Effects of Alfalfa Saponins on Growth Performance, Intestinal Flora, Tissue Antioxidant Capacity and mRNA Expression of Related Enzymes in Weaned Piglets (Postprint)

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Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of alfalfa saponins on growth performance, intestinal microbiota, tissue antioxidant capacity, and mRNA expression of related enzymes in weaned piglets. Twenty-four Large White × Landrace crossbred weaned piglets with an average body weight of 8 kg were selected and randomly divided into 2 groups, with 3 replicates per group and 4 piglets per replicate. The control group was fed a basal diet, while the alfalfa saponin group was fed a basal diet supplemented with 0.25% alfalfa saponins. The adaptation period was 10 days, and the experimental period was 30 days. The results showed that: 1) Compared with the control group, dietary supplementation with alfalfa saponins significantly increased the average daily gain of weaned piglets ($P<0.05$) and significantly decreased the feed-to-gain ratio ($P<0.05$). 2) Compared with the control group, dietary supplementation with alfalfa saponins significantly decreased the pH of the duodenum and cecum of piglets ($P<0.05$) and significantly increased the number of lactic acid bacteria in the duodenum, jejunum, and ileum ($P<0.05$). 3) Compared with the control group, dietary supplementation with alfalfa saponins significantly increased the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) in the liver and kidney of piglets ($P<0.05$), and significantly increased the mRNA expression of GSH-Px in the liver and jejunum and the mRNA expression of CAT in the duodenum and ileum of piglets ($P<0.05$). In conclusion, alfalfa saponins can improve the growth performance of weaned piglets, enhance their tissue antioxidant capacity, and effectively improve their intestinal microbiota.

Full Text

Effects of Alfalfa Saponin on Growth Performance, Intestinal Microflora, Antioxidant Ability and Related Enzyme mRNA Expression in Tissues of Weaned Piglets

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Abstract

This experiment was conducted to investigate the effects of alfalfa saponin on growth performance, intestinal microflora, tissue antioxidant ability and related enzyme mRNA expression in weaned piglets. A total of 24 crossbred (Landrace × Large White) weaned piglets with an average body weight of 8 kg were randomly assigned to 2 groups with 3 replicates per group and 4 piglets per replicate. The control group was fed a basal diet, while the alfalfa saponin group received the basal diet supplemented with 0.25% alfalfa saponin. The experiment consisted of a 10-day pre-trial period followed by a 30-day formal trial period. The results showed that: (1) Compared with the control group, dietary alfalfa saponin significantly increased average daily gain ($P < 0.05$) and significantly decreased feed-to-gain ratio ($P < 0.05$) in weaned piglets. (2) Dietary alfalfa saponin significantly reduced pH values in the duodenum and cecum ($P < 0.05$) and significantly increased Lactobacillus counts in the duodenum, jejunum and ileum ($P < 0.05$). (3) Dietary alfalfa saponin significantly increased the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) in the liver and kidney ($P < 0.05$), and significantly upregulated GSH-Px mRNA expression in the liver and jejunum as well as CAT mRNA expression in the duodenum and ileum ($P < 0.05$). In conclusion, alfalfa saponin can improve growth performance, enhance tissue antioxidant capacity and effectively modulate intestinal microflora in weaned piglets.

Keywords: alfalfa saponin; weaned piglet; growth performance; intestinal microflora; antioxidant ability; mRNA expression level

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Introduction

Alfalfa (*Medicago sativa*) is the most widely cultivated forage crop with a long history of cultivation, primarily due to its advantages of being rich in protein, minerals and vitamins, with good protein quality, balanced amino acid composition and high lysine content. Alfalfa resources contain many potential active components, such as saponins, flavonoids and polysaccharides. Saponins, as

