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Original Research Article

Effects of soybean raffinose on growth performance, digestibility, humoral immunity and intestinal morphology of growing pigs

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ABSTRACT

There are appreciable does of raffinose in soybean, but the impacts of raffinose on pigs are poorly investigated. We used 2 experiments to investigate the influence of soybean raffinose on growth performance, digestibility, humoral immunity and intestinal morphology of growing pigs. In Exp. 1, a total of 30 crossbred (Duroc × Landrace × Yorkshire) barrows (21.93 ± 0.43 kg) were randomly divided into 3 groups, and were fed with the control diet, the control diets supplemented with 0.2% and 0.5% raffinose, respectively, for 21 d. Results showed that the addition of 0.2% or 0.5% raffinose reduced ($P < 0.05$) average daily feed intake (ADFI), average daily gain (ADG) and nutrient digestibility, and dietary 0.5% raffinose increased the ratio of feed to gain ($P < 0.05$). For serum indexes, dietary 0.5% raffinose decreased growth hormone and increased glucagon-like peptide-2, immunoglobulin G, tumor necrosis factor-α (TNF-α) and interleukin-6 concentration ($P < 0.05$). In Exp. 2, a total of 24 crossbred barrows (38.41 ± 0.45 kg) were randomly divided into 3 groups, and were fed with the control diet (ad libitum), the raffinose diet (0.5% raffinose, ad libitum), and the control diet in the same amount as the raffinose group (feed-pair group) for 14 d, respectively. Compared with the control diet, dietary 0.5% raffinose decreased ADFI ($P < 0.05$). Intriguingly, the raffinose group had lower ADG than the feed-pair group, lower nutrient digestibility, lower amylase activity in duodenum, lower amylase, lipase and trypsin activities in jejunum and higher TNF-α concentration in serum compared with the other 2 groups, and a higher ratio of villus height to crypt depth compared with the control group ($P < 0.05$). These results showed that soybean raffinose could reduce feed voluntary intake and body gain while improving intestinal morphology without a significant negative influence on immunity. Taken together, dietary raffinose could decrease growth performance by reducing both feed intake and nutrient digestibility while inducing humoral immune response of growing pigs.

1. Introduction

Soybean is a superiorly and widely used protein source for swine (García et al., 2016; Karr et al., 2005). However, the antinutritional factors of soybean are known to have various negative influences on swine (Choct et al., 2010; Friesen et al., 1993; Pluske et al., 1997). Soybean antinutritional factors could be partly eliminated by routine thermal treatment, but heat-stable antinutritional factors including soybean oligosaccharide still exist afterwards (Ruiz et al., 2020). It has been reported that soybean oligosaccharide could take up about 10% of soybean (Macrae et al., 1993). A further way to remove soybean oligosaccharide used in industry is through alcohol or ethanol extraction, which ultimately increases

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feed cost considerably (Ruiz et al., 2020). Previous research has established that soybean oligosaccharide decreases growth performance, nutrient digestibility and causes flatulence symptom in pigs (Smiricky et al., 2002, 2003). Soybean oligosaccharide is mainly composed of raffinose, stachyose and sucrose (Macrae et al., 1993). Zhang et al. (2003) demonstrated that addition of 1% and 2% stachyose had negative effects on the growth performance of piglets. However, no research has been conducted concerning the influence of raffinose on swine.

Raffinose, a trisaccharide synthesized from galactose, fructose and glucose, is an important component of soybean oligosaccharides. Raffinose takes up about 12.6 mg/g of dry soybean seed (Chelakkot et al., 2018; Kuo et al., 1988). Pigs cannot digest raffinose in the small intestine as they lack α-galactosidase to hydrolyze the α-1,6-linkage of raffinose (NRC, 2012). Previous studies have observed some effects and functions of raffinose on different animal models. Raffinose can decrease body weight and plasma lipid of rats (Tortuero et al., 1997), produce large volumes of gas in vitro fermentation of swine fecal microflora (Zhou et al., 2012) and increase the cecal weight of rats (Ishizuka et al., 2009). To the best of our knowledge, there is no systematic and fundamental research about the effect of raffinose supplementation on pigs.

Therefore, the present experiment was designed to detect the influence of raffinose on growth performance, digestibility, humoral immunology and intestinal morphology of growing pigs.

