Effects of Dietary Alfalfa Meal Supplementation on the Colonic Microbiota and Its Metabolites in Growing Pigs

Authors: Wang Jiawei, Zhang Lei, Zhu Jiaoyue, Wang Yubo, YIN Jingdong, Zhu Weiyun

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

Abstract

This experiment aimed to investigate the effects of insoluble fiber derived from alfalfa meal on the colonic microbiota and its fermentation metabolites in growing pigs. Twenty-four castrated male Duroc × Landrace × Large White pigs with a body weight of (24.8$±$0.7) kg were randomly divided into 4 groups and fed diets containing 0 (control group), 5%, 10%, and 15% alfalfa meal, respectively, with 6 replicates per group and 1 pig per replicate. After a 28-day experimental period, the pigs were slaughtered and samples were collected to determine serum fatty acid composition, short-chain fatty acid concentrations in colonic digesta, and microbial composition. The results showed that feeding alfalfa meal significantly reduced the feed-to-gain ratio of growing pigs (P<0.05), but had no significant effect on average daily gain and average daily feed intake (P>0.05). 16S rDNA V3 region sequencing of the colonic microbiota indicated that alfalfa meal had no significant effect on the porcine colonic microbiota (P>0.05), but significantly increased the concentrations of total short-chain fatty acids, acetate, and butyrate in colonic digesta (P<0.05). With increasing dietary alfalfa meal content, serum polyunsaturated fatty acid content was significantly increased (P<0.05), while saturated fatty acid and monounsaturated fatty acid contents were significantly decreased (P<0.05). These results suggest that insoluble fiber derived from alfalfa meal does not affect the structure of the colonic microbial community, but enhances the fermentation activity of colonic microorganisms and short-chain fatty acid production, thereby exerting a regulatory effect on fatty acid composition in pigs.

Full Text

Effects of Dietary Alfalfa Meal Supplementation on the Colonic Microbiota and Its Metabolites in Growing Pigs

Wang Jiawei^1 Zhang Lei^2 Zhu Jiaoyue^2 Wang Yubo^2 Yin Jingdong[2*] Zhu Weiyun[1*]

(1. Laboratory of Gastrointestinal Microbiology, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095; 2. State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193)

Abstract: This experiment was conducted to investigate the effects of insoluble fiber derived from alfalfa meal on the colonic microbiota and its fermentation metabolites in growing pigs. Twenty-four Duroc × Landrace × Large White castrated male pigs with a body weight of (24.8$±$0.7) kg were selected and randomly divided into 4 groups. The pigs were fed diets containing 0 (control group), 5%, 10%, and 15% alfalfa meal, respectively, with 6 replicates per group and 1 pig per replicate. After 28 d of the experiment, samples were collected after slaughter to determine serum fatty acid composition, concentrations of short-chain fatty acids in colonic digesta, and microbial composition. The results showed that dietary alfalfa meal significantly reduced the feed-to-gain ratio of growing pigs (P<0.05), but had no significant effect on average daily gain or average daily feed intake (P>0.05). Sequencing results for the 16S rDNA V3 region of the colonic microbial community showed that alfalfa meal had no significant effect on the colonic microbial community of pigs (P>0.05), but significantly increased the concentrations of total short-chain fatty acids, acetic acid, and butyric acid in colonic digesta (P<0.05). As the alfalfa meal content in the diet increased, serum polyunsaturated fatty acid content increased significantly (P<0.05), whereas saturated fatty acid and monounsaturated fatty acid contents decreased significantly (P<0.05). These results suggest that insoluble fiber derived from alfalfa meal does not affect the structure of the colonic microbial community, but enhances the fermentative activity of colonic microorganisms and the production of short-chain fatty acids, and has a role in regulating the fatty acid composition of the pig body.

Key words: growing pig; alfalfa meal; insoluble fiber; short-chain fatty acids; colonic microbiota; fatty acids

Chinese Library Classification No.: S811.6

Dietary fiber plays an increasingly important role in human health and livestock production[1-2], but the regulatory effects of different types of fiber on the body are not entirely the same. Alfalfa is often used as a high-quality green feed supplement for pigs raised under extensive systems, and is especially com-

monly used as a feed fiber source to prevent constipation in sows. The present study will help develop alfalfa into a healthy, high-value-added insoluble dietary fiber product to improve the nutrition and health of humans and pigs. Fiber in feed can be fermented by anaerobic bacteria in the hindgut of pigs and mainly produces metabolic products such as short-chain fatty acids (SCFA), including acetic acid, propionic acid, and butyric acid[3-4]. To date, studies on dietary fiber have mostly focused on soluble fibers such as inulin, guar gum, and arabinoxylan[5-8]. In pig production, alfalfa meal is often used as a fiber source in pig diets, but insoluble