natural substances with unique biological activities, have been reported to possess antioxidant, immunomodulatory, anti-cancer and lipid-lowering functions, thus attracting widespread attention. Wu et al. [1] investigated the effects of different supplementation levels of betaine and alfalfa saponin on growth performance of weaned piglets, finding that both betaine and alfalfa saponin significantly affected average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G), with optimal effects observed at dietary supplementation levels of 0.08% betaine and 0.25% alfalfa saponin. Yalinkilic et al. [2] fed mice with extracts from horse chestnut (*Aesculus hippocastanum*), spinach (*Spinacia oleracea*) and alfalfa containing saponins, and found that these extracts could significantly enhance antioxidant capacity and protect cells from X-ray damage. However, previous research on alfalfa has mainly focused on the nutritional value and processing utilization of hay and meal products, primarily for dairy cow feeding, while studies on alfalfa saponin in piglet diets remain limited. Therefore, this experiment aimed to investigate the effects of alfalfa saponin on growth performance, intestinal microflora, tissue antioxidant capacity and related enzyme mRNA expression in weaned piglets, providing a scientific basis for its rational application in practical production.

1. Materials and Methods

1.1 Experimental Material

The alfalfa saponin used in this experiment was provided by Hebei Baoen Biotechnology Co., Ltd., with the following composition: 62.00% saponins, 10.97% flavonoids, 8.12% polysaccharides, 7.11% moisture, and 11.80% unknown factors. Saponins are generally hygroscopic, bitter-tasting, and mucosal irritants. They are typically water-soluble, readily soluble in hot water, hot methanol and hot ethanol, but insoluble in low-polarity organic solvents such as ether. Most steroidal saponins are neutral, while most triterpenoid saponins are acidic. The saponin product used in this experiment was neutral with a pH of 7.04.

1.2 Experimental Design and Diets

A single-factor completely randomized design was adopted. Based on the principles of similar age, parity and body weight, 24 healthy Duroc × Landrace crossbred piglets weighing approximately 8.0 kg were selected as experimental animals and randomly divided into 2 groups with 3 replicates per group and 4 piglets per replicate. The control group was fed a basal diet, while the alfalfa saponin group received the basal diet supplemented with 0.25% alfalfa saponin. The composition and nutrient levels of the basal diet are presented in Table 1 .

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

Content	Items
Ingredients	
Wheat	
Soybean meal	
Oil and fat	
Fish meal	
Whey powder	
Limestone	
$CaHPO_4$	
NaCl	
Lys	
Thr	
Premix[1]	
Total	
Nutrient levels[2]	
CP	
DE/(MJ/kg)	
Ca	
Lys	
Met+Cys	
Thr	

[1]Premix provided the following per kg of diet: VA 5,500 IU, VD_3 500 IU, VE 66.1 IU, VB_{12} 28.2 μg, VB_2 5.1 mg, VB_3 12.6 mg, VB_5 29.8 mg, choline 540 mg, Mn 40 mg, Zn 120 mg, Fe 130 mg, Cu 150 mg, Co 1 mg, Se 0.25 mg, I 4.5 mg.
[2]Nutrient levels were calculated values.

1.3 Feeding Conditions and Management

The experiment consisted of a 10-day pre-trial period and a 30-day formal trial period. Piglets were allowed free access to feed and water, with feeding times at 06:00, 10:00, 14:00 and 18:00 daily. During the pre-trial period, all groups received the same basal diet. During the formal trial period, the control and alfalfa saponin groups were fed their respective diets, with daily feed intake and health status recorded. The pig house temperature was maintained at approximately 25 °C with humidity between 65% and 75%. Pens were cleaned once daily, and immunization and disinfection were performed according to conventional farm procedures. At the end of the experiment, piglets were fasted (with free access to water) for 12 hours before weighing to calculate ADFI, ADG and F/G for each group. Diarrhea rate (%) was calculated as: 100 × [(number of diarrheic piglets × diarrhea days) / (total number of piglets × trial days)].

1.4 Sample Collection

Six piglets from each group (2 per replicate) were selected and euthanized by jugular venous bloodletting. The abdominal cavity was opened, and the intestinal tract was isolated. The same segments of duodenum, jejunum, ileum and cecum were collected and ligated. Intestinal contents were collected for microbial enumeration (*Escherichia coli* and *Lactobacillus*) and pH measurement. Subsequently, samples were taken from the same locations in the liver, spleen and kidney, and sections of duodenum, jejunum, ileum and cecum were collected. All samples were rinsed with physiological saline, blotted dry with gauze, wrapped in aluminum foil, immediately frozen in liquid nitrogen, and stored at -80 °C.