2. Materials and methods

All experimental procedures and animal care were accomplished in accordance with the Guide for the Care and Use of Laboratory Animals provided by the Institutional Animal Care Advisory Committee for Sichuan Agricultural University. The Animal Experimental Committee of Sichuan Agricultural University approved the protocol used in this experiment under permit number CD-SYXK-2017-015. The experiment was conducted at the Animal Experiment Center of Animal Nutrition Institute, Sichuan Agricultural University.

2.1. Animal, diet and experiment design

In Exp. 1, a total of 30 crossbred (Duroc × Landrace × Yorkshire) barrows (21.93 ± 0.43 kg) were randomly allotted to 3 treatments with 5 replicates (pens) per treatment and 2 pigs per pen. The experimental diets were based on maize and low-saccharide soybean meal (saccharide content about 1.5%). Raffinose treatments were supplemented with 0.2% and 0.5% raffinose to substitute for wheat bran in the basal diet. Raffinose was obtained from Sino-Leader Biotech (Beijing, China) and the purity was over 99.0%. Raffinose concentration in the diet was measured with High-Performance Liquid Chromatography according to Kumar et al. (2010). The raffinose concentration was 0.041%, 0.243% and 0.525% in the control diet, dietary 0.2% raffinose and dietary 0.5% raffinose diet, respectively. The diets were formulated to meet or exceed nutrient requirements for pigs weighing 25 to 50 kg recommended by National Research Council (NRC, 2012). Ingredients and composition of the diets are presented in Table 1. The feeding condition was in an environmentally controlled building. Throughout the experiment, the house was maintained at 24 to 26 °C, humidity was kept around 65%, and daily cleaning and hygiene were maintained to control the ammonium concentration and prevent disease. Pigs had availability to feed and water ad libitum.

Based on the results of raffinose supplementation decreased average daily feed intake (ADFI) of growing pigs in Exp.1, we conducted Exp. 2 to investigate whether the effect of raffinose is due to only feed intake or to more than feed intake. In the second trial, a total of 24 crossbred (Duroc × Landrace × Yorkshire) barrows were randomly allotted to 3 treatments with 8 replicates per treatment and 1 pig per pen. The initial average body weight of pigs was 38.41 ± 0.45 kg. Three treatments included the control group, feed-pair group and diet supplemented with 0.5% raffinose group. Pigs fed with the basal diet and the raffinose diet were allowed to feed ad libitum, and the feed-pair group was fed the control diet in the same amount as the raffinose group. The trial lasted for 14 d. Experimental management and environment were consistent with that in Exp.1.

Table 1
Feed ingredients and nutrient contents of experimental diets.

Item	Content
Ingredients, g/kg	
Corn	751.0
Soybean meal	139.5
Soybean oil	17.0
Wheat bran	30.0
Fish meal	45.0
Limestone	2.6
Dicalcium phosphate	4.0
NaCl	2.5
L-Lys·HCl	3.5
DL-Met	1.0
Thr (98.5%)	0.7
Trp (98.0%)	0.2
Choline chloride	0.5
Vitamin premix[1]	0.5
Mineral premix[2]	2.0
Total	1,000.0
Nutrient levels[3], %	
Digestible energy, Mcal/kg	3.40
Crude protein	15.74
Ca	0.52
Total P	0.50
Available P	0.32
D-Lys	0.98
D-Met	0.35
D-Thr	0.59
D-Trp	0.17

[1] Provided the following per kilogram of complete diet: 6,000 IU of vitamin A; 400 IU of vitamin D_3; 10 IU of vitamin E; 2 mg of vitamin K_3; 0.8 mg of vitamin B_1; 6.4 mg of riboflavin; 2.4 mg of vitamin B_6; 0.2 mg of folic acid; 14 mg of nicotinic acid; 10 mg of pantothenic acid; 12 μg of vitamin B_{12}.
[2] Provided the following per kilogram of complete diet: 60 mg of Fe (as ferrous sulfate); 4 mg of Cu (as copper sulfate); 2 mg of Mn (as manganese sulfate); 60 mg of Zn (as zinc sulfate); 0.2 mg of I (as KI); 0.2 mg of Se (as Na_2SeO_3).
[3] All data were calculated values.

