et al., 2015). Studies have reported that Artemisia argyi has strong antioxidative and free radical scavenging capacity (Wu, 2010; Tan et al., 2012; Wu and Extraction, 2008). Wu (2010) reported that dried Folium Artemisia argy and fresh mugwort volatile oil within the range of test concentrations enhanced ability of scavenging the free radical 1,1-diphenyl-2-picrylhydrazyl radical (·DPPH), hydroxyl radical (·OH) and superoxide anion free radical  $(O_2^-)$ , and fresh Artemisia argyi essential oil has a stronger ability of scavenging free radical. Hu et al. (2015) demonstrated that different concentrations of Artemisia argyi polysaccharide has the ability to remove  $\cdot OH$ ,  $O_2^-$  and  $\cdot DPPH$ , and between the clearance rate and concentration there is a dose-dependent manner. Wu and Sun (2008) illustrated that flavonoids of Artemisia argyi can effectively remove the  $H_2O_2$ ,  $O_2^-$  and  $\cdot OH$ , and its antioxidant effect is much higher than that of vitamin C. In addition, Chu et al. (2015) reported that broilers supplemented with 1,000 mg/kg Artemisia argyi aqueous extract (AAE) can improve the serum superoxide dismutase (SOD) activity, and the content of malondialdehyde (MDA) in serum decreased. Thus it can be seen that Artemisia argvi antioxidation research mainly concentrate on the volatile oil, polysaccharide, flavonoids and other active ingredients, and AAE on antioxidant research is very rare. Especially, the effect of AAE on antioxidant function of intestinal tissue has not been reported. Therefore, the present study was conducted to investigate the effect of different levels of AAE on antioxidant function of broiler's small intestine, and hope to determine the appropriate supplemental dosage level of AAE in broiler diet.

# 2. Materials and methods

#### 2.1. Materials

Fresh green *Artemisia argyi* was collected from Huhhot in July. All plants were washed with distilled water and dried at room temperature in the shade. The plant was extracted with distilled water, the extraction was concentrated and lyophilized to obtain the powder, stored at -20 °C. The dose used in the experiment was calculated by air dry powder.

#### 2.2. Experiment design and management

This experiment was used single factorial random experiment, a total of 192 Arbor Acre (AA) broiler chickens (one-day-old) were randomly divided into 4 treatments by initial body weight and sex, and each treatment had 6 replicates with 8 chickens (4 females and 4 males) each. These 4r diets were formulated by adding AAE of 0,

500, 1,000 and 2,000 mg/kg to basal diets. The experiment lasted 42 days, containing 2 periods with 21 days each.

# 2.3. Basal diet and nutrition level

The basal diet was formulated according to the nutrient requirements recommended by the NY/T 33-2004 (Agricultural Industry Standards of People's Republic of China) for broilers and actual situation in Inner Mongolia, its composition and nutritional levels are shown in Table 1.

#### 2.4. Feeding and management

The feeding experiment was carried out in Inner Mongolia Agricultural University. The start of the experiment, chicken house, the surrounding environment and test equipment had carried out strict disinfection. Experimental diets and water were available *ad libitum* during the experimental period. A total of 192 Arbor Acre (AA) broiler chickens (one-day-old) of similar body weight were selected and randomly assigned to 1 of 4 dietary treatments with 6 replicate cages of 8 chicks per cage. Each cage was 100 cm × 100 cm (1 m<sup>2</sup> per 8 birds). The temperature was set at 36 °C during the first day, 34 °C during the first week, and was gradually reduced by 3 °C per week to reach a minimum 22 °C at 28 days of age. Relative humidity was between 65% and 75%. During the whole experiment period, air ventilation schedule was maintained. The experiments were conducted in accordance with the guidelines of Animal Care and Use Committee of Inner Mongolia Agricultural University.