Received: 2016-03-03
Funding: National 973 Program funding support (2013CB127302, 2012CB124702); Beijing Municipal Undergraduate Innovation Training Program (2015BJ049)
Author biography: Wang Jiawei (1990–), female, Manchu, Chengde, Hebei, master' s student, engaged in research on animal nutrition and gastrointestinal microbiology. E-mail: jviwang@foxmail.com
* Corresponding authors: Yin Jingdong, research fellow, doctoral supervisor, E-mail: yinjd@cau.edu.cn; Zhu Weiyun, professor, doctoral supervisor, E-mail: zhuweiyun@njau.edu.cn

fiber [9]; however, the effects on the structure of the colonic digesta microbiota and its fermentation products in pigs have not yet been reported. Therefore, in this study, different proportions of alfalfa meal were added to the diet to investigate the effects of insoluble fiber on growth performance, the colonic digesta microbial community, its metabolites, and serum fatty acid composition in growing pigs, with the aim of determining whether dietary insoluble fiber can alter the intestinal microbial community and microbial fermentation metabolites.

1 Materials and Methods

1.1 Experimental animals and experimental design

This experiment was conducted at the Fengning Animal Experimental Base of the Feed Industry Centre of the Ministry of Agriculture. Twenty-four Duroc × Landrace × Yorkshire crossbred castrated male pigs, 60 days of age and with a body weight of (24.8 ± 0.7) kg, were selected. A single-factor completely randomized block design was used, with pigs divided into 4 groups. The supplemental levels of alfalfa meal were 0 (control group), 5%, 10%, and 15%, respectively. Each group had 6 replicates, with 1 pig per replicate. Before the formal experiment began, pigs were allowed to adapt for 3 d, and the formal experimental period lasted 28 d. During the experiment, each pig was housed in an individual metabolic cage and was fed once daily at 08:00 and 16:00, with free access to feed and water. The pig house was cleaned twice daily and disinfected regularly.

The diet formulas were designed and prepared according to the nutrient requirements for growing pigs (25–50 kg) recommended by the NRC (2012). The net energy level and the ileal standardized digestible amino acids of all essential amino acids in the diets met the requirements of growing pigs. The composition and nutrient levels of the diets in the control and experimental groups are shown in Table 1. No antibiotics were added to the diets.

Table 1 Composition and nutrient levels of the diets (as-fed basis) %

Items	Control	Alfalfa meal supplemental level/%		
		5	10	15
Ingredients				
Corn	71.10	64.97	58.00	52.54
Soybean meal	25.00	24.60	25.00	24.00
Alfalfa meal	0.00	5.00	10.00	15.00
Soybean oil	0.52	2.20	3.99	5.58
Limestone	1.16	1.00	0.88	0.72
$CaHPO_4$	0.70	0.70	0.65	0.65
L-Lys · HCl	0.24	0.24	0.21	0.22
L-Thr	0.08	0.08	0.06	0.07
DL-Met	0.07	0.08	0.08	0.09
L-Try	0.02	0.02	0.02	0.02

Item				
Premix1)	1.11	1.11	1.11	1.11
Total	100.00	100.00	100.00	100.00
Nutrient levels2)				
DM	88.25	88.71	89.24	89.75
NE/(MJ/kg)	10.36	10.36	10.36	10.36
CP	16.93	17.54	17.89	17.61
SDF	1.82	1.51	2.04	2.18
IDF	12.66	13.02	14.82	16.80
TDF	14.48	14.52	16.86	18.98
Lys	1.12	1.16	1.11	1.17
Met+Cys	0.60	0.64	0.65	0.71
Try	0.18	0.20	0.19	0.18
Thr	0.76	0.80	0.77	0.80

1) The premix provided the following per kg of the diet: VA 20 000 IU, VD3 2 000 IU, VE 40 IU, VK3 2 mg, VB1 2 mg, VB2 4 mg, VB6 3 mg, VB12 0.06 mg, nicotinic acid 20 mg, pantothenic acid 12 mg, folic acid 1.60 mg, biotin 0.14 mg, Fe 76.50 mg, Cu 140 mg, Zn 50 mg, Mn 19.50 mg, I 0.50 mg, Se 0.40 mg, NaCl

3 g, antioxidants 0.05 mg, choline chloride 1.20 g, sweetener 0.1 g, feed flavor 0.08 g, phytase 0.10 g.