2.2. Sampling

The sampling process followed laboratory procedures described by Zheng et al. (2017). At the beginning and end of the trial period, body weight was measured individually after overnight fasting (12 h), and feed consumption of each pen was recorded daily for the calculation of average daily weight gain (ADG), ADFI and feed-to-gain (F:G) ratio. Feed diet was sampled and stored at −20 °C until analyzed for chemical analysis. Fecal collection lasted for 4 d at the end of the experiment. Feces were collected from every pen immediately after excretion and placed in sample bags with 10% hydrochloric acid to fix excreta nitrogen. Then samples were kept under −20 °C. On d 14 of Exp. 2, after overnight starvation, the 24 pigs' blood samples (10 mL/pig) were collected from the jugular vein and immediately frozen at −20 °C after centrifugation (3,500 × *g* for 10 min at 4 °C) for lab analysis. After weighing and blood collection, the 24 fasted pigs were slaughtered by electrical stunning and

exsanguination. Subsequently, small intestinal sections were removed to isolate duodenum and jejunum. Approximately 2 cm of jejunum were cut immediately and preserved in 10% formalin solution for morphology analysis and goblet cell counting. Meanwhile, the digesta of duodenum and the rest of the parts of jejunum were collected with cryovial tubes and frozen in liquid nitrogen, and lastly kept frozen at −80 °C until digestive enzyme activity could be measured.

2.3. Digestibility measurement

The digestibility measurement process was according to Liu et al. (2018). Diets and fecal samples were milled through a 40-mesh screen (to 1 mm). Samples were used for the measurement of dry matter (DM) (method 930.15), crude protein (CP) (method 990.03), and crude fat (CF) (method 945.16) according to AOAC (2007). Gross energy (GE) was detected by an adiabatic calorimeter (Parr Instrument Co., Moline, IL, USA). To measure apparent total tract digestibility (ATTD) of nutrients, acid insoluble ash (AIA) was chosen as the endogenous indicator. The equation for ATTD calculation was:

$$\text{ATTD (\%)} = 1 - [(\text{AIA}_{\text{diet}} \times \text{Nutrient}_{\text{feces}})/(\text{AIA}_{\text{feces}} \times \text{Nutrient}_{\text{diet}})] \times 100$$

2.4. Digestive enzyme activity

The frozen digesta samples of duodenum and jejunum were weighed at about 1.5 g each, and then homogenized in 9 volume (wt/vol) of pre-cooled physiological saline. The homogenate was centrifuged at 4,000 × *g* for 10 min at 4 °C, and the supernatant was collected to analyze amylase, lipase and trypsin activities. Protein concentration was measured using a Coomassie blue kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). Activities of trypsin, lipase, and amylase of supernatant were measured with specific kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) combined with a spectrophotometer (SpectraMax M2, USA) as stated in the manufacturer's instructions. The activities of these enzymes were normalized with protein concentration as indicated in the instructions.

2.5. Serum hormone and immunity parameters

Measurement of growth hormone (GH), adiponectin (ADP), glucagon-like peptide-2 (GLP-2), insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α), and interleukin (IL), including IL-1, IL-6, IL-10, were measured by using porcine-specific ELISA kits (Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China). Immunoglobulin (Ig), including IgG, IgM, IgA, were measured through the automatic biochemical analyzer (Model 3100; Hitachi, Tokyo, Japan).

2.6. Intestinal morphology analysis and cell counting

The jejunum morphology analysis and goblet cell counting were determined according to Mao et al. (2015). Briefly, 2-cm jejunal segments were dehydrated and embedded in paraffin wax before transverse sections were prepared, and then stained with hematoxylin-eosin and Periodic Acid Schiff (PAS) to visualize intestinal morphology and goblet cells respectively. The measurement of villus height, crypt depth and goblet cells were detected with Image-Pro Plus software (Version 6.0, Media Cybernetics, USA).

2.7. Statistical analysis

Descriptive statistics was performed to evaluate whether the data were normally distributed using the statistical software SPSS 22.0 Statistical Software (SPSS Inc., Chicago, USA). Then one-way ANOVA and Tukey's test were used to evaluated differences of the normal distributed data among groups in SPSS 22.0 Statistical Software (SPSS Inc., Chicago, USA) as Zhang et al. (2019) stated. Significance was considered at $P < 0.05$. Tendency was considered at $0.05 \leq P < 0.10$.

