Dietary addition of *Artemisia argyi* reduces diarrhea and modulates the gut immune function without affecting growth performances of rabbits after weaning¹

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ABSTRACT: The present study was conducted to investigate the effect of *Artemisia argyi* on the production performance and intestinal barrier of rabbits. Weaned Hyla rabbits (30 d, $n = 160$) of similar body weight were divided into 4 groups (40 rabbits per treatment), and they were fed a control diet or fed an experimental diet supplemented with 3%, 6%, or 9% *A. argyi*. The results showed that the dietary supplementation with *A. argyi* did not affect the rabbits' food intake and body weight gain regardless of the inclusion level but decreased the diarrhea rate and diarrhea index ($P < 0.05$). Dietary addition of *A. argyi* increased the small intestine length and villus height/crypt depth, regardless of the inclusion level ($P < 0.05$). Compared with the control, the *A. argyi* supplementation increased the gene

expression of zonula occludens 1 (**ZO-1**) and claudin 1 in all segments of the small intestine and regardless of the level of *A. argyi* ($P < 0.05$). In the duodenum, a dietary supplementation with 6% and 9% *A. argyi* increased the immunoglobulins A (**IgA**) content ($P < 0.05$). In the jejunum, the *A. argyi* supplementation decreased interleukin 2 (**IL2**) and IL6 content regardless of the inclusion level ($P < 0.05$). In the ileum, a 3% *A. argyi* addition decreased IL2 content, whereas a 6% *A. argyi* addition decreased IL6 content ($P < 0.05$). Furthermore, 6%–9% *A. argyi* supplementation increased the IgA content in the ileum ($P < 0.05$). In conclusion, dietary addition of *A. argyi* reduces diarrhea and modulates the gut immune function without affecting growth performances of rabbits.

Key words: *Artemisia argyi*, diarrhea, intestinal health, rabbit weaning

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INTRODUCTION

Infectious diseases of the digestive system currently account for 70% of all rabbit diseases (Carabaño et al., 2008). The percentage has increased further due to epizootic rabbit

enteropathy, which can cause up to 60% mortality, especially for the weaned rabbits (Fann et al., 2001; Carabaño et al., 2008). Rabbit diarrhea may be mainly caused by pathogens (e.g., enteropathogenic *Escherichia coli* and pasteurella) and stress caused by the weaning process, feed replacement, or changes in climate. Antibiotics have been widely used to control mortality but the corresponding veterinary treatments have increased by 2.5 fold, reducing the benefit margin for many farms. For this reason, some antibiotic substitutes (e.g., herbs and plant extracts) have been found and studied by many researchers. *Artemisia argyi*, the Chinese mugwort, is an aromatic plant in the genus *Artemisia*, which is native China, Japan, and far-eastern Siberia. This plant

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contains many potentially bioactive compounds such as coumarins, flavonoids, glycosides, polyacet-ylenes, sterols, monoterpenes, triterpenes, essen-tial oils, and sesquiterpene lactones (Zhang et al., 2013). Some compounds have presented many bio-activities such as antiulcer (Yoon et al., 2011), anti-diabetic (Adams et al., 2012), anti-inflammatory (Cai, 2001), and anticancer (Adams et al., 2006) activities in humans or mice. It has been used as a traditional herbal medicine for the treatment of microbial infections, inflammatory diseases, hepa-titis, diarrhea, cancer, malaria, and circulatory sys-tem and metabolism disorders (Adams et al., 2006; Bao et al., 2013; Khan et al., 2012). The *A. argyi* leaf polysaccharides were capable of enhancing the Concanavallin A-induced T cell proliferation (Lan et al., 2010). A water-soluble polysaccha-ride, isolated from *A. argyi*, significantly inhib-ited the growth of the sarcoma 180 transplanted tumors and prolonged the survival time of the tumor-bearing mice (Bao et al., 2013). *Artemisia argyi* or its extracts have been used as a feedstuff or additive to enhance the body metabolism and improve production performance in broilers and pigs (Kim et al., 2009; Chu et al., 2016). However, there is little available information about its use as feedstuff in rabbit production and its effect on the intestinal barrier. Based on the previous research, we hypothesized that *A. argyi* could improve the intestinal health of weaned rabbits. The present experiment was conducted to investigate the func-tion of *A. argyi* on the production performance and gut immune function of rabbits and to identify the effect of *A. argyi* as a feed resource in rabbit production.