1.5 Measurement Indicators

1.5.1 pH Measurement The pH of duodenal, jejunal, ileal and cecal contents was measured directly using a pH meter.

1.5.2 Enumeration of *E. coli* and *Lactobacillus* in Intestinal Contents
Preparation of intestinal content dilutions: Collected intestinal contents were mixed uniformly. Under sterile conditions, 0.5 g was placed in tube No. 1 containing 4.5 mL sterile physiological saline and vortexed for 20 minutes. Five additional sterilized tubes were prepared, each containing 4.5 mL sterile physiological saline. Then 0.5 mL from tube No. 1 was transferred to tube No. 2, vortexed for 5 minutes, and serially diluted to 10^{-6}.

Inoculation and cultivation of *E. coli* and *Lactobacillus*: *E. coli* and *Lactobacillus* were cultured on eosin methylene blue (EMB) and MRS agar plates, respectively, using the colony counting method. Three appropriate dilutions were selected with two replicates each. Starting from the highest dilution and working near an alcohol lamp, 100 µL of mixed dilution was dropped from 2–3 cm above each selective medium and spread with a bent glass rod. *E. coli* plates were incubated at 37 °C for 24 hours before colony counting. *Lactobacillus* plates were anaerobically incubated in a CO_2 incubator at 37 °C for 48 hours before colony counting. Bacterial counts per gram of sample were calculated and expressed as lg(CFU/g) of intestinal content.

1.5.3 Determination of Tissue Antioxidant Capacity Liver, kidney and spleen samples (0.3–0.5 g) were weighed and placed in small beakers on an ice bath. Ice-cold 0.9% physiological saline was added at a tissue-to-saline ratio of 1:9 (w/v). Tissue blocks were quickly minced with scissors and homogenized in a glass homogenizer in an ice-water mixture for 6–10 minutes until fully homogenized. The homogenate was centrifuged at 3,500 r/min for 15 minutes, and the supernatant was carefully collected, aliquoted and stored in a low-temperature refrigerator. Superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity, total antioxidant capac-

ity (T-AOC) and malondialdehyde (MDA) content were measured according to the instructions of Nanjing Jiancheng Bioengineering Institute.

1.6 Primer Design and RT-PCR Analysis

1.6.1 Primer Design Using porcine SOD, GSH-Px and CAT DNA sequences from GenBank as templates and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene, primers were designed using Primer 5.0 software. Designed primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Before use, primer powder was centrifuged at 12,000 r/min for 1 minute, then ddH_2O was added according to the ratio indicated on the tube to prepare a 100 μmol/L working solution, which was stored at -20 °C. Primer sequences and parameters are shown in Table 2 .

Table 2 Sequences and parameters of primers

Gene	GenBank Accession No.	Sequences (5 –3)
GAPDH	NM_{001206359}.1	F: GTCGGTTGTGGATCT-GACCTR: AGCTTGACGAAGTG-GTCGTT
SOD	NM_{001190422}.1	F: GAGACCTGGGCAAT-GTGACTR: CCAAAC-GACTTCCAGCATTT
GSH-Px	NM_{214201}.1	F: AGAAGTGTGAGGT-GAATGGCR: CCCGAGAGTAGCACTG-TAAC
CAT	NM_{214301}.2	F: GAGCACGTTGGAAA-GAGGACR: GGCTGTGGATAAAG-GATGGA

1.6.2 Total RNA Extraction and Detection Appropriate amounts of tissue were ground in liquid nitrogen, and total RNA was extracted using the Trizol method. The concentration and purity of RNA samples were measured, with all samples showing absorbance ratios between 1.8 and 2.0, indicating purity suitable for molecular biology experiments. The total RNA was reverse-transcribed using a 10 μL reaction system, and the cDNA products were stored at -20 °C for later use.

1.6.3 RT-PCR Amplification The mRNA expression of SOD, GSH-Px and CAT was determined using RT-PCR with fluorescent dye. A 20 μL reaction system was prepared according to the kit instructions, with three replicates per

sample. Cycling conditions were: pre-denaturation at 95 °C for 60 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 58 °C for 30 s, and extension at 72 °C for 20 s. Using GAPDH expression as the reference, relative gene expression levels were calculated using the 2^(-ΔΔCt) method.