3. Results

3.1. Experiment 1

3.1.1. Growth performance

The effects of different raffinose inclusion levels on growth performance are shown in Table 2. Diet supplemented with 0.2% and 0.5% raffinose decreased ADFI and ADG compared with the control group ($P < 0.05$). Diet supplemented with 0.5% raffinose increased F:G ratio compared with the control ($P < 0.05$), whereas there is no significant difference among 0.2% raffinose group and the other 2 groups.

3.1.2. ATTD

The effects of different raffinose inclusion levels on ATTD are shown in Table 3. Compared with the control group, animals fed with 0.2% and 0.5% raffinose had a significant decrease in digestibility of DM, CP, GE and CF ($P < 0.05$).

3.1.3. Serum hormones

Table 4 illustrated the effects of different raffinose inclusion levels on serum hormones. The ADP and IGF-1 concentration of serum were not unaffected by raffinose treatments compared with the control ($P > 0.10$). In comparison to the control, GH concentration of serum was decreased by 0.5% raffinose treatment ($P < 0.05$) but unaffected by 0.2% raffinose treatment. Intriguingly, the concentration of GLP-2 in serum was increased by 0.5 raffinose treatment ($P < 0.05$) but unaffected by 0.2% raffinose treatment compared with the control group.

3.1.4. Humoral immunity

It can be seen from Table 5 that raffinose treatment did not affect IgM, IgA, IL-1 and Il-10 concentration in the serum compared with the control treatment ($P > 0.10$). Diet supplemented with 0.5% raffinose significantly increased IgG, TNF-α and IL-6 concentration of serum compared with the control group ($P < 0.05$), whereas 0.2% raffinose group had no impacts on these indexes compared with the control.

Table 2
Effects of soybean raffinose on growth performance of growing pigs in Exp.1.[1]

Item	Control	0.2% raffinose	0.5% raffinose	SEM	*P*-value
ADFI, g	1,558.36[a]	1,278.92[b]	1,315.87[b]	46.51	0.02
ADG, g	899.52[a]	582.38[b]	542.86[b]	54.53	<0.01
F:G ratio	1.74[b]	2.25[ab]	2.60[a]	0.15	0.04

ADFI = average daily feed intake; ADG = average daily gain; F:G ratio = feed gain ratio.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 5 replicates per treatment.

Table 3
Effects of soybean raffinose on apparent total tract digestibility of growing pigs in Exp.1 (%).[1]

Item	Control	0.2% raffinose	0.5% raffinose	SEM	*P*-value
DM	84.19[a]	74.46[b]	76.68[b]	1.31	<0.01
CP	74.15[a]	59.41[b]	63.76[b]	2.07	<0.01
GE	83.10[a]	69.88[b]	72.13[b]	1.76	<0.01
CF	59.41[a]	30.58[b]	28.59[b]	4.30	<0.01

DM = dry matter; CP = crude protein; GE = gross energy; CF = crude fat.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 5 replicates per treatment.

Table 4
Effects of soybean raffinose on serum hormones of growing pigs in Exp.1.[1]

Item	Control	0.2% raffinose	0.5% raffinose	SEM	*P*-value
GH, μg/L	17.00[a]	15.96[ab]	14.89[b]	0.35	0.03
ADP, μg/L	83.72	81.01	83.32	2.10	0.86
GLP-2, pmol/L	5.07[b]	5.50[ab]	5.82[a]	0.13	0.04
IGF-1, μg/L	9.77	9.47	8.99	0.17	0.16

GH = growth hormone; ADP = adiponectin; GLP-2 = glucagon-like peptide-2; IGF-1 = insulin-like growth factor-1.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 5 replicates per treatment.

Table 5
Effects of soybean raffinose on humoral immunity of growing pigs in Exp.1.[1]

Item	Control	0.2% raffinose	0.5% raffinose	SEM	*P*-value
IgG, g/L	3.34[b]	3.74[ab]	4.08[a]	0.12	0.02
IgM, μg/mL	30.68	33.00	35.60	1.12	0.21
IgA, μg/mL	41.39	42.67	45.28	0.98	0.27
TNF-α, pg/mL	334.97[b]	432.68[ab]	482.59[a]	25.49	0.04
IL-1, ng/L	111.68	116.00	118.16	2.36	0.56
IL-6, ng/L	773.18[b]	807.04[ab]	924.67[a]	25.69	0.03
IL-10, ng/L	170.87	175.39	182.58	3.02	0.30

IgG = immunoglobulin G; IgM = immunoglobulin M; IgA = immunoglobulin A; TNF-α = tumor necrosis factor-α; IL-1 = interleukin-1; IL-6 = interleukin-6; IL-10 = interleukin-10.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 5 replicates per treatment.