# 2.5. Sample collection and processing

#### 2.5.1. Sample collection

Two broilers were chosen randomly from each replicate group at 21 and 42 day of age which had approximately average weight of the group, and the birds were slaughtered by bleeding the left jugular vein. With medical alcohol cleaning and disinfection of the chest and abdomen, the duodenum, jejunum and ileum tissue were

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

Item	1 to 3 weeks	4 to 6 weeks
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
NaCl	0.37	0.37
Lysine (98%)	0.05	0.03
Methionine	0.19	0.10
Premix <sup>1</sup>	0.71	0.71
Total	100	100
Nutrients levels <sup>2</sup>		
ME, MJ/kg	12.62	12.87
Crude protein	21.84	19.95
Crude fat	5.42	5.56
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

<sup>1</sup> Premix provided the following per kilogram of diet: VA 6,141.5 IU; VD<sub>3</sub> 1,789.2 IU; VE 7.99 mg; VK 1.82 mg; VB<sub>1</sub> 0.65 mg; VB<sub>2</sub> 3.93 mg; VB<sub>6</sub> 2.08 mg; VB<sub>12</sub> 0.01 mg; niacin 18.06 mg; calcium pantothenate 6.65 mg; folic acid 0.59 mg; biotin 0.07 mg; holine 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.

<sup>2</sup> Crude protein was measured value, while others were all calculated values.

removed. After rinsing clean with physiological saline, the samples were packaged with tin foil paper and stored at -20 °C until analysis of antioxidant index.

# 2.5.2. Sample processing

The duodenum, jejunum and ileum, which were approximately 3 cm in the middle segment, were taken and rinsed clean with the precooling physiological saline. After filter paper bloted, the samples were accurately weighed and prepared into nine times volume of physiological saline, made of 10% tissue homogenate grinding and slurry at low temperatures. The samples were centrifuged at 1,346 g for 10 min at room temperature, and the supernatant was removed and stored at -20 °C until analysis of antioxidant index.

# 2.6. Testing index and method

Total antioxidant capacity (T-AOC), catalase (CAT), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and Total superoxide dismutase (T-SOD) were measured using commercial assay kits provided by Nanjing Jiancheng Bioengineering Institute: Xanthine oxidase method; GSH-Px: DTNB direct chromatometry; CAT: Ammonium molybdate method; MDA: TBA method), and the total protein content was determined by Coomassie brilliant blue method.

#### 2.7. Statistical analysis

The data were preliminary collated by Excel 2007, and the 4 treatment means were compared by ANOVA, using the Duncan's multiple range tests, and P < 0.05 was considered to be statistically significant, and P < 0.01 was considered to be extremely significant difference.

# 3. Result

# 3.1. Changes of T-AOC level in small intestine

As Table 2 shows, compared with the control, the T-AOC level of ileum in 2,000 mg/kg group was significantly increased at 21 days of age (P < 0.05), while the other group compared with the control group, there was no significant difference between feeding AAE and without AAE. At 42 days of age, the T-AOC level of duodenum showed no significant difference; supplementation of 500 mg/kg AAE significantly increased the T-AOC level of jejunum and ileum (P < 0.05); and the T-AOC level of jejunum and ileum in 1,000 mg/kg group was significantly increased (P < 0.01).

#### 3.2. Changes of CAT activity in small intestine

As Table 3 shows, compared with the control, at 21 days of age, the CAT activity of small intestine in 500 mg/kg group was significantly increased (P < 0.01); supplementation of 1,000 and 2,000 mg/kg AAE significantly increased the CAT activity of duodenum and ileum (P < 0.05). At 42 days of age, the CAT activity of duodenum was no significant difference; broilers fed the diets containing 1,000 mg/kg AAE had higher jejunum CAT activity (P < 0.05); but the CAT activity of ileum in 2,000 mg/kg group was significantly decreased (P < 0.05).

# 3.3. Changes of GSH-Px activity in small intestine

As Table 4 shows, compared with the control, at 21 days of age, supplementation of 500 mg/kg AAE significantly increased the GSH-Px activity of duodenum (P < 0.05) and significantly increased the GSH-Px activity of jejunum (P < 0.01); supplementation of 1,000 mg/kg AAE significantly increased the GSH-Px activity of duodenum and jejunum (P < 0.05) and significantly increased the ileum GSH-Px activity (P < 0.01); but the GSH-Px activity of small intestine in 2,000 mg/kg group showed no significant change. At 42 days of age, the GSH-Px activity of duodenum was not significantly different; the GSH-Px activity of ileum in 500 mg/kg group and the jejunum GSH-Px activity in 1,000 mg/kg group were significantly increased (P < 0.05); but the GSH-Px activity of jejunum in 2,000 mg/kg group was significantly reduced (P < 0.05).