1.7 Data Processing

Experimental data were analyzed using SPSS 19.0 statistical software with t-tests. Differences were considered significant at $P < 0.05$. Results are expressed as "mean ± standard deviation."

2. Results

2.1 Effects of Alfalfa Saponin on Growth Performance and Diarrhea Rate of Weaned Piglets

As shown in Table 3 , dietary alfalfa saponin increased ADFI and reduced diarrhea rate in weaned piglets, but these differences were not significant ($P > 0.05$). The alfalfa saponin group showed significantly higher ADG ($P < 0.05$) and significantly lower F/G ($P < 0.05$) compared with the control group.

Table 3 Effects of alfalfa saponin on growth performance and diarrhea rate of weaned piglets

Items	Control group	Alfalfa saponin group
ADG/g	492.97 ± 22.54	545.92 ± 13.71
ADFI/g	769.24 ± 46.06	803.95 ± 33.82
F/G	1.56 ± 0.04	1.47 ± 0.02
Diarrhea rate/%	9.62 ± 6.53	3.06 ± 1.92

In the same row, values with different small letter superscripts indicate significant difference ($P < 0.05$). The same applies below.

2.2 Effects of Alfalfa Saponin on Intestinal pH and Microflora of Weaned Piglets

As shown in Table 4 , dietary alfalfa saponin significantly reduced pH values in the duodenum and cecum ($P < 0.05$) but had no significant effect on pH in the jejunum and ileum ($P > 0.05$). Alfalfa saponin reduced *E. coli* counts in the duodenum, jejunum, ileum and cecum, but the differences were not significant ($P > 0.05$). Compared with the control group, the alfalfa saponin group showed significantly increased *Lactobacillus* counts in the duodenum, jejunum and ileum ($P < 0.05$), with a non-significant increase in the cecum ($P > 0.05$).

Table 4 Effects of alfalfa saponin on intestinal pH and microflora of weaned piglets

Items	Control group	Alfalfa saponin group
pH		
Duodenum	6.05 ± 0.04	5.93 ± 0.04
Jejunum	6.49 ± 0.11	6.36 ± 0.19
Ileum	6.48 ± 0.10	6.41 ± 0.58
Cecum	6.58 ± 0.12	5.97 ± 0.29
***E. coli*/[lg(CFU/g)]**		
Duodenum	7.15 ± 0.64	6.36 ± 0.79
Jejunum	7.24 ± 0.72	6.66 ± 0.29
Ileum	7.36 ± 0.51	6.28 ± 0.91
Cecum	6.37 ± 0.43	6.62 ± 0.16
***Lactobacillus*/[lg(CFU/g)]**		
Duodenum	8.39 ± 0.12	8.87 ± 0.09
Jejunum	8.46 ± 0.19	8.94 ± 0.05
Ileum	8.43 ± 0.07	8.92 ± 0.06
Cecum	8.43 ± 0.34	8.79 ± 0.16

2.3 Effects of Alfalfa Saponin on Antioxidant Ability in Tissues of Weaned Piglets

As shown in Table 5 , dietary alfalfa saponin reduced MDA content and increased SOD activity and T-AOC in the liver, kidney and spleen, but these differences were not significant ($P > 0.05$). Compared with the control group, the alfalfa saponin group showed significantly increased GSH-Px activity in the liver and kidney ($P < 0.05$), while GSH-Px activity in the spleen decreased non-significantly ($P > 0.05$). CAT activity in the liver, kidney and spleen was higher in the alfalfa saponin group, with significant increases observed in the liver and kidney ($P < 0.05$).