3.2. Experiment 2

3.2.1. Growth performance

As shown in Table 6, diet supplemented with 0.5% raffinose significantly inhibited ADFI compared with the control group. Feed-pair group decreased ADG compared with the control group, whereas 0.5% raffinose group decreased ADG compared with both control and feed-pair groups ($P < 0.05$). In addition, animals fed with 0.5% raffinose supplemented diet had a significantly higher F:G ratio compared with both control and feed-pair treatments ($P < 0.05$).

Table 6
Effects of soybean raffinose on growth performance of growing pigs in Exp.2.[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
ADFI	2,161.97[a]	1,940.30[b]	1,943.21[b]	30.65	<0.01
ADG	1,069.23[a]	960.58[b]	896.16[c]	20.77	<0.01
F:G ratio	2.03[b]	2.02[b]	2.18[a]	0.03	0.049

ADFI = average daily feed intake; ADG = average daily gain; F:G ratio = feed-to-gain ratio.
[a, b, c] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

3.2.2. ATTD

As exhibited in Table 7, the feed-pair group had no impacts on DM, CP, GE and CF compared with the control group. However, the 0.5% raffinose treatment significantly decreased digestibility of DM, CP, GE and CF compared with the other 2 groups ($P < 0.05$).

3.2.3. Digestive enzymes

It can be seen in Table 8 that 0.5% raffinose treatment significantly decreased amylase activity ($P < 0.05$) whereas it did not affect lipase and trypsin activities ($P > 0.10$) compared with other 2 groups in duodenum. In jejunum, dietary 0.5% raffinose reduced lipase activity compared with the other 2 groups, and lessened amylase and trypsin activities compared with the control group ($P < 0.05$). The feed-pair group had no significant difference on digestive enzymes in duodenum or jejunum compared with the control group.

3.2.4. Serum hormones

Table 9 showed that diet supplemented with 0.5% raffinose significantly decreased GH level and increased GLP-2 concentration compared with the control group ($P < 0.05$). The feed-pair group had no significant difference on serum hormones compared with the control group.

3.2.5. Humoral immunity

Data in Table 10 reflected that 0.5% raffinose treatment significantly increased IgG, IgA and TNF-α concentration in comparison to the control group ($P < 0.05$) and did not affect IL-1, IL-6 or IL-10 concentration compared with the other 2 groups ($P > 0.05$).

3.2.6. Intestinal morphology

It can be seen from Table 11 that 0.5% raffinose significantly elevated villus height in comparison to the feed-pair group, increased ratio of villus height to crypt depth compared with the control group ($P < 0.05$) and tended to elevate goblet cell numbers ($0.05 \leq P < 0.10$). However, the crypt depth was not altered by either the feed-pair or raffinose treatments in comparison to the control ($P > 0.10$).

4. Discussion

For the first time, we investigated the influence of dietary raffinose on the growth performance of pigs in this study. In Exp.1, we found that the addition of 0.2% and 0.5% raffinose depressed growth performance of growing pigs. Previous research investigating the effects of raffinose on rats was inconsistent. Tortuero et al. (1997) found that dietary raffinose induced a decrease of body weight in rats, whereas Ishizuka et al. (2009) found that supplementation of raffinose did not influence body weight gain of rats. Both researchers did not observe the negative effect of raffinose on feed intake. The difference between present and previous research might be related to the different feed ingredients and

Table 7
Effects of soybean raffinose on apparent total tract digestibility of growing pigs in Exp.2 (%).[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
DM	82.25[a]	82.38[a]	74.29[b]	0.88	<0.01
CP	74.27[a]	72.30[a]	65.23[b]	1.51	0.02
GE	79.63[a]	79.32[a]	74.16[b]	0.68	<0.01
CF	46.91[a]	48.12[a]	23.60[b]	2.88	<0.01

DM = dry matter; CP = crude protein; GE = gross energy; CF = crude fat.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