2) NE was a calculated value, and the others were measured values.

1.2 Slaughter and Sample Collection

On day 28 of the formal trial, blood was collected from the anterior vena cava of each experimental pig; serum was prepared and stored at −20 °C for analysis of fatty acid composition. After weighing, all experimental pigs were slaughtered. The abdominal cavity was opened, the colon was separated, and colonic digesta were collected for determination of SCFA concentrations and microbial composition.

1.3 Determination of Growth Performance

Each experimental pig was weighed at the start of the formal trial and on day 28, and the average daily gain (ADG) was calculated. The feed intake of each experimental pig was recorded, and average daily feed intake (ADFI) and the feed/gain ratio (F/G) were calculated.

1.4 Determination of SCFA Concentrations in Colonic Digesta

After thawing, 1 g of the digesta sample was taken, 3 mL of 50 mmol/L sulfuric acid solution was added, and the mixture was left at 4 °C for 30 min, then centrifuged at 20 000×g

Centrifuge for 10 min, filter the supernatant into a sample vial, and analyze SCFA concentrations by gas chromatography. The chromatographic column was 30 m in length, with an inner diameter of 0.32 mm and a film thickness of 0.5 μm; the injector and detector temperatures were 260 and 280 °C, respectively; the carrier gas was nitrogen, with a flow rate of 2.5 mL/min.

1.5 Analysis of the microbiota in colonic digesta

Genomic DNA was extracted from 100 mg of sample using the Power Soil DNA Isolation Kit from Mobio (USA), following the manufacturer' s instructions. The extracted DNA was used as the template to amplify the V3 hypervariable region of 16S rDNA by PCR. The primer sequences were as follows: forward primer, 338-CCTACGGGAGGCAGCAG-355; reverse primer, 502-ATTACCGCGGCTGCTGG-518. The PCR amplification system (25 μL) consisted of 2×Master Mix 12.5 μL, 1.5 μL each of the forward and reverse primers, DNA template 2.5 μL, and sterile double-distilled water 7 μL. The PCR amplification conditions were: 94 °C for 5 min; 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 30 s for 25 cycles; finally, extension at 72 °C for 10 min, followed by storage at 4 °C. The purified PCR products were subjected to Illumina MiSeq sequencing.

The sequencing data were compared and analyzed against the existing 16S rDNA database, and sequences with adapters and low-quality sequences were removed. QIIME software was used to count the sequences for each sample, and then UCLUST software was used to cluster sequences at a 97% similarity level, yielding operational taxonomic unit (OTU) sequences. Based on OTUs, species richness in the samples was calculated at two taxonomic levels: phylum and genus.

1.6 Analysis of fatty acid composition in serum

A 1 mL serum sample was placed in a hydrolysis tube, and 4 mL of acetyl chloride/methanol solution and 1 mL of C11:0 internal standard solution (1.0 mg/mL) were added. After the cap was tightly screwed on, the tube was kept in an 80 °C water bath for 2 h. After the solution cooled, 5 mL of 7% potassium carbonate solution was added for neutralization. The lipid-phase solution was transferred into a sample vial, and fatty acid composition was analyzed by gas chromatography. The chromatographic column was 60 m in length, with an inner diameter of 0.25 mm and a film thickness of 0.25 μm; the injector and detector temperatures were 260 and 270 °C, respectively; the carrier gas was nitrogen, with a flow rate of 2 mL/min.

1.7 Statistical analysis

After SCFA and fatty acid data were organized using Excel 2013, analysis of variance was performed using the GLM procedure in SAS 9.3 statistical software. Microbial relative abundance data were analyzed using the Mann-Whitney U test. $P<0.05$ was considered a significant difference, and $0.05<P<0.10$ was considered a trend toward significance.

2 Results and analysis

2.1 Effects of alfalfa meal on growth performance of growing pigs

As shown in Table 2, under the conditions of this experiment, the addition of alfalfa meal to the diet had no significant effect on final body weight, ADG, or ADFI in growing pigs ($P>0.05$). However, compared with the control group, alfalfa meal supplementation extremely significantly reduced F/G ($P<0.01$), while there was no significant difference in F/G among the experimental groups ($P>0.05$).

Table 2 Effects of alfalfa meal on growth performance of growing pigs

Table 2 Effects of alfalfa meal on the growth performance of growing pigs

Items	Control	5	10	15	SEM	Linear	Quadratic
Initial BW/kg	25.13	24.70	24.72	24.58	0.20	0.08	0.46

Items	Control	5	10	15	SEM	Linear	Quadratic
Final BW/kg	46.52	46.48	46.58	46.02	0.91	0.74	0.77
ADG/g	712	726	728	714	14	0.91	0.36
ADFI/g	1 438	1 406	1 410	1 368	24	0.08	0.85
F/G	2.04a	1.94b	1.95b	1.93b	0.02	<0.01	0.09

Alfalfa meal supplemental level/%; *P*-value.