Table 5 Effects of alfalfa saponin on antioxidant ability in tissues of weaned piglets

Items	Control group	Alfalfa saponin group
Liver		
MDA/(nmol/mg prot)	3.17 ± 0.53	3.02 ± 0.49
SOD/(U/mg prot)	138.28 ± 5.21	147.70 ± 14.21
GSH-Px/(U/mg prot)	79.71 ± 8.65	102.25 ± 4.38
CAT/(U/mg prot)	658.23 ± 44.55	863.02 ± 53.29
T-AOC/(U/mg prot)	3.59 ± 0.01	3.82 ± 0.57
Kidney		
MDA/(nmol/mg prot)	1.80 ± 0.13	1.62 ± 0.34
SOD/(U/mg prot)	166.44 ± 9.60	175.09 ± 16.01
GSH-Px/(U/mg prot)	96.39 ± 7.36	128.12 ± 9.45
CAT/(U/mg prot)	441.63 ± 50.43	658.42 ± 31.20

Items	Control group	Alfalfa saponin group
T-AOC/(U/mg prot)	2.66 ± 0.47	2.82 ± 0.33
Spleen		
MDA/(nmol/mg prot)	2.00 ± 0.10	1.94 ± 0.27
SOD/(U/mg prot)	23.03 ± 12.02	43.76 ± 23.34
GSH-Px/(U/mg prot)	38.23 ± 9.90	37.10 ± 1.26
CAT/(U/mg prot)	36.38 ± 15.62	44.96 ± 8.06
T-AOC/(U/mg prot)	0.28 ± 0.05	0.29 ± 0.01

2.4 Effects of Alfalfa Saponin on mRNA Expression of SOD, GSH-Px and CAT in Tissues of Weaned Piglets

As shown in Table 6 , dietary alfalfa saponin had no significant effect on SOD mRNA expression in the liver, duodenum, jejunum or ileum ($P > 0.05$). Dietary alfalfa saponin significantly increased GSH-Px mRNA expression in the liver and jejunum ($P < 0.05$) but had no significant effect in the duodenum or ileum ($P > 0.05$). Compared with the control group, the alfalfa saponin group showed significantly increased CAT mRNA expression in the duodenum and ileum ($P < 0.05$), with a trend toward increased expression in the liver and jejunum ($P > 0.05$).

Table 6 Effects of alfalfa saponin on mRNA expression of SOD, GSH-Px and CAT in tissues of weaned piglets

Items	Control group	Alfalfa saponin group
Liver		
SOD	1.00 ± 0.27	1.08 ± 0.23
GSH-Px	1.00 ± 0.03	1.07 ± 0.02
CAT	1.00 ± 0.16	1.22 ± 0.16
Duodenum		
SOD	1.00 ± 0.14	1.01 ± 0.18
GSH-Px	1.00 ± 0.34	1.03 ± 0.07
CAT	1.00 ± 0.01	1.50 ± 0.16
Jejunum		
SOD	1.00 ± 0.16	0.97 ± 0.19
GSH-Px	1.00 ± 0.05	1.50 ± 0.05
CAT	1.00 ± 0.15	1.13 ± 0.30
Ileum		
SOD	1.00 ± 0.06	1.01 ± 0.13
GSH-Px	1.00 ± 0.26	0.97 ± 0.16
CAT	1.00 ± 0.04	1.34 ± 0.09

3. Discussion

3.1 Effects of Alfalfa Saponin on Growth Performance and Diarrhea Rate of Weaned Piglets

Research results on the effects of alfalfa saponin on livestock growth performance have been inconsistent over the years, possibly due to differences in animal species, physiological status and extraction sources of alfalfa saponin. Xu [3] reported that dietary alfalfa saponin increased ADFI and ADG in weaned piglets. Cao [4] studied the effects of alfalfa meal on piglet growth performance and found that ADG was higher in the treatment group than in the control group. Hou et al. [5] added 30, 60 and 90 mg/kg alfalfa saponin to laying hen diets and found that egg production rate and egg weight were higher than those of the control group, with a certain degree of reduction in F/G. Wang et al. [6] reported that appropriate supplementation levels of alfalfa saponin (0.25%-0.50%) could improve piglet growth performance. The results of this experiment showed that dietary alfalfa saponin increased ADFI and reduced diarrhea rate in weaned piglets, with significantly higher ADG and significantly lower F/G compared with the control group. These findings indicate that dietary supplementation with appropriate amounts of alfalfa saponin can improve growth performance and reduce diarrhea rate in weaned piglets.