MATERIALS AND METHODS

Experimental Protocol and Sample Collection

At 35 d of age, 160 weaned rabbits (Hyla, male-female ratio of 1:1) with similar body weight ($1,388 \pm 8.5$ g) were divided into 4 treatments (40 rabbits per treatment), and they were fed a con-trol diet or fed an experimental diet supplemented with 3%, 6%, or 9% *A. argyi* (cultivated in Hubei Province, purchased from Anguo Herbal Market, China) powder (the doses of *A. argyi* refer to dry matter basis). The experiment lasted for 37 d. Rabbits were individually housed in homemade plastic cages ($60 \times 40 \times 40$ cm). Temperature and lighting were maintained according to commercial conditions (20 to 23 °C, 12 light/12 dark). The diets were formulated according to the recommendation

of de Blas and Mateos (1998), and the feed was made into 4-mm pellets by using steam. The ingredients and chemical composition of the diet are listed in Table 1. All rabbits had free access to feed and water during the rearing period. All study procedures were approved by the Shandong Agricultural University Animal Care and Use Committee (SDAUA-2016-132) and were in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). At the end of the trial, 32 rabbits (72 d of age, 8 rabbits per treat-ment, male-female ratio of 1:1) were electrically stunned and slaughtered by exsanguination. The whole intestine from the pylorus to the ileocecal junction was carefully removed from each rabbit, and the length of the small intestine was recorded. Then, 2- and 4-cm segments were from the distal duodenum (between the U-bending location and treitz lig), mid-jejunum (between the treitz lig and Meckel's diverticulum), and mid-ileum (between the Meckel's diverticulum and ileocecal junction). The 2-cm intestinal segments were flushed gently with ice-cold phosphate-buffered saline (PBS, pH 7.4) and then placed in 10% fresh, chilled formalin solution for histological and immunohistochem-ical measurements. The 4-cm intestinal segments were opened longitudinally, and the contents were flushed with ice-cold PBS. Mucosa was collected by scraping using a sterile glass microscope slide, and then rapidly frozen in liquid nitrogen and stored at -80 °C until analysis.

Growth Performance and Diarrhea

Body weight and feed consumption of in-dividual rabbits were recorded daily at 8:00 am. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were cal-culated for all rabbits ($n = 40$). The number of rab-bits with diarrhea was recorded daily throughout the study. Fecal consistency scores (0 normal; 1 soft feces stools; 2 soft/liquid stools; 3 liquid stools) were determined by 2 trained people with no prior knowledge of the dietary treatment allocation (Isolaure et al., 1990). The incidence of diarrhea was calculated as (the total number of rabbits with diarrhea/the total number of all experimental rab-bits) $\times 100\%$. The diarrhea index was calculated as the total score of diarrhea/total number of rab-bits. The incidence and index of diarrhea were cal-culated daily. The final incidence and index were obtained by repeated measures analysis in whole experimental period ($n = 37$).

Table 1. Ingredients and chemical composition of the experimental diet on DM basis

Ingredients, %	Experimental group			
	Control	3%	6%	9%
Bean pulp	13	13	13	13
Corn	14	14	14	14
Corn germ dregs	10	10	10	10
Alfalfa meal	22	22	22	22
Premix ¹	4	4	4	4
Peanut vine	22	21	19	18
Wheat bran	15	13	12	10
<i>Artemisia argyi</i> powder ²	0	3	6	9
Measured chemical composition				
Digestible energy, MJ/kg	8.37	8.19	7.88	7.90
Crude protein, %	16.60	16.56	16.60	16.59
Crude fiber, %	16.89	17.07	17.13	16.90
Crude fat, %	2.74	2.74	2.58	2.62
Dry matter, %	91.30	91.08	91.08	91.22
Acid detergent fiber, %	18.35	18.14	18.21	18.24
Neutral detergent fiber, %	37.06	36.58	36.18	36.19
Acid detergent lignin, %	3.16	3.47	3.34	3.36
Calcium, %	1.43	1.42	1.46	1.49
Phosphorus, %	0.65	0.65	0.63	0.64