#### 3.4. Changes of T-SOD activity in small intestine

As Table 5 shows, compared with the control, at 21 days of age, supplementation of 500 mg/kg AAE significantly increased the T-SOD activity of duodenum and jejunum (P < 0.05). However, the T-SOD activity of small intestine in 1,000 and 2,000 mg/kg group showed no significant difference. At 42 days of age, supplementation of 500 mg/kg AAE significantly increased the T-SOD activity of duodenum (P < 0.05) and significantly increased the jejunum T-SOD activity (P < 0.01); the T-SOD of ileum in 1,000 mg/kg group was higher than that in the control group (P < 0.01); broilers supplemented with 2,000 mg/kg AAE had higher T-SOD activity of the ileum tissue (P < 0.05).

# 3.5. Changes in the content of lipid peroxidation products in small intestine

As Table 6 shows, the content of MDA in each treatment group was lower than that in the control group. As the statistical data show, compared with the control, at 21 days of age, the MDA content of duodenum in 500 mg/kg addition group was significantly reduced

#### Table 2

Time	Intestinal tissue	Artemisia argyi aqueous extract supplemental levels, mg/kg				
		0	500	1,000	2,000	
21 d	Duodenum Jejunum Ileum	$\begin{array}{c} 1.10 \pm 0.077 \\ 2.39 \pm 0.132 \\ 2.82 \pm 0.159^{\rm b} \end{array}$	$\begin{array}{c} 1.00 \pm 0.061 \\ 2.50 \pm 0.266 \\ 2.44 \pm 0.227^{\mathrm{b}} \end{array}$	$\begin{array}{c} 1.06 \pm 0.127 \\ 2.69 \pm 0.165 \\ 2.71 \pm 0.494^{\rm b} \end{array}$	$\begin{array}{c} 1.01 \pm 0.074 \\ 2.15 \pm 0.589 \\ 3.52 \pm 0.604^{a} \end{array}$	0.276 0.260 0.023
42 d	Duodenum Jejunum Ileum	$\begin{array}{c} 0.91 \pm 0.072 \\ 0.68 \pm 0.090^{c} \\ 0.77 \pm 0.045^{c} \end{array}$	$\begin{array}{c} 0.94 \pm 0.046 \\ 0.86 \pm 0.096^{\rm b} \\ 0.94 \pm 0.140^{\rm b} \end{array}$	$\begin{array}{c} 0.83 \pm 0.091 \\ 1.12 \pm 0.163^{a} \\ 1.12 \pm 0.102^{a} \end{array}$	$\begin{array}{c} - & - \\ 0.82 \pm 0.094 \\ 0.74 \pm 0.059^{bc} \\ 0.90 \pm 0.026^{bc} \end{array}$	0.126 <0.001 0.005

a,b,c In the same row, values with no letter or the same superscript letters mean no significant difference (P > 0.05), while adjacent different letters mean significant difference (P < 0.05), and the interphase letters mean extremely significant difference (P < 0.01).

Table 3
Effects of Artemisia argyi aqueous extract on catalase (CAT) levels (U/mg prot) of broiler's small intestine.

Time	Intestinal tissue	Artemisia argyi aqueous extract supplemental levels, mg/kg				
		0	500	1,000	2,000	
21 d	Duodenum	$0.62 \pm 0.200^{\circ}$	$3.57 \pm 0.468^{a}$	$1.25 \pm 0.179^{b}$	$1.27 \pm 0.433^{b}$	<0.001
	Jejunum	$2.53 \pm 0.695^{bc}$	$4.79 \pm 0.465^{a}$	$3.60 \pm 0.496^{b}$	$2.34 \pm 0.951^{\circ}$	0.003
	Ileum	$1.35 \pm 0.255^{\circ}$	$2.82 \pm 0.065^{a}$	$1.71 \pm 0.100^{b}$	$1.78 \pm 0.160^{b}$	< 0.001
42 d	Duodenum	$0.84 \pm 0.079$	$0.90 \pm 0.286$	$0.69 \pm 0.177$	$0.98 \pm 0.144$	0.284
	Jejunum	$1.94 \pm 0.299^{\circ}$	$1.94 \pm 0.051^{\circ}$	$3.20 \pm 0.363^{a}$	$1.77 \pm 0.286^{\circ}$	< 0.001
	Ileum	$2.72 \pm 0.437^{a}$	$2.39 \pm 0.164^{ab}$	$2.34 \pm 0.294^{ab}$	$1.86 \pm 0.209^{b}$	0.045

