

Effects of probiotics, oligosaccharides, and berberine combinations on growth performance of pigs

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Abstract: In order to find alternative feed additives to replace antibiotics for safe animal production, combinations of probiotics, oligosaccharides, and berberine were used in this study. Ninety 60-day-old pigs were assigned to 9 groups, with 10 pigs for each group. Group 1 was the control, group 2 had antibiotics added, and groups 3–9 had different levels of combinations added. The experimental period was 60 days. The results indicated that average daily gain and feed conversion ratio had no significant differences among the 9 groups ($P > 0.05$); however, the diarrhea rates in the control group and the high probiotic groups with low or high oligosaccharides additions were higher than those in the other groups ($P < 0.05$). High probiotics with high oligosaccharides addition was the best group for improvement in the apparent nutrient digestibility, followed by the antibiotics and individual high probiotics addition groups, which were better than the other groups ($P < 0.05$). Addition of berberine could significantly reduce *E. coli* counts in pig feces compared with the other groups ($P < 0.05$), while the fecal counts of lactic acid bacteria in groups given probiotics and oligosaccharides or berberine were higher than in the control group ($P < 0.05$). The fecal lipase, protease, and amylase activity was also improved by the combination additions ($P < 0.05$). It was concluded that the combinations of probiotics, oligosaccharides, and berberine had the same effect as antibiotics on reducing diarrhea rates and improving gut microflora for pigs.

Key words: Probiotics, oligosaccharides, berberine, pig, growth performance

1. Introduction

Antibiotics have been prohibited as feed additives in many countries due to superbug appearance as well as their creation of toxicity, drug resistance, and residues in animal products. It becomes more and more important to seek alternatives to substitute for antibiotics. Probiotics can balance the host gut microflora and improve animal production by filling the role of competitive exclusion for creating an optimal microflora (1). Prebiotics such as oligosaccharides have been defined as selectively fermented ingredients that allow specific changes both in the composition and/or activity of the microflora to benefit host well-being and health (2). It was reported that probiotics and prebiotics have good cooperation in regulating gut microflora and improving animal production (3). Berberine is an isoquinoline alkaloid found in many plants including the families Berberidaceae and Ranunculaceae, such as in *Berberis aquifolium*, *Berberis aristata*, *Coptis chinensis*, and so on. Berberine possesses multiple pharmacological activities such as anti-diarrheal, antibiotic, antihyperlipidemic, anti-inflammatory,

antiproliferative, and anti-diabetic functions, and it is also a good alternative to replace antibiotics (4,5). There have been no reports on the cooperation among probiotics, prebiotics, and berberine to date, so it is useful to study their cooperating functions for antibiotic substitution and food safety as feed additives in animal production.

2. Materials and methods

2.1. Materials

Probiotics contained *Bacillus subtilis*, *Lactobacillus casei*, and *Pichia anomala* (formerly named *Hansenula anomala*) at a ratio of 2:3:2 as in the previous report from our laboratory (6). The microbial counts in the combinations were 7×10^9 colony forming units per gram (CFU/g). Oligosaccharides (98% effective concentration, Alltech Co., Ltd., Beijing, China), berberine (98% effective concentration, Shanghai Yisha Biological Technology Co., Ltd., Shanghai, China), and aureomycin (15% effective concentration, Shanghai Dubang Biological Technology Co., Ltd., Shanghai, China) were purchased from the market.

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2.2. Experimental design, animals, diets, and feeding management

Ninety 60-day-old castrated pigs with initial body weight of 22.58 ± 2.18 kg (Duroc \times Landrace \times Pietrain) were assigned to 9 groups, with 10 pigs for each group and 2 pigs in each pen (4 m²). Every pig had its own identification code for determination of average daily gain. The preliminary period was 5 days and the experimental period was 60 days. The diets were prepared according to the recommended standard (7). The feed compositions and nutrient levels are listed in Table 1.

The pigs were weighed at the time of the initial and terminal experiment, and they were fasted for 12 h before weighing. Feed and water were given ad libitum. Watery feces were considered as diarrhea, which was recorded daily. Feed intake in each group was recorded once a week. The temperature in the shed was 23–35 °C during the trial.