Table 8
Effects of soybean raffinose on digestive enzymes of growing pigs in Exp.2.[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
Duodenum					
Amylase, U/g prot	291.38[a]	314.35[a]	219.22[b]	14.13	<0.01
Lipase, U/mg prot	292.05	315.98	230.19	21.87	0.27
Trypsin, 10^3 U/mg prot	6.28	6.91	4.44	0.56	0.16
Jejunum					
Amylase, U/g prot	318.14[a]	286.01[ab]	213.31[b]	16.69	0.03
Lipase, U/mg prot	282.97[a]	264.72[a]	182.35[b]	17.80	0.04
Trypsin, 10^3 U/mg prot	12.50[a]	11.48[ab]	7.84[b]	0.79	0.03

[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

Table 9
Effects of soybean raffinose on serum hormones of growing pigs in Exp.2.[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
GH, μg/L	10.40[a]	9.88[ab]	8.42[b]	0.29	0.01
ADP, μg/L	54.18	51.81	46.83	1.73	0.21
GLP-2, pmol/L	3.99[b]	4.15[ab]	4.63[a]	0.10	0.02
IGF-1, μg/L	10.43	9.84	9.46	0.22	0.21

GH = growth hormone; ADP = adiponectin; GLP-2 = glucagon-like peptide-2; IGF-1 = insulin-like growth factor-1.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

Table 10
Effects of soybean raffinose on humoral immunity of growing pigs in Exp.2.[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
IgG, μg/mL	3.69[b]	3.67[ab]	4.34[a]	0.11	0.01
IgM, μg/mL	36.56[ab]	34.71[b]	42.21[a]	1.27	0.03
IgA, μg/mL	18.52[b]	20.32[ab]	26.56[a]	1.29	0.03
TNF-α, pg/mL	428.81[b]	447.60[b]	547.11[a]	12.64	<0.01
IL-1, ng/L	266.40	259.38	289.40	5.64	0.07
IL-6, ng/L	834.72	927.57	982.78	27.28	0.08
IL-10, ng/L	186.39	200.93	203.02	5.52	0.43

IgG = immunoglobulin G; IgM = immunoglobulin M; IgA = immunoglobulin A; TNF-α = tumor necrosis factor-α; IL-1 = interleukin-1; IL-6 = interleukin-6; IL-10 = interleukin-10.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

Table 11
Effects of soybean raffinose on jejunum morphology of growing pigs in Exp.2.[1]

Item	Control	Feed-pair	0.5% raffinose	SEM	*P*-value
Villus height, μm	470.65[ab]	441.50[b]	543.29[a]	16.69	0.03
Crypt depth, μm	249.07	218.92	209.27	9.35	0.19
V:C ratio	1.95[b]	2.34[ab]	2.66[a]	0.12	0.03
Goblet cell numbers	24.68	22.83	35.97	2.53	0.08

V:C ratio = villus height-to-crypt depth ratio.
[a, b] Means in the same row with different superscript differ at $P < 0.05$.
[1] Values are the means of 8 replicates per treatment.

nutrient requirements due to different species. Meanwhile, the ATTD of CP, CF, DM and GE was decreased in raffinose supplementation groups. Prior studies have observed that limited feed intake could affect nutrient digestibility (Le et al., 2014). To investigate whether the decrease of nutrient digestibility was induced by raffinose per se or by the decreased feed intake, we designed a feed-pair group in Exp.2. Intriguingly, the results showed that even under the same amount of ADFI, the raffinose group had noteworthy lower ADG than the feed-pair group, indicating that the decrease of nutrient digestibility was induced by raffinose instead of lessened feed intake. It is known that nutrient digestibility is closely correlated with digestive enzymes (De et al., 2010). In Exp. 2, we found that dietary 0.5% raffinose decreased amylase activity in duodenum and lipase activity in jejunum, which partially explained the decreased nutrient digestibility of the raffinose group. Moreover, GH concentration was decreased in 0.5% raffinose group compared with the control group in both experiments, suggesting that the anabolic metabolism was decreased and thus depressed the growth performance of the raffinose group. Based on the interesting results found on the growth performance of growing pigs, we were further interested in whether raffinose would influence intestinal morphology or not.