¹The following premix ingredients were added to each kg of diet: vitamin A, 8 000 IU; vitamin D3, 1 000 IU; vitamin E, 50 mg; vitamin K3, 2.3 mg; thiamine, 1.75 mg; riboflavin, 6.9 mg; niacin, 28.45 mg; pantothenic acid, 6.7 mg; biotin, 2.75 mg; folic acid, 0.6 mg; vitamin B12, 2.2 mg; choline, 420 mg; lysine, 1.5 g; methionine, 1.5 g; copper, 50 mg; iron, 100 mg; manganese, 30 mg; magnesium, 150 mg; iodine, 0.1 mg.

²The composition of *A. argyi* powder: energy value 17.62 MJ/Kg; crude protein, 15.70%; crude fiber, 7.29%; crude fiber, 16.64%.

Histological Examination

The mucosa samples were processed, embedded, and stained according to the procedures of [Hu et al. \(2013\)](#). In brief, samples were fixed and embedded in paraffin, sectioned at a thickness of 5 µm and stained with haematoxylin and eosin. The villus height (the distance from the villus tip to crypt mouth) and the associated crypt depth (the distance from the crypt mouth to base) were measured in 5 slides for each sample. In each slide, 10 well-oriented villus and crypts were analyzed at a low magnification (40×) with a light microscope (using IMAGEJ 1.43d software; Research Services Branch, National Institute of Mental Health, Bethesda, MD).

Cytokine and Immunoglobulin Levels

Mucosa samples were disrupt and homogenized in PBS buffer (1-g samples: 9 mL PBS). After centrifuging at a speed of 5,000 × *g* for 10 min, the supernatant was isolated. The immunoglobulins A (**IgA**) and immunoglobulins G (**IgG**) concentrations were measured with an immunoturbidimetry using the commercial diagnostic kits (Name: IgA Assay Kit and IgG Assay Kit, Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Immunoglobulins in the supernatant combined, respectively, the IgA or IgG antibody, antigen-antibody complex produced turbidity. Turbidity was monitored spectrophotometrically at 415 nm with an automated reader (BioTek, Winooski, VT). The levels of interleukins (interleukin 2 [**IL2**], IL-6, IL-8, and IL-10) and IFNγ were determined in supernatant using double-antibody sandwich Enzyme-Linked Immunosorbent Assay (Jiancheng Bioengineering Institute, Nanjing, P.R. China). Optical density value at 450 nm was measured by a Bio-Rad ELISA reader (Bio-Rad, Richmond, CA). Duplicate assays were performed for both sample and standard testing. The sensitivities for IL-2, IL-6, IL-8, IL-10, and IFNγ were 2, 0.25, 7.5, 10, and 8 ng/L respectively.

RNA Isolation and Analysis

Total RNA extraction and quantitative reverse transcription-PCR (**qRT-PCR**) of mucosa samples were performed as described previously ([Liu et al., 2014](#); [Wang et al., 2017](#)). Total RNA was isolated by the guanidinium isothiocyanate method with Trizol Reagent (Invitrogen, San Diego, CA). The quality of RNA after DNase treatment was tested by electrophoresis on an agarose gel and the quantity of RNA was determined using a biophotometer (Eppendorf,