The diets were mash feed, and the experimental design was as follows:

Group 1: Basal diet + 0.2% wheat bran (control)

Group 2: Basal diet + 0.1% aureomycin + 0.1% wheat bran (antibiotic)

Group 3: Basal diet + 0.05% probiotics + 0.15% wheat bran (low probiotic)

Group 4: Basal diet + 0.10% probiotics + 0.10% wheat bran (high probiotic)

Group 5: Basal diet + 0.05% probiotics + 0.05% oligosaccharides + 0.10% wheat bran (low probiotic + low prebiotic)

Group 6: Basal diet + 0.05% probiotics + 0.10% oligosaccharides + 0.05% wheat bran (low probiotic + high prebiotic)

Group 7: Basal diet + 0.10% probiotics + 0.05% oligosaccharides + 0.05% wheat bran (high probiotic + low prebiotic)

Group 8: Basal diet + 0.10% probiotics + 0.10% oligosaccharides (high probiotic + high prebiotic)

Group 9: Basal diet + 0.05% probiotics + 0.05% oligosaccharides + 0.02% berberine + 0.08% wheat bran (low probiotic + low prebiotic + berberine)

2.3. Determination of nutrient digestibility

After the feeding experiment, a following 3-day metabolic experiment was carried out. Each of 5 pigs in each group was put in a metabolic cage. Fresh feces were collected and measured immediately after discharge without contamination from the cage bottom for each pig for 3 days, and 35% of the feces were kept at -20 °C each time. Finally, the 3-day feces of each pig were mixed. Fecal samples for nutrient digestibility measurements were dried at 65 °C, subsequently ground through 40-mesh sieves, and mixed to determine the concentrations of nutrients and 4 N hydrochloric acid (HCl) insoluble ashes. Crude protein, fat, calcium (Ca), and phosphorus (P) contents in diets and feces were estimated with Kjeldahl, ether extract, potassium permanganate, and ammonium molybdate protocols, respectively (8). Amino acid concentrations in feedstuffs and diets were measured with an automatic amino acid analyzer (Biochrom, UK). The

Table 1. Feed compositions and nutrient levels (%).

Feed ingredients	Compositions	Nutrients	Nutrient levels
Corn meal	66.00	Digestive energy (MJ/kg)	11.59
Soybean meal	20.00	Crude protein	16.65
Wheat bran	10.00	Calcium	0.79
Calcium phosphate dibasic	1.20	Total phosphorus	0.58
Limestone	1.20	Available phosphorus	0.28
Lysine-HCl	0.24	Lysine	0.90
DL-methionine	0.02	Methionine + cysteine	0.54
NaCl	0.32		
Premix compound	1.00		

Note: premix compound provided the following amounts per kilogram of complete diet: vitamin A 5000 IU, vitamin D 3450 IU, vitamin E 60 IU, vitamin K 4.5 mg, vitamin B₁₂ 0.028 mg, vitamin B₁ 8.75 mg, vitamin B₆ 1.7 mg, niacin 35 mg, pantothenic acid 13 mg, folic acid 0.85 mg, biotin 0.47 mg, choline 500 mg, Cu 150 mg, Zn 100 mg, Fe 130 mg, Mn 30 mg, I 0.35 mg, Se 0.25 mg. "Digestive energy" was calculated based on the digestive energy concentrations in the raw materials, and other nutrients were analyzed.

nutrient apparent digestibility was determined by using the endogenous indicator protocol (8). The calculation was made as follows: nutrient apparent digestibility = $100 - (100 \times \text{indicator content in feed} / \text{indicator content in feces} \times \text{nutrient content in feces} / \text{nutrient content in feed})$.

2.4. Determination of the counts of *E. coli* and lactic acid bacteria in pig feces

One gram of fresh feces without contamination from each of five pigs in each group was diluted at different folds (from 10^{-1} to 10^{-9}) with 0.9% sterile physiological saline for *E. coli* incubation or with anaerobic dilution fluid for lactic acid bacteria incubation (9) and then vortexed completely. Two hundred microliters was taken from each mixture and put on eosin methylene blue agar plates for determining *E. coli* counts, or injected into anaerobic Hungate tubes with Man-Rogosa-Sharpe medium for determining lactic acid bacteria counts (9). Each mixture was used in triplicate. The microbes were incubated at 37 °C for 48 h, and only the colonies between 10 and 100 per plate or tube were counted. The counts of bacteria were expressed as natural logarithm (lg).