In the same row, values with different lowercase superscript letters indicate significant differences ($P<0.05$). The same applies below.

2.2 Effects of alfalfa meal on SCFA concentrations in colonic digesta of growing pigs

As shown in Table 3, alfalfa meal significantly increased the concentrations of total SCFA (linear $P<0.01$), acetate (linear $P<0.01$) and valerate (quadratic $P<0.01$), as well as the concentrations of butyrate, isobutyrate, isovalerate and total branched-chain fatty acids (BCFA) (linear $P<0.01$; quadratic $P<0.01$); propionate concentration also tended to increase (linear $P=0.07$). When the alfalfa meal supplemental level was 10%, the concentrations of total SCFA, propionate, butyrate, valerate and total BCFA were the highest.

Table 3 Effects of alfalfa meal on SCFA concentration in colonic digesta mmol/kg

Items	Control	5	10	15	SEM	Linear	Quadratic
Total SCFA	34.21b	35.47b	43.90a	40.80a	1.68	<0.01	0.21
Total BCFA	1.85c	2.33b	3.24a	2.40b	0.14	<0.01	<0.01
Acetate	18.36b	17.98b	21.55a	22.11a	1.02	<0.01	0.65
Propionate	9.66	9.27	10.68	10.56	0.47	0.07	0.77
Butyrate	3.59c	4.79bc	6.94a	4.82b	0.40	<0.01	<0.01
Valerate	0.75c	1.08b	1.51a	0.90bc	0.10	0.08	<0.01
Isobutyrate	0.64c	0.87bc	1.32a	0.94b	0.08	<0.01	<0.01
Isovalerate	1.21b	1.45b	1.92a	1.46b	0.10	<0.01	<0.01

2.3 Effects of alfalfa meal on the colonic microbial community

As shown in Table 4, Firmicutes was the most predominant phylum, with a relative abundance of 79.84% in the control group and 81.28% in the 10% alfalfa meal supplemental group. The second most predominant phylum was

Bacteroidetes, with a relative abundance of 8.33% in the control group and 7.56% in the 10% alfalfa meal supplemental group. The phyla with the next highest relative abundances were Proteobacteria, Actinobacteria, Acidobacteria, and Tenericutes; their relative abundances in the control group were 2.90%, 2.67%, 1.20%, and 0.97%, respectively, and those in the 10% alfalfa meal supplemental group were 1.61%, 1.65%, 0.39%, and 2.90%, respectively. Statistical analysis of the relative abundances of the microbial communities at the phylum and genus levels in the control group and the 10% alfalfa meal supplemental group using the Mann–Whitney U test showed that microbial communities with relative abundance above 0.1% did not differ significantly at either the phylum or genus level (P>0.05).

Table 4 Effects of alfalfa meal on relative abundance of colonic microbiota at the phylum and genus levels %

Taxa	Control group	10% alfalfa meal supplemental group	P-value
Phylum level			
Firmicutes	79.84	81.28	0.69
Bacteroidetes	8.33	7.56	0.89
Proteobacteria	2.90	1.61	1.00
Actinobacteria	2.67	1.65	0.69
Acidobacteria	1.20	0.39	0.69
Tenericutes	0.97	2.90	0.11
Gemmatimonadetes	0.67	0.19	1.00
Spirochaetes	0.48	0.33	0.89
Chloroflexi	0.39	0.13	1.00

Taxon			
Verrucomicrobia	0.16	0.11	0.89
Planctomycetes	0.16	0.06	0.89
Nitrospirae	0.13	0.04	0.69
Cyanobacteria	0.13	0.11	0.89
Chlamydiae	0.03	0.48	0.20
GAL15	0.11	0.02	0.11
Genus level			
Turicibacter	11.38	5.09	0.69
Lactobacillus	5.57	7.32	0.49
Clostridium	2.29	1.55	0.69
Blautia	1.84	1.16	0.20
Faecalibacterium	1.59	2.06	1.00
Prevotella	1.42	1.58	0.69