Germany). Reverse transcription was performed as the direction of TaKaRa cDNA synthesis kit described previously (TaKaRa Biotechnology, Co., Ltd. Dalian, P. R. China). RT reaction system (10 μ L) containing 500 ng total RNA, 5 mmol/L $MgCl_2$, 1 μ L RT buffer, 1 mmol/L dNTP, 2.5 U avian myeloblastosis virus, 0.7 nmol/L oligo d(T), and 10 U Ribonuclease inhibitor were performed at 65 °C for 2 min, 42 °C for 30 min, and 70 °C for 15 min. RT-PCR analysis was conducted using the Applied Biosystems 7500 RT-PCR System (Applied Biosystems, Foster, CA). Each RT-reaction served as a template in a 20- μ L PCR reaction containing 0.2 μ mol/L of each primer and SYBR green master mix (Takara Biotechnology, Co., Ltd. Dalian, P. R. China). All the genes sequences were from NCBI, and primer-set sequences are described in Table 2. RT-PCR reactions were performed at 95 °C for 10 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 40 s. A standard curve of pooled samples was plotted to calculate the efficiency of the RT-PCR primers. The relative amount of mRNA of a gene was calculated according to the 2 delta delta CT method (Livak and Schmittgen, 2001) using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as normalizing gene and the control group as calibrator group. On the basis of the CT values, *GAPDH*

mRNA expression was stable across the treatments ($P > 0.1$).

Statistical Analysis

All data were analyzed using general statistical linear mode (GLM) procedure of SAS. Differences among treatments were examined using Duncan's multiple range test and were considered to be significant at $P < 0.05$.

RESULTS

Effects of *A. argyi* Supplementation on Production Performance in Rabbits

Compared with the control, a dietary supplementation with the *A. argyi* did not affect the ADG, ADFI, or FCR (Table 3), but it decreased the diarrhea rate and diarrhea index ($P < 0.05$). Besides, addition of 6% *A. argyi* decreased further the diarrhea rate and diarrhea index compared with the 3% *A. argyi* addition group ($P < 0.05$). There was no significant difference between 6% *A. argyi* addition group and 9% *A. argyi* addition group. The rabbits fed *A. argyi* had a greater small intestine length than control ($P < 0.05$).

Table 2. Gene-specific primers used for the analysis of rabbit gene expression

Gene	Genebank	Primer sequences (5'-3')	Product size (bp)
<i>claudin1</i>	NM_001089316	F: GTCTTCGACTCTTTGCTGAATCT R: CAATGACAGCCATCCGCATC	169
<i>occludin</i>	XM_008262318	F: CTTGCCTGGGACAGAACCTA R: AGCCATAACCGTAGCCGTAA	121
<i>ZO-1</i>	XM_008269782	F: GACTGATGCGAAGACGTTGA R: GCAGAATGGATGCTGTCAGA	117
<i>GAPDH</i>	NM_001082253	F: TGCCACCCACTCCTCTACCTTCG R: CCGGTGGTTTGAGGGCTCTTACT	163

ZO-1 = zonula occludens 1; *GAPDH* = glyceraldehyde 3-phosphate dehydrogenase.

Table 3. Effects of *A. argyi* powder supplementation on growth performance and feed intake in rabbits

	Experimental group				RMSE	<i>P</i>
	Control	3%	6%	9%		
Average daily feed intake, g/d	168.6	167.6	169.6	168.6	4.94	0.3966
Average daily gain, g/d	46.96	46.25	46.25	47.01	5.20	0.8706
Feed conversion ratio	3.63	3.63	3.68	3.60	0.34	0.8521
Diarrhea rate, %	23.50 ^a	11.14 ^b	6.78 ^c	6.56 ^c	1.89	0.0005
Diarrhea index	0.39 ^a	0.21 ^b	0.13 ^c	0.13 ^c	0.37	0.0007
Small intestine length, cm	351.1 ^b	398.5 ^a	405.8 ^a	391.1 ^a	13.32	0.0021

For average daily feed intake, average gain, and feed conversion ratio, $n = 40$; for diarrhea rate and diarrhea index, $n = 37$; for small intestine length, $n = 8$.