a.b.c. In the same row, values with no letter or the same superscript letters mean no significant difference (*P* > 0.05), while adjacent different letters mean significant difference (*P* < 0.05), and the interphase letters mean extremely significant difference (*P* < 0.01).

#### Table 4

Effects of Artemisia argyi aqueous extract on glutathione peroxidase (GSH-Px) activity (U) of broiler's small intestine.

Time	Intestinal tissue	Artemisia argyi aqueous extract supplemental levels, mg/kg				
		0	500	1,000	2,000	
21 d	Duodenum	$6.69 \pm 0.187^{b}$	$8.84 \pm 0.972^{a}$	$9.44 \pm 0.982^{a}$	$7.23 \pm 0.991^{b}$	0.013
	Jejunum	$4.67 \pm 0.436^{\circ}$	$36.20 \pm 1.574^{a}$	$12.02 \pm 2.103^{b}$	$2.13 \pm 0.348^{\circ}$	< 0.001
	lleum	$11.71 \pm 0.795^{\circ}$	$12.94 \pm 1.053^{\circ}$	$26.71 \pm 4.446^{a}$	$13.73 \pm 1.648^{\circ}$	< 0.001
42 d	Duodenum	$7.35 \pm 0.188$	$6.03 \pm 0.927$	$6.38 \pm 0.097$	$7.05 \pm 1.236$	0.228
	Jejunum	$6.67 \pm 0.237^{b}$	$6.89 \pm 1.550^{b}$	$8.94 \pm 1.710^{a}$	$4.80 \pm 0.534^{\circ}$	0.004
	Ileum	15.39 ± 1.611 <sup>bc</sup>	$20.23 \pm 1.771^{a}$	$16.15 \pm 1.512^{b}$	$12.80 \pm 1.581^{\circ}$	< 0.001

<sup>a,b,c</sup> In the same row, values with no letter or the same superscript letters mean no significant difference (P > 0.05), while adjacent different letters mean significant difference (P < 0.05), and the interphase letters mean extremely significant difference (P < 0.01).

#### Table 5

Effects of Artemisia argyi aqueous extract on total superoxide dismutase (T-SOD) activity (U/mg prot) of broiler's small intestine.

Time	Intestinal tissue	Artemisia argyi aqueous extract supplemental levels, mg/kg				P-value
		0	500	1,000	2,000	
21 d	Duodenum Jejunum	$\begin{array}{c} 254.92 \pm 6.779^{b} \\ 124.56 \pm 8.244^{b} \end{array}$	$291.61 \pm 15.718^{a} \\ 154.01 \pm 15.007^{a}$	$270.72 \pm 13.320^{ab} \\ 142.96 \pm 10.905^{ab}$	$268.21 \pm 13.492^{ab} \\ 119.79 \pm 12.302^{b}$	0.041 0.025
42 d	lleum Duodenum	$\begin{array}{c} 58.18 \pm 4.460 \\ 275.63 \pm 4.248^{b} \end{array}$	$\begin{array}{c} 59.59 \pm 9.354 \\ 319.43 \pm 25.423^{a} \end{array}$	$\begin{array}{c} 64.69 \pm 4.451 \\ 296.07 \pm 11.549^{ab} \end{array}$	$\begin{array}{c} 63.49 \pm 10.734 \\ 290.00 \pm 3.588^{b} \end{array}$	0.671 0.032
	Jejunum Ileum	$202.61 \pm 8.602^{\circ}$ 162.72 \pm 6.469^{\circ}	$\begin{array}{l} 251.69 \pm 3.431^{a} \\ 165.49 \pm 29.113^{bc} \end{array}$	$\begin{array}{c} 208.97 \pm 16.011^c \\ 227.90 \pm 4.971^a \end{array}$	$\begin{array}{c} 208.97 \pm 3.713^c \\ 196.87 \pm 9.335^{ab} \end{array}$	<0.001 <0.001

<sup>a,b,c</sup>In the same row, values with no letter or the same superscript letters mean no significant difference (P > 0.05), while adjacent different letters mean significant difference (P < 0.05), and the interphase letters mean extremely significant difference (P < 0.01).