3.2 Effects of Alfalfa Saponin on Intestinal pH and Microflora of Weaned Piglets

Intestinal pH is an important factor affecting microbial survival and reproduction, as well as a primary regulator of digestive enzyme secretion and activity. Therefore, maintaining a stable and appropriate intestinal pH is essential for digestive health and normal digestive-absorptive function. Research has reported that high intestinal pH in weaned piglets not only adversely affects small intestinal digestive enzyme activity but also promotes proliferation of pathogenic bacteria, ultimately causing diarrhea. Lupton et al. [7] and Kashiwagura et al. [8] reported that pH can affect cell development, with changes in intracellular pH inducing cell division and promoting DNA synthesis, while lower pH helps maintain intact morphological structure of intestinal mucosa and promotes mucosal cell proliferation. In this experiment, alfalfa saponin significantly reduced duodenal and cecal pH in piglets, possibly by promoting secretion of gastric acid and digestive fluids, or by enhancing *Lactobacillus* populations, which would lower intestinal pH.

The balance of the intestinal microecosystem significantly affects normal intestinal physiology and whole-body metabolism. Reduced *E. coli* populations can decrease diarrhea and other gastrointestinal diseases in piglets [9], while beneficial bacteria such as *Lactobacillus* can play a role in the defense system against pathogenic invasion through their metabolites and antimicrobial substances [10]. Studies have reported that plant saponins possess antibacterial activity [11-12]. Jin et al. [13] found that total saponins from soapberry showed strong antibac-

terial activity against *E. coli*. Rose root total saponins also exhibited varying degrees of inhibition against *E. coli* [14]. Previous studies have shown that the pentacyclic triterpenoid saponin monomer Bp3 from *Bupleurum* had antifungal effects against fluconazole-resistant *Candida albicans* strains [15]. Li et al. [16] reported that treatment with alfalfa saponin extract inhibited the growth of both *E. coli* and *Bacillus subtilis*. Wu [17] studied the effects of alfalfa saponin supplementation in weaned piglet diets and found that alfalfa saponin significantly inhibited *E. coli* and *Salmonella*, reducing diarrhea rate to some extent. The results of this experiment indicate that alfalfa saponin slightly inhibited *E. coli* proliferation while promoting *Lactobacillus* growth, suggesting that alfalfa saponin may help maintain intestinal microecological balance.

3.3 Effects of Alfalfa Saponin on Tissue Antioxidant Capacity and Related Enzyme mRNA Expression in Weaned Piglets

Antioxidant capacity is a self-protective mechanism against free radical damage. Antioxidant enzymes can enhance defense and immune functions, with their activity reflecting the oxidative-antioxidative status of the organism [18]. Appropriate concentrations of reactive oxygen species are essential for resisting certain harmful bacteria and for many metabolic processes including cell signal transduction [19]. Under normal physiological conditions, nutritional status and free radical content maintain a dynamic balance; once this balance is disrupted, excessive free radicals cause oxidative damage to cells [20]. MDA is a product of lipid peroxidation; GSH-Px reflects the ability to scavenge oxygen free radicals and is a major component of the antioxidant defense system; SOD is an enzyme that protects cell membrane structure and function [21]; CAT promotes decomposition of hydrogen peroxide (H_2O_2), thereby protecting cells from free radical damage [22]; T-AOC is a comprehensive indicator reflecting the functional status of the antioxidant system, representing the combined effects of various antioxidant enzymes [23]. Enhancing exogenous antioxidant intake or increasing endogenous antioxidant production can reduce free radical damage to cell membranes and effectively scavenge free radicals [24].