Effects of *A. argyi* Supplementation on Intestinal Morphology in Rabbits

The *A. argyi* supplementation in the diet did not alter the villus height in all segments of the small intestine (Table 4). Compared with the control, the 6% *A. argyi* supplementation decreased the crypt depth in the duodenum and ileum ($P < 0.05$). In the duodenum and the ileum, no difference was observed for the 3% and 9% *A. argyi* addition groups. The dietary *A. argyi* supplementation decreased the crypt depth in the jejunum for all *A. argyi* incorporation levels ($P < 0.05$) but increased the villus height/crypt depth in all intestinal segments ($P < 0.05$).

Effects of *A. argyi* Supplementation on Gene Expression of Physical Barrier in Rabbits

Compared with the control, the *A. argyi* supplementation increased the expression of zonula occludens 1 (*ZO-1*) and claudin 1 gene (Table 5, $P < 0.05$) while did not change the gene expression of occludin in any segment of the small intestine and regardless of the level of *A. argyi*.

Effects of *A. argyi* Supplementation on Cytokine and Immunoglobulin Levels in Rabbits

As shown in Table 6, IgA content significantly increased in the group with 6% and 9% *A. argyi* supplementation in the duodenum (Table 6, $P < 0.05$), but not in the group with 3% *A. argyi* supplementation. The *A. argyi* treatment did not affect the IL2, IL6, IL8, IL10, IgG, and IFN γ content regardless of the inclusion level. In the jejunum, the dietary *A. argyi* supplementation decreased the IL2 and IL6 content ($P < 0.05$) but did not alter the IL8, IL10, IgA, IgG, and IFN γ content regardless of the inclusion level. In the ileum, IL2 levels were decreased in the 3% group compared with the control group, whereas no significant differences were observed for other inclusion levels ($P < 0.05$). IL6 levels were decreased in the 6% group compared with all other experimental groups ($P < 0.05$). Compared with the control, the 6% to 9% *A. argyi* addition in diet increased the IgA content ($P < 0.05$). Regardless of the level of *A. argyi*, the levels of IL8, IL10, IgG, and IFN γ in ileum were not changed.

Table 4. Effects of *A. argyi* powder supplementation on intestinal morphology of rabbits ($n = 8$)

Intestinal sites		Experimental group				RMSE	<i>P</i>
		Control	3%	6%	9%		
Duodenum	Villus height, μm	675.3	768.9	717.8	746.1	143.2	0.5693
	Crypt depth, μm	129.2 ^a	127.3 ^a	104.9 ^b	124.1 ^a	16.33	0.0193
	Villus height/crypt depth	5.26 ^c	6.62 ^{ab}	7.06 ^a	6.44 ^b	1.04	0.0279
Jejunum	Villus height, μm	682.2	700.7	694.3	769.3	107.0	0.3947
	Crypt depth, μm	138.1 ^a	103.6 ^b	100.3 ^b	110.1 ^b	18.89	0.0037
	Villus height/crypt depth	5.07 ^b	7.22 ^a	7.20 ^a	7.25 ^a	1.09	0.0015
Ileum	Villus height, μm	486.2	542.7	536.3	579.9	94.5	0.2632
	Crypt depth, μm	109.9 ^a	97.4 ^{ab}	89.3 ^b	102.9 ^{ab}	15.50	0.0746
	Villus height/crypt depth	4.57 ^b	5.79 ^a	6.13 ^a	5.95 ^a	0.76	0.0012

Table 5. Effects of *A. argyi* powder supplementation on related gene expression of physical barrier in intestinal tract ($n = 8$)

Intestinal sites		Experimental group				RMSE	<i>P</i>
		Control	3%	6%	9%		
Duodenum	<i>ZO-1</i>	1.00 ^c	1.69 ^a	1.63 ^{ab}	2.07 ^a	0.66	<0.001
	<i>Occludin</i>	1.00	1.52	1.34	1.49	0.47	0.2373
	<i>Claudin1</i>	1.00 ^c	1.35 ^b	1.36 ^b	1.79 ^a	0.74	0.0432
Jejunum	<i>ZO-1</i>	1.00 ^b	2.20 ^a	2.20 ^a	2.03 ^a	0.53	0.0043
	<i>Occludin</i>	1.00	1.27	1.39	1.38	0.44	0.4435
	<i>Claudin1</i>	1.00 ^b	2.55 ^a	2.63 ^a	3.25 ^a	0.93	0.0186
Ileum	<i>ZO-1</i>	1.00 ^c	1.33 ^{ab}	1.51 ^a	1.52 ^a	0.45	0.0269
	<i>Occludin</i>	1.00	1.14	1.21	1.35	0.51	0.6911
	<i>Claudin1</i>	1.00 ^c	1.57 ^{ab}	1.42 ^b	1.89 ^a	0.87	0.0315