 Table 6

 Effects of Artemisia argyi aqueous extract on malondialdehyde (MDA) levels (nmoL/mg prot) of broiler's small intestine.

Time	Intestinal tissue	Artemisia argyi aqueous extract supplemental levels, mg/kg				
		0	500	1,000	2,000	
21 d	Duodenum Jejunum Ileum	$\begin{array}{c} 1.11 \pm 0.157^{a} \\ 1.60 \pm 0.160^{a} \\ 1.45 \pm 0.060^{a} \end{array}$	$\begin{array}{c} 0.37 \pm 0.073^c \\ 1.55 \pm 0.331^a \\ 1.08 \pm 0.233^a \end{array}$	$\begin{array}{c} 0.59 \pm 0.081^{\rm b} \\ 0.99 \pm 0.172^{\rm b} \\ 0.64 \pm 0.189^{\rm b} \end{array}$	$\begin{array}{r} 0.54 \pm 0.001^{\rm bc} \\ 1.08 \pm 0.175^{\rm b} \\ 1.09 \pm 0.284^{\rm a} \end{array}$	<0.001 0.019 0.010
42 d	Duodenum Jejunum Ileum	$\begin{array}{c} 1.41 \pm 0.172^{c} \\ 0.31 \pm 0.092^{a} \\ 0.65 \pm 0.075^{a} \end{array}$	$\begin{array}{c} 1.29 \pm 0.164^{ab} \\ 0.16 \pm 0.028^{b} \\ 0.52 \pm 0.032^{b} \end{array}$	$\begin{array}{c} 1.09 \pm 0.112^{b} \\ 0.18 \pm 0.032^{b} \\ 0.44 \pm 0.080^{bc} \end{array}$	$\begin{array}{c} 0.59 \pm 0.160^{a} \\ 0.23 \pm 0.027^{ab} \\ 0.37 \pm 0.025^{c} \end{array}$	<0.001 0.014 <0.001

 $^{a,b,c}$  In the same row, values with no letter or the same superscript letters mean no significant difference (P > 0.05), while adjacent different letters mean significant difference (P < 0.05), and the interphase letters mean extremely significant difference (P < 0.01).

(P < 0.01), but the MDA content of jejunum and ileum had no significant difference; supplementation of 1,000 mg/kg AAE significantly reduced the small intestine MDA content (P < 0.05); the MDA content of duodenum and jejunum in 2,000 mg/kg group was significantly reduced (P < 0.05), however, the MDA content of ileum had no significant difference. At 42 days of age, the MDA content of small intestine in 500 and 1,000 mg/kg group was significantly reduced (P < 0.05); the duodenum and ileum MDA content in 2,000 mg/kg group was significantly decreased (P < 0.01), but the MDA content of jejunum had no significant difference.

# 4. Disscussion

# 4.1. Effects of AAE on the level of T-AOC of small intestine in broilers

Total antioxidant capacity is a main index to measure the total antioxidant level of enzymatic system and non-enzymatic system (Wang et al., 2009). Its antioxidant role mainly through three ways: 1) elimination of free radicals and reactive oxygen species to prevent lipid peroxidation; 2) degradation of peroxidation products and blocking the oxidative chain; 3) removal of metal ions from the catalytic reaction. The present experiment showed that the diets containing AAE can improve the total antioxidant capacity of small intestine in broiler, and the 1,000 mg/kg treatment group had the highest level of small intestine T-AOC at 42 days of age. However, there are not a lot of reports about the effects of Artemisia plants on total antioxidant capacity of small intestine in broilers. This finding may be caused from Artemisia argyi containing bioactive substances, such as flavonoids (Wu and Extraction, 2008) and polysaccharides (Lan et al, 2010; Xiong, 2011) which can remove excess free radicals of intestine tissue and raise the T-AOC level of small intestine and protect the normal function of the small intestine epithelial cells.