2.5. Determination of protease, amylase, and lipase activity in feces

Five grams of feces was mixed with 45 mL of 0.9% physiological saline in a 250-mL conical flask, shaken at $250 \times g$ for 30 min, and then filtrated with four-fold gauze. The filtrate was centrifuged at $12,000 \times g$ for 15 min. Enzyme activities in the supernatant were measured. Starch and tyrosine were used as the substrates for determining amylase (10) and protease activity (11), respectively, and the esterification method was used to determine lipase

activity (12). One unit of enzyme was defined as the amount of enzyme that catalyzed the release of 1 μmol of product per minute under the assay conditions.

2.6. Statistical analysis

Experimental data were expressed as means and standard errors. The data were analyzed using the ANOVA procedures of the Statistical Analysis Systems Institute (SAS 6.12; <http://www.sas.com/rnd/>). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of probiotics, oligosaccharides, and berberine on pig growth performance

Table 2 shows that the average daily gain (ADG, $P > 0.05$), daily feed intake (DFI, $P > 0.05$), and feed conversion ratio (FCR, $P > 0.05$) had no significant differences among the different groups; however, the diarrhea rates in the control group and high probiotic groups with low or high oligosaccharides addition were higher than those in the other groups ($P < 0.05$).

3.2. Effect of the combinations on nutrient digestibility

Table 3 indicates that the group with high probiotics and oligosaccharides addition was the best group for improvement of the apparent digestibility of crude protein, crude fat, calcium, and phosphorus, followed by the antibiotics and individual high probiotics addition groups, which were better than the other groups ($P < 0.05$). Most parameters of nutrient digestibility in the other experimental groups had no significant changes compared with the control group ($P > 0.05$).

Table 2. Effects of the combinations on pig growth performance.

Groups	Initial weight, kg	Final weight, kg	ADG, g	DFI, kg	FCR	Diarrhea rates, %
Control	22.30 ± 1.31	58.03 ± 7.44	600.00 ± 121.40	1.58 ± 0.16	2.64 ± 0.26	6.00 ± 0.84 ^A
Antibiotic	22.88 ± 3.01	62.70 ± 7.60	663.67 ± 111.00	1.65 ± 0.18	2.49 ± 0.24	2.50 ± 0.32 ^B
Low probiotic	22.68 ± 1.30	58.60 ± 6.05	590.33 ± 91.39	1.60 ± 0.17	2.71 ± 0.28	3.67 ± 0.45 ^{BC}
High probiotic	22.58 ± 2.03	59.30 ± 7.23	628.67 ± 115.03	1.65 ± 0.16	2.62 ± 0.26	2.33 ± 0.33 ^B
Low probiotic + low prebiotic	22.90 ± 2.96	60.80 ± 5.46	631.67 ± 66.37	1.62 ± 0.16	2.57 ± 0.25	2.83 ± 0.31 ^B
Low probiotic + high prebiotic	22.68 ± 2.88	57.12 ± 5.10	577.00 ± 71.50	1.54 ± 0.15	2.67 ± 0.27	1.17 ± 0.15 ^B
High probiotic + low prebiotic	22.60 ± 3.97	60.14 ± 9.95	662.00 ± 85.31	1.63 ± 0.17	2.47 ± 0.26	4.83 ± 0.52 ^{AC}
High probiotic + high prebiotic	22.40 ± 2.58	59.34 ± 5.50	615.67 ± 66.87	1.63 ± 0.16	2.65 ± 0.28	4.17 ± 0.49 ^{AC}
Low probiotic + low prebiotic + berberine	22.24 ± 3.57	60.74 ± 8.54	643.33 ± 98.80	1.60 ± 0.15	2.48 ± 0.26	2.00 ± 0.24 ^B

Note: each value represents the mean ± SE of 5 replicates per treatment. Different letters in the same columns represent significant differences ($P < 0.05$), while the same letters or values without letters in the same columns are insignificantly different ($P > 0.05$).

Table 3. Effects of the combinations on nutrient digestibility (%).