ZO-1 = zonula occludens 1.

Table 6. Effects of *A. argyi* supplementation on cytokine and immunoglobulin levels in intestinal tract ($n = 8$; units: ng/L for IL2, IL6, IL10, and IFN γ ; μ g/L for IgA and IgG)

Intestinal sites		Experimental group				RMSE	P
		Control	3%	6%	9%		
Duodenum	IL2	19.72	21.41	16.67	20.23	3.44	0.4832
	IL6	159.1	56.8	123.5	147.3	83.39	0.4001
	IL8	69.07	60.46	47.43	69.50	39.57	0.7882
	IL10	97.30	113.5	123.7	144.3	57.55	0.7297
	IgA	22.31 ^b	20.30 ^b	92.29 ^a	89.60 ^a	43.65	0.0396
	IgG	2.53	3.50	3.28	7.86	3.97	0.1865
	IFN γ	118.6	97.35	62.29	108.2	66.64	0.6388
Jejunum	IL2	45.40 ^a	30.08 ^b	28.93 ^b	31.23 ^b	6.32	0.0329
	IL6	180.3 ^a	108.5 ^b	76.35 ^b	105.5 ^b	38.90	0.0050
	IL8	106.6	86.85	68.6	101.0	32.61	0.4332
	IL10	187.6	156.1	119.5	103.7	93.46	0.4339
	IgA	18.84	16.53	15.98	23.99	7.34	0.3584
	IgG	7.87	7.32	6.23	7.44	2.79	0.8066
	IFN γ	108.06	103.07	72.20	86.07	57.27	0.7398
Ileum	IL2	44.39 ^a	16.08 ^b	35.51 ^{ab}	35.11 ^{ab}	10.85	0.0019
	IL6	158.6 ^a	149.2 ^a	81.17 ^b	147.5 ^a	49.72	0.0022
	IL8	133.9	98.48	77.08	116.70	36.09	0.1137
	IL10	197.3	142.7	94.24	146.3	61.43	0.1417
	IgA	18.84 ^c	23.61 ^{bc}	43.53 ^a	37.46 ^{ab}	14.44	0.0248
	IgG	9.31	8.13	8.22	9.73	2.13	0.4211
	IFN γ	138.7	128.6	95.43	107.7	65.35	0.7431

IL = interleukin; IgA = immunoglobulins A; IgG = immunoglobulins G; IFN- γ = interferon- γ .

DISCUSSION

Effects of A. argyi on Performance and Intestine Development in Rabbits

This experiment indicated that dietary supplementation with *A. argyi* instead of peanut vine and wheat bran did not change the ADG, ADFI, and FCR in rabbits, which is similar to the results reported by Zhang et al. (2017) in broilers. Zhang et al. (2017) showed that the dietary supplementation with 1000 mg/kg *A. argyi* aqueous extract attenuated the decline of ADG and ADFI of broilers caused by a lipopolysaccharide (LPS) challenge but had no effect on the growth performance of broilers without a LPS challenge. These results suggested that *A. argyi* could be largely used as feedstuff in rabbit production without changing their production performance.