Taxon			
Ruminococcus	1.11	2.13	0.34
Lachnospira	1.04	1.12	0.34
Bulleidia	0.93	0.85	0.69
Streptococcus	0.88	1.44	1.00
Parabacteroides	0.47	0.37	0.89
Treponema	0.39	0.26	0.89
Phascolarctobacterium	0.38	0.33	0.69
Acidiphilium	0.33	0.12	0.69
Oscillospira	0.26	0.41	0.20
Coprococcus	0.20	0.21	1.00
p-75-a5	0.18	0.22	0.89
Propionibacterium	0.16	0.08	0.89
Agrobacterium	0.15	0.04	0.34
Bacillus	0.13	0.03	0.69
Bacteroides	0.13	0.23	0.49
Sphingomonas	0.13	0.06	1.00
Corynebacterium	0.12	0.04	0.49
YRC22	0.09	0.11	0.69
CF231	0.08	0.21	0.34

2.4 Effects of alfalfa meal on serum fatty acid composition

As shown in Table 5, compared with the control group, addition of alfalfa meal significantly reduced the contents of myristic acid (C14:0), palmitoleic acid (C16:1), oleic acid (C18:1n-9), eicosatrienoic acid (C20:3n-6), and docosahexaenoic acid (C22:6n-3) in the serum of experimental pigs (linear, $P<0.01$), as well as the contents of palmitic acid (C16:0), arachidic acid (C20:0), docosanoic acid (C22:0), and tetracosenoic acid (C24:1) (linear, $P<0.01$; quadratic, $P<0.05$). However, it significantly increased the contents of linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), and eicosapentaenoic acid (C20:5n-3) (linear, $P<0.01$). With increasing alfalfa meal content in the diet, the contents of saturated fatty acids (SFA) (linear, $P<0.01$; quadratic, $P<0.01$) and monounsaturated fatty acids (MUFA) decreased significantly (linear, $P<0.01$), whereas the contents of polyunsaturated fatty acids (PUFA) and the PUFA/SFA value increased significantly (linear, $P<0.01$).

Table 5 Effects of alfalfa meal on composition of serum fatty acid in growing pigs %

Items	Control	5	10	15	SEM	Linear	Quadratic
Myristic acid (C14:0)	0.62a	0.49b	0.48b	0.49b	0.04	0.04	0.11
Palmitic acid (C16:0)	19.78a	17.87b	16.92c	18.38b	0.24	<0.01	<0.01
Stearic acid (C18:0)	17.34	17.76	16.30	17.35	0.27	0.25	0.26
Arachidic acid (C20:0)	0.45a	0.28b	0.27b	0.28b	0.04	<0.01	0.02
Docosanoic acid (C22:0)	0.36a	0.19b	0.20b	0.20b	0.03	<0.01	<0.01
Tetracosanoic acid (C24:0)	1.97	2.00	1.98	2.00	0.15	0.95	0.95
SFA	40.51a	38.59b	36.15c	38.70b	0.47	<0.01	<0.01
Palmitoleic acid (C16:1)	0.87a	0.73ab	0.65ab	0.60b	0.08	0.03	0.59
Oleic acid (C18:1n-9)	17.98a						

Item							
Monounsaturated fatty acids MUFA	19.50a	17.86ab	17.22b	16.37b	0.60	<0.01	0.52
Linoleic acid (C18:2n-6)	23.81c	28.05b	31.46a	33.17a	1.09	<0.01	0.26
Linolenic acid (C18:3n-3)	0.64c	1.34b	1.85a	2.05a	0.14	<0.01	0.09

Item							
Docosatrienoic acid (C20:3n-6)	0.75a	0.84a	0.54b	0.46b	0.06	<0.01	0.19
Docosatetraenoic acid (C20:4n-6)	9.70c	8.85	8.33	8.71	0.39	0.06	0.13
Eicosapentaenoic acid EPA (C20:5n-3)	0.29c	0.41bc	0.50b	0.74a	0.05	<0.01	0.25
Docosahexaenoic acid DHA (C22:6n-3)	1.33c	1.15b	0.84b	0.88b	0.11	<0.01	0.30
Polyunsaturated fatty acids PUFA	36.51d	40.63b	43.53ab	46.01a	1.04	<0.01	0.44
Polyunsaturated fatty acids/saturated fatty acids PUFA/SFA	0.90d	1.05b	1.21a	1.19a	0.03	<0.01	0.08

3 Discussion

As shown in Table 1, the contents of soluble fiber in the diets of the 4 groups were relatively low and basically consistent, whereas the level of insoluble fiber was relatively high, accounting for about 90% of total fiber and increasing linearly. Therefore, the fiber in alfalfa meal was mainly insoluble fiber, and the experimental effects were mainly derived from differences in the level of insoluble fiber in the diets.