Groups	Crude protein	Crude fat	Ca	P
Control	87.17 ± 0.99 ^D	69.50 ± 7.68 ^C	76.62 ± 1.40 ^C	75.35 ± 1.11 ^D
Antibiotic	90.55 ± 0.92 ^{ABC}	79.59 ± 3.19 ^B	84.94 ± 2.25 ^B	90.81 ± 1.75 ^{AB}
Low probiotic	83.75 ± 2.38 ^E	64.19 ± 6.16 ^C	76.80 ± 4.27 ^C	81.72 ± 3.28 ^C
High probiotic	90.68 ± 1.45 ^{AB}	78.30 ± 4.29 ^B	86.57 ± 2.47 ^B	86.93 ± 2.56 ^B
Low probiotic + low prebiotic	89.03 ± 2.28 ^{CD}	80.09 ± 6.55 ^B	75.02 ± 6.26 ^C	77.08 ± 5.77 ^D
Low probiotic + high prebiotic	88.04 ± 3.00 ^D	78.65 ± 3.00 ^B	78.79 ± 4.09 ^C	77.99 ± 4.37 ^{CD}
High probiotic + low prebiotic	87.94 ± 1.61 ^D	63.12 ± 6.68 ^C	77.96 ± 2.81 ^C	77.14 ± 2.96 ^D
High probiotic + high prebiotic	91.49 ± 0.57 ^A	88.34 ± 2.57 ^A	90.46 ± 1.26 ^A	90.34 ± 1.27 ^{AB}
Low probiotic + low prebiotic + berberine	88.41 ± 0.99 ^{CD}	65.62 ± 3.03 ^C	77.57 ± 3.10 ^C	76.95 ± 2.17 ^D

Note: each value represents the mean ± SE of 5 replicates per treatment. Different letters in the same columns represent significant differences ($P < 0.05$), while the same letters or values without letters in the same columns are insignificantly different ($P > 0.05$).

3.3. Effect of the combinations on microflora and enzyme activity in pig feces

Table 4 shows that the addition of berberine could significantly reduce *E. coli* counts in pig feces compared with the other groups ($P < 0.05$), indicating that berberine had a strong ability to inhibit *E. coli* proliferation. In addition, the fecal counts of lactic acid bacteria in groups treated with probiotics and oligosaccharides or with berberine were higher than in the control group ($P < 0.05$), implying that the combinations could improve pig gastrointestinal microflora.

Table 4 also indicates that the fecal lipase activity in all the groups treated with combinations was increased compared with the control and antibiotic groups ($P <$

0.05), fecal protease activity in the group treated with low doses of probiotics and oligosaccharides was higher than that in the control and low-dose probiotics groups ($P < 0.05$), and fecal amylase activity in the group with high probiotic addition was higher than that in the group with low probiotic addition ($P < 0.05$).

4. Discussion

Many studies have showed that probiotics are the best substitutes for antibiotics (13–15). The reasons are that probiotics such as lactobacilli can help the animal's growth as well as improve the animal's bodily resistance to infectious agents by equilibrating gut microflora and stimulating the immune system (16). Probiotics have

Table 4. Enzyme activity and microbial counts in pig feces.

Groups	Protease activity, U/g	Amylase activity, U/g	Lipase activity, U/g	<i>E. coli</i> , CFU/g	Lactic acid bacteria, CFU/g
Control	22.02 ± 13.14 ^B	96.46 ± 22.15 ^{AB}	8.18 ± 0.69 ^E	7.35 ± 0.46 ^{ABC}	8.46 ± 0.34 ^B
Antibiotic	30.51 ± 12.00 ^{AB}	99.55 ± 9.69 ^{AB}	8.74 ± 0.70 ^{DE}	7.17 ± 0.47 ^{ABC}	9.01 ± 0.84 ^{AB}
Low probiotic	23.40 ± 10.04 ^B	77.43 ± 25.39 ^B	9.67 ± 0.69 ^{BC}	7.11 ± 0.18 ^{ABC}	9.25 ± 0.55 ^{AB}
High probiotic	37.01 ± 22.91 ^{AB}	116.01 ± 5.49 ^A	10.53 ± 1.11 ^{ABC}	6.49 ± 0.47 ^{CD}	9.07 ± 0.39 ^{AB}
Low probiotic + low prebiotic	48.24 ± 17.12 ^A	96.72 ± 19.02 ^{AB}	10.68 ± 0.36 ^A	6.98 ± 0.52 ^{BC}	9.45 ± 0.36 ^A
Low probiotic + high prebiotic	33.85 ± 6.99 ^{AB}	97.49 ± 23.86 ^{AB}	10.28 ± 0.74 ^{ABC}	7.73 ± 0.52 ^A	9.61 ± 0.87 ^A
High probiotic + low prebiotic	33.94 ± 12.94 ^{AB}	95.43 ± 18.04 ^{AB}	9.57 ± 0.41 ^{CD}	7.53 ± 0.16 ^{AB}	9.48 ± 0.66 ^A
High probiotic + high prebiotic	27.54 ± 10.94 ^{AB}	82.06 ± 37.21 ^{AB}	10.57 ± 0.48 ^{AB}	7.64 ± 0.36 ^{AB}	9.39 ± 0.97 ^A
Low probiotic + low prebiotic + berberine	31.53 ± 8.10 ^{AB}	93.12 ± 17.77 ^{AB}	10.26 ± 0.73 ^{ABC}	6.20 ± 1.11 ^D	9.47 ± 0.65 ^A