Numerous studies have demonstrated that most saponins possess certain antioxidant capacities [25]. Liu et al. [26] reported that ginsenosides could enhance antioxidant enzyme activity and alleviate induced lung oxidative damage in mice. Shi et al. [27] found that alfalfa saponin significantly increased GSH-Px and SOD activities while reducing MDA content in piglet tissues. Sun et al. [28] discovered through in vitro antioxidant assays that triterpenoid saponins from sour jujube fruit could scavenge DPPH radicals, showing significant antioxidant effects. Guan et al. [29] found that a pentacyclic triterpenoid saponin compound exhibited strong scavenging capacity against DPPH and hydroxyl radicals. Huang et al. [30] reported that daidzein could induce SOD expression in rat heart, brain and liver tissues, enhance free radical scavenging activity and significantly reduce MDA content. In this experiment, alfalfa saponin reduced MDA content and increased T-AOC and SOD activity in the liver, kidney and

spleen; GSH-Px and CAT activities in the liver and kidney were significantly higher in the alfalfa saponin group; and alfalfa saponin significantly increased CAT mRNA expression in the duodenum and ileum and GSH-Px mRNA expression in the liver and jejunum. These results indicate that alfalfa saponin can enhance free radical scavenging or reduce free radical production, thereby improving tissue antioxidant capacity.

4. Conclusion

Alfalfa saponin can improve growth performance and tissue antioxidant capacity, modulate intestinal microbiota and promote intestinal health in weaned piglets.

References

[1] WU Zhimin, GENG Zhongcheng, WANG Xiuna, et al. Effects of betaine and alfalfa saponin on growth performance of weaned piglets[J]. Journal of Heilongjiang Bayi Agricultural University, 2010, 22(3): 58-60.

[2] YALINKILIC O, ENGINAR H. Effect of X-radiation on lipid peroxidation and antioxidant systems in rats treated with saponin-containing compounds[J]. Photochemistry and Photobiology, 2008, 84(1): 236-242.

[3] XU Bing. Study on the effects and mechanism of alfalfa saponin on growth performance, digestive physiology and immunity of weaned piglets[D]. Master' s thesis. Zhengzhou: Henan Agricultural University, 2008.

[4] CAO Yanan. Weight gain experiment of adding alfalfa meal to piglet diets[J]. Shandong Journal of Animal Science and Veterinary Medicine, 2008, 29(11): 8.

[5] HOU Yonggang, CHEN Hui, HUANG Renlu, et al. Effects of alfalfa saponin on production performance, carcass indices, cholesterol and serum lipids of laying hens[J]. Chinese Journal of Animal Science, 2009, 45(17): 30-33.

[6] WANG Yanhua, WANG Chengzhang, GAO Yongge, et al. Effects of alfalfa saponin on production performance, digestibility and blood biochemical indices of weaned piglets[J]. Acta Prataculturae Sinica, 2009, 18(5): 115-122.

[7] LUPTON J R, JACOBS L R. Fiber supplementation results in expanded proliferative zones in rat gastric mucosa[J]. The American Journal of Clinical Nutrition, 1987, 46(6): 980-984.

[8] KASHIWAGURA T, DEUTSCH C J, TAYLOR J, et al. Dependence of gluconeogenesis, urea synthesis, and energy metabolism of hepatocytes on intracellular pH[J]. Journal of Biological Chemistry, 1984, 259(1): 237-243.

[9] SARRAZIN E, FRITZSCHE C, BERTSCHINGER H U. Main virulence factors in Escherichia coli isolates from swine over two weeks old with edema disease and/or E. coli diarrhea[J]. Schweizer Archiv für Tierheilkunde, 2000, 142(11): 625-630.

[10] SERVIN A L. Antagonistic activities of Lactobacilli and Bifidobacteria against microbial pathogens[J]. FEMS Microbiology Reviews, 2004, 28(4): 405-440.

[11] PENG Qian, XU Rong, YIN Ning, et al. Study on antibacterial activity of Gleditsia sinensis saponin and tea saponin[C]//Proceedings of the Forestry Biomass Chemistry and Engineering Academic Symposium. Nanjing: Chinese Society of Forestry, 2009.

[12] YU Zhihui, DING Xuezhi, XIA Liqiu, et al. Antibacterial activity and mechanism of total saponins from Chinese onion (Allium chinense)[J]. Food Science, 2013, 34(15): 75-80.

[13] JIN Qiu, XI Limin, ZHANG Xinxin, et al. Study on antibacterial activity of Sapindus mukorossi saponin[J]. Bulletin of Science and Technology, 2014, 30(3): 35-38.