3.1 Effects of alfalfa meal on growth performance of growing pigs

An appropriate fiber content can increase ADG and ADFI in pigs[10], but when the fiber content in the diet is too high, it reduces the digestibility of nutrients throughout the intestinal tract of pigs[9], thereby affecting growth performance. Xu Xiangyang et al.[11] reported that feeding alfalfa meal at levels of 5% and

10% significantly increased ADG in growing pigs and significantly reduced F/G, but had no significant effect on ADFI; when 15% and 20% alfalfa meal were added to the diet, ADG and ADFI in pigs were not affected.

The results of this study showed that alfalfa meal had no significant effect on ADG or ADFI in growing pigs, but improved F/G, which is similar to previous findings. Thus, although alfalfa meal replaced part of the corn and soybean meal in the diet in this experiment, with the maximum inclusion level being 15%, as long as the dietary net energy and standardized ileal digestible amino acid levels met the nutritional requirements of pigs, alfalfa meal did not affect the growth performance of growing pigs.

3.2 Effects of alfalfa meal on fecal SCFA concentrations in the colon of growing pigs

Dietary fiber in the small intestine of monogastric animals cannot be digested, but in the hindgut it can be fermented by intestinal microorganisms and produce large amounts of SCFA[3-4]. SCFA mainly include acetic acid, propionic acid, and butyric acid, which together account for approximately 95% of total SCFA[12]. SCFA participate in host energy metabolism and nutrient transformation and exert specific physiological functions; for example, butyric acid is the main energy source for the colonic epithelium. After being absorbed by the colonic epithelium, it can provide pigs with 5%-20% of their energy.

as substrates for gluconeogenesis; after acetate is taken up by the liver, it enters peripheral tissues through the blood circulation and is taken up and utilized[^{14}]. In addition, SCFA can lower the pH in the large intestine, thereby preventing the proliferation of pathogenic bacteria[^5,^{15}] and promoting the proliferation of colonic epithelial cells[^3]. It has also been reported that SCFA, by activating free fatty acid receptor (FFAR) 2 or FFAR3, induce endocrine L cells to produce and release peptide YY and glucagon-like peptide-1[^{16}-^{17}], thereby regulating satiety in animals[^{18}-^{19}].

The concentration of SCFA is an important indicator reflecting the intensity of hindgut fermentation activity[^{20}]. Insoluble fiber has important effects on hindgut fermentation activity and on the production and absorption of SCFA[^{21}]. In this experiment, as the level of alfalfa meal in the diet increased linearly, the level of insoluble fiber increased linearly, and the concentrations of total SCFA, acetate, and butyrate in colonic digesta also increased linearly; among them, the 10% and 15% alfalfa meal supplementation groups showed significant increases compared with the control group. This is similar to the findings of Chen et al.[^9]. When the level of alfalfa meal supplementation was 10%, the concentration of SCFA produced was the highest; when alfalfa meal supplementation was increased to 15%, the level of insoluble fiber in the diet was 16.8%, which affected the fermentation efficiency of the hindgut microbiota, resulting in a significant decrease in butyrate concentration compared with the 10% alfalfa meal supplementation group. In addition, when the alfalfa meal

supplementation level was 5%, its insoluble fiber level was basically the same as that of the control group, and therefore did not affect the concentrations of acetate, propionate, and butyrate in colonic digesta.

3.3 Effects of alfalfa meal on the colonic microbiota

It has been suggested that resistant starch is also classified as insoluble fiber[^{22}]; however, because it is highly fermentable, it can markedly alter the microbial community structure in the hindgut of pigs and produce large amounts of SCFA[^{23}-^{24}]. At present, there have been no reports on the effects of alfalfa meal on the colonic microbiota of pigs. This study found that alfalfa meal had no significant effect on the colonic microbiota of growing pigs. Insoluble fibers from different sources, together with their different physicochemical properties, also differ in their effects on the fermentation activity of hindgut microorganisms in animals. In the present study, although the microbial community structure did not differ significantly, the concentration of SCFA in colonic digesta in the experimental group was significantly higher than that in the control group, suggesting that insoluble fiber from alfalfa may enhance the metabolic activity of the colonic microbiota.