Keepers pursue high growth rate and shortened feeding period by increasing dietary digestible energy and protein levels. This may cause serious intestinal problems, especially for the postweaned rabbits. The rate of diarrhea in postweaned rabbits could be up to 50%, which induces a high death rate (Li and Gu, 2003). In our study, the diarrhea rate in rabbits of the control group was 22.5%, but the

dietary *A. argyi* addition significantly decreased the diarrhea rate and diarrhea index. The result was in agreement with a previous study in mice (Bao et al., 2013). These results suggest that dietary supplementation of *A. argyi* could decrease diarrhea and improve intestinal health in animal. The small intestine is the primary tissue responsible for the terminal digestion and absorption of dietary nutrients (Shimizu, 1999). In the present study, the *A. argyi* treatment increased the ratio of villus height to crypt depth and small intestine length, indicating a critical role of *A. argyi* in the development of the intestine and protection against intestinal atrophy in vivo. This was in agreement with the findings in broiler chicks by Chu et al. (2016), indicating that *A. argyi* extract could affect the development of enterocyte of broilers and increase the villus height to crypt depth.

Effects of A. argyi on Physical Barriers in Rabbits

The intestinal epithelial cells are joined at their apical side by tight junctions. The tight junctional complexes form the narrowest distance between plasma membranes of 2 cells, thus excluding the influx of bacteria through paracellular routes (Turner, 2009). The transmembranous junctional

proteins (e.g., claudins and occludin) are linked to intracellular ZO, which are bridges to cytoskeletal actin and myosin filaments (Turner, 2006; Ivanov et al., 2010). RT-PCR analysis of the small intestine mucosa showed that the *A. argyi* addition increased the gene expression of *ZO-1* and claudin 1, indicating that there may be improvement of the intestinal barrier mechanism. The results were in line with the previous study, indicating extract from *A. argyi* leaf reduced the permeability in glacial acetic acid-induced abdominal blood capillary in mice (Ge et al., 2016)

Effects of *A. argyi* on the Intestinal Immunological Barrier in Rabbits

The small intestine is an important component of the mucosal immune system and performs important and unique immune functions. IgA is the predominant immunoglobulin isotype in the mucosal tissue and is responsible for the defense of mucosal homeostasis, influencing the development of systemic immunity (Mestecky et al., 1999). In the present study, the IgA content was increased after the 6% and 9% *A. argyi* treatments in duodenum and ileum, indicating a critical role of *A. argyi* in the maintenance of IgA production in vivo. But dietary addition *A. argyi* did not affect the IgA content in jejunum, which is in accordance with the study in pigs (Chu and Song, 2012). A dietary addition of *Artemisia montana* did not affect the IgA level in serum (Chu and Song, 2012). These results imply that the regulation of *Artemisia* on IgA is related to the segment of the small intestine and species.

IgG is another important immunoglobulin, which promotes immune cells to devour pathogens and neutralize bacterial toxins. Zhang et al. (2017) found that a daily addition of *A. argyi* aqueous extract did not affect the IgG section in the small intestine of broilers. In line with our present study, dietary addition of *A. argyi* did not regulate the intestinal IgG production. These results imply that *A. argyi* may not be involved in the regulation of intestinal IgG production. In fattening pigs, feeding *A. montana* Pampan increased the IgG level in serum (Chu and Song, 2012), indicating that it is different for different varieties of *Artemisia* in regulating IgG production.

In a previous study, *A. argyi* significantly attenuated the LPS-induced increase in serum IL-2 and IL-6 in broilers (Zhang et al., 2017). In our study, the dietary *A. argyi* supplementation significantly decreased the IL-2 and IL-6 content in

the jejunum and ileum, but not in the duodenum, indicating that *A. argyi* could regulate the immune response, with the major regulative sites in rabbits occurring in the posterior segment of the small intestine. Additionally, IL-8, IL-10, and IFN γ in the small intestine were not significantly altered after the *A. argyi* treatment, indicating that IL-8, IL-10, and IFN γ may not be the key factors in *A. argyi* regulating the immune response.

In conclusion, dietary addition of *A. argyi* reduces diarrhea and modulates the gut immune function without affecting growth performances of rabbits after weaning. These results provide a basis for a new approach to preventing intestinal mucosal injury and the resulting clinical problems.

Conflict of interest statement. None declared.

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