[14] Qimai Guli · Ashamu, Maliyamu · Afanlimiti, Miliban · Huojiaheimaitti, et al. Extraction and antibacterial effect of total saponins from wild rose root[J]. Chinese Journal of Spectroscopy Laboratory, 2013, 30(6): 3045-3051.

[15] GAO Qingyun, SHEN Liangliang, SHI Weimin, et al. Study on antifungal activity of Bp3 against fluconazole-resistant Candida albicans[J]. Journal of Tongji University (Medical Edition), 2009, 30(1): 40-43.

[16] LI Bo, SUN Tianying, YU Xinyang. Study on antibacterial activity and antioxidant properties of alfalfa saponin[J]. Food Research and Development, 2013(2): 1-2, 33.

[17] WU Zhimin. Effects of betaine and alfalfa saponin on growth performance and immune function of weaned piglets[D]. Master' s thesis. Daqing: Heilongjiang Bayi Agricultural University, 2010.

[18] RUDNEVA I I. Blood antioxidant system of Black Sea elasmobranch and teleosts[J]. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 1997, 118(2): 255-260.

[19] MITTAL C K, MURAD F. Activation of guanylate cyclase by superoxide dismutase and hydroxyl radical: a physiological regulator of guanosine 3' ,5' -monophosphate formation[J]. Proceedings of the National Academy of Sciences of the United States of America, 1977, 74(10): 4360-4364.

[20] YU Zepeng, LE Guowei, SHI Yonghui, et al. Effects of different zinc sources on growth and antioxidant capacity of weaned mice[J]. Animal Husbandry and Veterinary Medicine, 2005, 37(4): 1-3.

[21] LIU Wenfei, LIU Weilong, ZHAN Xiuan, et al. Effects of different forms of methionine on production performance, immune indices and antioxidant function of broiler breeder chickens[J]. Chinese Journal of Animal Nutrition, 2013, 25(9): 2118-2125.

[22] SOYLU E M, SOYLU S, KURT S. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent Phytophthora infestans[J]. Mycopathologia, 2006, 161(2): 119-128.

[23] ZHANG Jinlong, XU Lili, LI Yanfei. Oxidative and antioxidant characteristics of immune tissues in chicks with encephalomalacia[J]. China Animal Husbandry and Veterinary Medicine, 2007, 34(2): 42-44.

[24] ZHANG Jingjing, SHI Shaohui, BA Weisong, et al. Effect of Ginkgo biloba extract on antioxidant capacity in rabbits with renal acute ischemia-reperfusion injury[J]. Medical Research and Education, 2005, 22(1): 4-5.

[25] SPARG S G, LIGHT M E, VAN STADEN J. Biological activities and distribution of plant saponins[J]. Journal of Ethnopharmacology, 2004, 94(2/3): 219-243.

[26] LIU Baojian, WANG Cunlian, XU Mingju, et al. Effects of ginsenoside Rb1 on antioxidant enzymes in mice with H9N2 influenza virus-induced acute lung injury[J]. Acta Laboratorium Animalis Scientia Sinica, 2014, 22(1): 11, 38-43.

[27] SHI Yinghua, WANG Chengzhang, XU Bing, et al. Effects of alfalfa saponin on growth performance and antioxidant properties of weaned piglets[J]. Acta Agrestia Sinica, 2010, 18(5): 735-739.

[28] SUN Yanfang, LIANG Zongsuo, LIU Zheng, et al. Study on antibacterial and antioxidant activities of triterpenoid saponins from sour jujube fruit[J]. Science and Technology of Food Industry, 2012, 33(6): 139-142.

[29] GUAN Fuqin, LIU Min, SHAN Yu, et al. Study on antioxidant activity of pentacyclic triterpenoids from Lonicera macranthoides[J]. Lishizhen Medicine and Materia Medica Research, 2013, 24(6): 1315-1317.

[30] HUANG Yushan, ZENG Jing, HUANG Yuping, et al. Effects of daidzein on SOD and MDA in heart, brain and liver tissues of rats[J]. West China Journal of Pharmaceutical Sciences, 2007, 22(2): 138-139.

Note: Figure translations are in progress. See original paper for figures.