3.4 Effects of alfalfa meal on serum fatty acid composition

This study found that feeding alfalfa meal significantly increased the PUFA content and PUFA/SFA ratio in pig serum, while reducing the contents of MUFA and SFA; these results are similar to previous findings in rabbits[^{25}]. Some studies suggest that the increase in PUFA content may be a protective effect of certain antioxidant components in the diet[^{26}]. The insoluble fiber in alfalfa meal can improve the body' s antioxidant capacity by increasing the activities of superoxide dismutase and glutathione peroxidase and by reducing malondialdehyde content[^{10}]. In addition, alfalfa meal contains trace amounts of medicarpin and tricin and other substances, which also have positive effects on improving the body' s antioxidant capacity[^{10}]. The antioxidant mechanism of dietary fiber may be that it interferes with the absorption and synthesis of bile acids, affects bile acid metabolism, and reduces the accumulation of lipids in serum, thereby alleviating lipid peroxidation in the body[^{25}].

4 Conclusion

Insoluble fiber derived from alfalfa meal can significantly reduce the F/G of growing pigs and significantly increase the colonic digesta' s

SCFA concentrations, but did not affect the structure of the colonic microbiota.

When the alfalfa meal supplementation level was 10%, the concentrations of butyric acid, valeric acid, total SCFA, and total BCFA were the highest.

Insoluble fiber derived from alfalfa meal increased the PUFA content and the PUFA/SFA ratio in pig serum, and reduced the contents of MUFA and SFA;

this is of great significance for regulating the fatty-acid composition of pigs.

References:

[1] HOTAMISLIGIL G S. Inflammation and metabolic disorders[J]. Nature, 2006, 444(7121): 860-867.

[2] JHA R, BERROCOSO J D. Review: dietary fiber utilization and its effects on physiological functions and gut health of swine[J]. Animal, 2015, 9(9): 1441-1452.

[3] HIJOVA E, CHMELAROVA A. Short chain fatty acids and colonic health[J]. Bratislavske Lekarske Listy, 2007, 108(8): 354-358.

[4] WONG J M W, DE SOUZA R, KENDALL C W C, et al. Colonic health: fermentation and short chain fatty acids[J]. Journal of Clinical Gastroenterology, 2006, 40(3): 235-243.

[5] NIELSEN T S, LÆRKE H N, THEIL P K, et al. Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs[J]. British Journal of Nutrition, 2014, 112(11): 1837-1849.

[6] LANGE K, HUGENHOLTZ F, JONATHAN M C, et al. Comparison of the effects of five dietary fibers on mucosal transcriptional profiles, and luminal microbiota composition and SCFA concentrations in murine colon[J]. Molecular Nutrition & Food Research, 2015, 59(8): 1590-1602.

[7] OHASHI Y, SUMITANI K, TOKUNAGA M, et al. Consumption of partially hydrolysed guar gum stimulates *Bifidobacteria* and butyrate-producing bacteria in the human large intestine[J]. Beneficial Microbes, 2015, 6(4): 451-455.

[8] HAN K H, KOBAYASHI Y, NAKAMURA Y, et al. Comparison of the effects of longer chain inulins with different degrees of polymerization on colonic fermentation in a mixed culture of swine fecal bacteria[J]. Journal of Nutritional Science and Vitaminology, 2014, 60(3): 206-212.

[9] CHEN L, ZHANG H F, GAO L X, et al. Effect of graded levels of fiber from alfalfa meal on intestinal nutrient and energy flow, and hindgut fermentation in growing pigs[J]. Journal of Animal Science, 2013, 91(10): 4757-4764.

[10] Wang Yanhua, Cheng Ningning, Zheng Airong, et al. Effects of alfalfa meal and alfalfa saponins on growth performance and antioxidant performance of fattening pigs.

[J]. *Chinese Journal of Animal Nutrition*, 2013, 25(12): 2981-2988.

[11] Xu Xiangyang, Wang Chengzhang, Yang Yuxin, et al. Effects of alfalfa meal on production performance and serum indices of growing pigs [J]. *Journal of Huazhong Agricultural University*, 2006, 25(2): 164-169.

[12] KIMURA I, INOUE D, HIRANO K, et al. The SCFA receptor GPR43 and energy metabolism [J]. *Frontiers in Endocrinology*, 2014, 5: 85.

[13] DEN BESTEN G, VAN EUNEN K, GROEN A K, et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism [J]. *The Journal of Lipid Research*, 2013, 54(9): 2325-2340.

[14] BLOEMEN J G, VENEMA K, VAN DE POLL M C, et al. Short chain fatty acids exchange across the gut and liver in humans measured at surgery [J]. *Clinical Nutrition*, 2009, 28(6): 657-661.

[15] ROY C C, KIEN C L, BOUTHILLIER L, et al. Short-chain fatty acids: ready for prime time? [J]. *Nutrition in Clinical Practice*, 2006, 21(4): 351-366.