Note: each value represents the mean ± SE of 5 replicates per treatment. Different letters in the same columns represent significant differences ($P < 0.05$), while the same letters or values without letters in the same columns are insignificantly different ($P > 0.05$).

been found to have the ability to inhibit pathogenic bacteria growth, keep gut microbial balance, and improve growth performance for animals (17). A previous study suggested that supplementation of the diets of pigs with oligosaccharides can improve daily gain and FCR due to their ability to stimulate beneficial bacterial growth and inhibit pathogenic bacterial growth (18). It has been proved that the cooperation of probiotics and oligosaccharides will benefit the animals (3), which is in agreement with the findings of this study. Berberine was proved to be able to reduce diarrhea by inhibiting a variety of gram-positive and gram-negative bacteria proliferations, especially for pathogenic *E. coli*, *Shigella*, and *Campylobacter* (19). Our previous study showed that the combination of berberine and probiotics was better than their individual applications for inhibiting *E. coli* proliferation in vitro (unpublished data), so berberine-alone application was ignored in this study. The lower fecal *E. coli* counts and diarrhea rates in the diet added with berberine in this study also proved that berberine had a strong ability to inhibit *E. coli* proliferation. This study also showed that berberine could cooperate with probiotics and oligosaccharides well for improving animal production and inhibiting *E. coli* proliferation. Even though there are some reports about probiotics, oligosaccharides, and berberine applications in animal production individually (1,2,4,13), combinations of them have not been reported. This research indicated that the three of them had good cooperation for improving pig health and growth performance.

Bacillus can produce a variety of enzymes such as protease and amylase to digest nutrients by activating

animal endogenous digestive enzymes or by supplying large amounts of exogenous enzymes (20). This study showed that combinations of probiotics, oligosaccharides, and/or berberine could significantly increase fecal lipase, protease, and amylase activity to some extent. The reason may be that the probiotics can produce these enzymes or stimulate endogenous excretion (21). It was reported that diets supplemented with probiotics could significantly improve carbohydrate digestibility in the small intestine (22). This study demonstrated that supplementation of high doses of probiotics and oligosaccharides combinations could replace antibiotics to increase nutrient digestibility, maybe due to the higher digestive enzymes and regulation of gut microflora.

Bacillus could promote the growth of *Lactobacillus* and *Streptococcus* within the gastrointestinal tract, and the cooperation of these bacteria could produce a large amount of organic acid to decrease the pH values in the intestine to inhibit propagation of pathogenic bacteria (23). This study showed that addition of probiotics, oligosaccharides, and berberine to pig diets could effectively promote the growth of lactic acid bacteria in the gut and reduce the proliferation of *E. coli*, thereby improving the gastrointestinal microbial balance, in agreement with a former report (24). The reason may be that *Lactobacillus* can produce hydrogen dioxide, acidic materials, and bacterins, which have the ability to inhibit pathogenic bacterial growth.

It can be concluded that combinations of probiotics, oligosaccharides, and/or berberine could completely replace antibiotics to improve pig growth performance and nutrient digestibility, and to reduce diarrhea rates. It will be a good kind of feed additive for safe animal production.

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