# 4.2. Effects of AAE on antioxidant enzyme activity of small intestine in broilers

Catalase, total superoxide dismutase and glutathione peroxidase are not only the main antioxidant enzymes that can removal the super oxygen free radical, superoxide and hydrogen peroxide, but also can reduce or prevent the formation of free radicals (Wang et al., 2012). In the process of animal metabolism,  $O_2^$ production and cleaning is a process of dynamic equilibrium. The elimination of O<sub>2</sub><sup>-</sup> is completely dependent on the whole antioxidant defense system of the body (Wang, 2010). Super oxygen anion and hydrogen ions generated hydrogen peroxide under the action of SOD; and then hydrogen peroxide eventually turns into H<sub>2</sub>O under the action of CAT or GSH-Px. Therefore, the synergistic effect of SOD and CAT or GSH-Px can effectively eliminate free radicals. The experimental results showed that diets supplemented with AAE can improve the antioxidant enzymes activity (CAT, T-SOD and GSH-Px), and consequently improve antioxidant capacity of small intestinal tissue. This means that AAE is a kind of effective free radical scavenger that can enhance the antioxidant capacity of small intestinal tissue. This may because of Artemisia argyi containing bioactive substances, such as flavonoids materials and polysaccharide has the very good radical scavenging capacity and antioxidant effect (Wu and Extraction, 2008; Lan et al., 2010).

The results of the above experiments were in agreement with previous studies. Chu et al. (2015) illustrated that the diets containing 1,000 mg/kg AAE improved the SOD activity in broiler chickens. Zhang et al. (2011) reported that added *Artemisia japonica* extract (AJE) improved the GSH-Px and SOD activity of liver in mice. He et al. (2009) concluded that *Artemisia argyi* volatile oil can enhance the serum SOD activity in mice. In addition, Repetto et al. (2003) demonstrated that *Dow moxa* extracts showed significant antioxidant effect *in vitro* and *in vivo*. Huh et al. (2003) illustrated that oral administration of 40 mg/kg DA-9601 (a kind of alcohol extract of *artemisia Asia*) can significantly reduce alcohol induced rat gastrointestinal mucosa hemorrhagic injury and lipid peroxidation.

#### 4.3. Effect of AAE on the MDA content of small intestine in broilers

Malondialdehyde is an animal body end product of lipid peroxidation that can cause proteins, nucleic acids and other vital macromolecules cross-linked polymer, and has cytotoxicity, meanwhile, its content can reflect the degree of cell damage. The present experiment showed that diets containing AAE significantly reduced the MDA content of small intestine in broilers. With the increase of AAE dosage, MDA content in small intestine tissue decreased. This means that the AAE can effectively reduce the lipid peroxidation, and protect the normal function of the small intestine. This finding was in agreement with the results of Qiao et al. (2011) and Chu et al. (2015).

#### 5. Conclusion

The test results showed that feeding AAE increased the antioxidant capabilities of small intestine tissue in broilers. At 21 days of age, supplementation of 500 mg/kg AAE improved the intestinal tissue antioxidant enzymes activity, especially the activity of CAT. At 42 days of age, the antioxidant function of small intestinal in 1,000 mg/kg group had the best promotion effect.