[16] SAMUEL B S, SHAITO A, MOTOIKE T, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41 [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2008, 105(43): 16767-16772.

[17] KIMURA I, OZAWA K, INOUE D, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43 [J]. *Nature Communications*, 2013, 4(5): 1829.

[18] PSICHAS A, SLEETH M L, MURPHY K G, et al. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion *via* free fatty acid receptor 2 in rodents [J]. *International Journal of Obesity*, 2015, 39(3): 424-429.

[19] ZHOU J, HEGSTED M, MCCUTCHEON K L, et al. Peptide YY and proglucagon mRNA expression patterns and regulation in the gut [J]. *Obesity*, 2006, 14(4): 683-689.

[20] SERENA A, JØRGENSEN H, BACH KNUDSEN K E. Digestion of carbohydrates and utilization of energy in sows fed diets with contrasting levels and physicochemical properties of dietary fiber [J]. *Journal of Animal Science*, 2008, 86(9): 2208-2216.

[21] SERENA A, JØRGENSEN H, BACH KNUDSEN K E. Absorption of carbohydrate-derived nutrients in sows as influenced by types and contents of dietary fiber [J]. *Journal of Animal*

Science, 2009, 87(1): 136-147.

[22] FUENTES-ZARAGOZA E, RIQUELME-NAVARRETE M J, SÁNCHEZ-ZAPATA E, et al. Resistant starch as functional ingredient: a review[J]. Food Research International, 2010, 43(4): 931-942.

[23] HAENEN D, ZHANG J, DA SILVA C S, et al. A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine[J]. The Journal of Nutrition, 2013, 143(3): 274-283.

[24] FANG L, JIANG X, SU Y, et al. Long-term intake of raw potato starch decreases back fat thickness and dressing percentage but has no effect on the longissimus muscle quality of growing-finishing pigs[J]. Livestock Science, 2014, 170: 116-123.

[25] DAL BOSCO A, MUGNAI C, ROSCINI V, et al. Effect of dietary alfalfa on the fatty acid composition and indexes of lipid metabolism of rabbit meat[J]. Meat Science, 2014, 96(1): 606-609.

[26] LIAO X D, WU R J, MA G, et al. Effects of *Clostridium butyricum* on antioxidant properties, meat quality and fatty acid composition of broiler birds[J]. Lipids in Health and Disease, 2015, 14(1): 36.

Effects of Dietary Alfalfa Meal Supplementation on Colonic Microbiota and Its Metabolites in Growing Pigs

WANG Jiawei^1 ZHANG Lei^2 ZHU Jiaoyue^2 WANG Yubo^2 YIN Jingdong^2^ *ZHU Weiyun^1*

(1. *Laboratory of Gastrointestinal Microbiology, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China*; 2. *State Key Lab of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China*)

Abstract: The present study aimed to investigate the effects of insoluble dietary fiber from alfalfa meal on colonic microbiota and its fermentation metabolites in growing pigs. In this experiment, 24 castrated male pigs (Duroc×Landrace×Yorkshire) with initial body weight of (24.8$±$0.7) kg were randomly divided into 4 groups with 6 replicates per group and 1 pig per replicate. Pigs in each group were fed one of four diets (0, 5%, 10% and 15% level of alfalfa meal). Pigs were slaughtered after 28 d for sampling. Then we determined serum fatty acid composition, short-chain fatty acid (SCFA) concentration and microbiota composition in colonic digesta. The results showed that diets with alfalfa meal significantly decreased feed to gain ratio (P<0.05), but had no significant effect on average daily gain and average daily feed intake of growing pigs (P>0.05). Analysis of colonic microbiota 16S rDNA V3 region revealed that alfalfa meal had no significant effect on microbiota composition (P>0.05), but significantly increased total SCFA, acetate and butyrate in colonic digesta of pigs (P<0.05). With the addition of alfalfa meal in diets, polyunsaturated fatty acid content was significantly increased (P<0.05), while the contents of saturated fatty acid and monounsaturated fatty acid were significantly decreased in the serum (P<0.05). The results indicate that insoluble dietary fiber from alfalfa meal has no effect on colonic microbiota structure, but enhances fermentation activity of colonic microbiota and SCFA producing, and also plays a role in regulating fatty acid profile of growing pigs.

Key words: growing pigs; alfalfa meal; insoluble dietary fiber; SCFA; colonic microbiota; fatty acid

*Corresponding author: YIN Jingdong, professor, E-mail: yinjd@cau.edu.cn; ZHU Weiyun, professor, E-mail: zhuweiyun@njau.edu.cn

(Responsible editor Tian Yeming)

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