It is known that intestinal development is closely related to pig growth performance (Torres et al., 2017). However, intestinal morphology analysis of Exp.2 interestingly showed that 0.5% raffinose supplementation enhanced villus height and ratio of villus height to crypt depth. Besides, the goblet cell number also had an increasing tendency. The main function of the goblet cell is to synthesize and secrete mucin to form a mucosal barrier to protect epithelial cells (Chelakkot et al., 2018). These results indicated that raffinose partly improved intestinal morphology and mucosal barrier function. It is well known that GLP-2 is an important intestinal growth promoter. It could stimulate the proliferation and inhibit the apoptosis of intestinal cells. Also, it could promote the growth of intestinal mucosa and the regeneration and repair of damaged intestinal mucosa (Ørskov et al., 2005). We tested serum GLP-2 concentration and found that it was increased in the 0.5% raffinose supplementation group in both experiments, which was consistent with the improvement of intestinal morphology. The explanation for the phenomenon might be a compensatory effect for the decreased digestibility caused by raffinose. The proliferation and differentiation of intestinal cells were promoted to increase nutrients availability. However, this is only our speculation and further research is needed to test this explanation. The beneficial effects of raffinose on intestinal development has already been found and used in poultry. Berrocoso et al. (2017) reported that in ovo injection of raffinose could increase villus height and the ratio of villus height to crypt depth of ileum without affecting the growth performance of broiler. It should be possible to find the optimal dose of raffinose in pigs that would promote intestinal development without decreasing growth performance. This will require further investigation.

Immunoglobulin is pivotal to reflect the immune function and the ability of an animal's body to recognize the invasion of various antigens (Elgert, 2009). Cytokines including TNF-α, IL-1, IL-6 and IL-10 are critical for the activation of the immune system (Oberholzer et al., 2000). It is well known that TNF-α, IL-1 and IL-6 are typical pro-inflammatory cytokines that trigger immune responses and IL-10 is a classical anti-inflammatory cytokine that inhibits immune responses (Fang et al., 2015). Prior study has reported that raffinose can act as an immunomodulator to relieve allergy reactions (Watanabe et al., 2004). In Exp.1, serum IgG, IL-6 and TNF-α concentration were elevated by the addition of 0.5% raffinose. In Exp.2, dietary 0.5% raffinose supplementation increased IgG, IgA and TNF-α levels. These results indicated that raffinose supplementation elicited a humoral immune response, suggesting raffinose transferred more nutrients from growth to the immune response, which might be another reason for bodyweight loss. Interestingly, TNF-α levels were elevated in both experiments. It is well known that TNF-α has a beneficial function in the activation of host defenses, and it can exert opposing effects like apoptosis and activation at the cellular level (Szatmary, 1999). Intriguingly, under a normal physiological situation, dietary raffinose could activate an immune response in fish (Hoseinifar et al., 2019; Karimi et al., 2020; Lin et al., 2011), chicken (Berrocoso et al., 2017) and pig (this study), whereas raffinose could inhibit an immune response under allergic

situations (Nagura et al., 2002; Sonoyama et al., 2005; Watanabe et al., 2004). The regulatory effects of raffinose under different physiological situation are still largely unknown, which warrant further investigation.

Kumar et al. (2010) reviewed that the content of raffinose in soybean could be as high as 1.3%. The content of soybean in maize-soybean diets of growing pigs is normally around 15-20%, therefore, the content of raffinose would reach 0.2% or even more. Our study found that the addition of 0.2% would already have a negative impact on growth performance on growing pigs, suggesting the importance of alleviating the negative effects of raffinose. A previous study (Trugo et al., 2000) found that the soybean raffinose component would not be changed by heat treatment. As a result, raffinose could not be eliminated during conventional thermal treatment of soybean. Therefore, the presence of anti-nutritional factor like raffinose still has side effects on piglets, especially young piglets, which reminds us that feed producers should pay careful attention to soybean meal when used for piglet feed. Possible ways to attenuate the negative effects were extraction with 80% ethanol aqueous solution, the addition of enzymes like α-galactosidase (Shang et al., 2018), and breeding low-oligosaccharide soy species (Sebastian et al., 2000).

5. Conclusion

The current study showed that soybean raffinose could inhibit voluntary feed intake, nutrient digestibility and induce humoral immune response, which ultimately reduce growing pigs' growth.

Author contributions

D. Chen and **B. Yu** were responsible for the conceptualization and manuscript revision. **Z. Zeng** and **Y. Zhang** were responsible for the experimental conduct and data analysis. **Z. Zeng** was responsible for the investigation and manuscript writing. **J. He**, **X. Mao** and **J. Yu** were responsible for the validation. **P. Zheng**, **J. Luo**, **Y. Luo** and **Z. Huang** were responsible for the supervision. All the authors have read and approved the final version of this manuscript.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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