#### References

- Chu WB, Shi BL, Yan SM, Zhang PF, Zhao F, Sun DS, et al. Effect of Artemisiae argyi extract on immune and antioxidative function in broilers. Chin J Animal Sci 2015;51(19):67–70.
- Guo XQ, Shan AS, Zhao Y, Yan CJ, Wang HY. Effect of aqueous extract of *Ligustrum lucidum* on antioxidant indices of AA broilers. Chin J Animal Nutr 2007;19(1): 81–5.
- He RX, Long XM, Liu XX. Effects of volatile oil from *Artemisiae argyi* on physiological and biochemical indexes in mice. J Traditional Chin Veterinary Med 2009;5: 15–7.
- Hu G, Yin MZ, Yu X, Chen CY. Effects of polysaccharides from *artemisiae argyi* folum on the antioxidation in vitro. Lishizhen Med Materia Medica Res 2015;11: 2650–1.
- Huh K, Kwon TH, Shin US, Kim WB, Ahn BO, Oh TY, et al. Inhibitory effects of DA-9601 on ethanol-induced gastrohemorrhagic lesions and gastric xanthine oxidase activity in rats. J Ethnopharmacol 2003;88(2–3):269–73.
- Kim JK, Shin EC, Lim HJ, Choi SJ, Kim CR, Suh SH, et al. Characterization of nutritional composition, antioxidative capacity, and sensory attributes of seomae mugwort, a native Korean variety of *Artemisia argyi* H. Lev. & Vaniot. J Anal Methods Chem 2015;2015(4):1–9.
- Lan Min-Bo, Zhang Yan-Hong, Zheng Ying, Yuan Hui-Hui, Zhao Hong-Li, Gao Feng. Antioxidant and immunomodulatory activities of polysaccharides from moxa (*Artemisia argyi*) leaf. Food Sci Biotechnol 2010;19(6):1463–9.
- Qiao GH, Zhou XH, Li Y, Zhang HS, Li JH, Wang CM, et al. Effect of several supplemental Chinese herbs additives on rumen fermentation, antioxidant function and nutrient digestibility in sheep. J Anim Physiol A Anim Nutr 2011;96(5): 930–8.
- Repetto M, Maria A, Guzman J, Giordano O, Llesuy S. Protective effect of Artemisia douglasiana Besser extracts in gastric mucosal injury. J Pharm Pharmacol 2003;55(4):551–7.
- Shen RL, Wang JD. Effect of Chinese herb additive on antioxidation and production performance in laying hens. Chin J Veterinary Sci Technol 2000;30(5):27–9.
- Tan B, Yan HN, Huang SY, Liao HX, Zhang Q. Study on the extraction of polysaccharides from Artemisiae argyi folum and the effects on scavenging of hydroxyl radicals. China Licens Pharm 2012;9(3):10–3.
- Wang HF. Effect of Astragalus polysaccharides on performance, antioxidant enzyme activity, immuntiy enginery and intestined microflora of lying hens. Master Thesis. Bao Ding: Agricultural University of HEBEI; 2010. p. 25–6.
- Wang PZ, Li Y, Chen X, Zhao W, Shu J, Zhang ZH. Effects of seabuckthorn flavonoids on the antioxidant capacity under heat stress in broilers. China Feed 2009;15: 27–9.
- Wang QX, Lin H, Jia HQ, Zhang ZT, Wang CK. Effects of Lactobacillin on serum biochemical indices and antioxidant function in broilers. Chin J Animal Nutr 2012;24(1):131–6.
- Wu GH. Research on extraction process and bioactivity of the volatile oil and polysaccharide from *Artemisia argyi*. Master Thesis. Shang Hai: East China University of Science and Technology; 2010. p. 19–24.

- Wu N. Extraction, purification and antioxidation of flavonoids from Artemisia argyi.
- Master Thesis. Wu Han: Huazhong Agricultural University; 2008. p. 54–61.
   Wu N, Sun ZD. Study on antioxidant activity in vitro and protection against DNA oxidative damage of flavonoids of Artemisiae argyi. Food Sci 2008;29(10): 47-50.
- Xiong ZW. Chemical composition, antioxidant and antibacterial activity of Artemisia lavandulaefolia. Master Thesis. Nan Chang: Nanchang University; 2011. p. 12–22.
- Zhang ZX, Zhang XP, Liu HJ, Shao JW, Yang KJ, Zhang XW. Studies on the allelopathic effects of artemisia lavandulaefolia. J Anhui Normal Univ Nat Sci 2006;29(6): 579-81.
- Zhang DH, Cheng PF, Ling L. Antioxidation and genetic toxicity of artenisia japonica extract. Nat Prod Res Dev 2011;23(1):39–42. Zhou F, Qin LP, Lian JF, Zhen QM. Chemical constituents, biological activities and
- plant resources of Folium Artemisia argyi. J Pharm Pract 2000;2:96-8.