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JAE HONG PARK

HYEOK MIN YUN

IN HO KIM

TÜBİTAK

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The effect of feeding Rhizopus oligosporus on growth performance, nutrient digestibility, blood profile, fecal microbiota, and fecal score in weanling pigs

Jae Hong PARK, Hyeok Min YUN, In Ho KIM*
Department of Animal Resource and Science, Dankook University, Cheonan, Republic of Korea

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Abstract: Piglets were allocated to 1 of 3 treatments by body weight and sex, and fed different levels of 0%, 0.15%, and 0.30% of *R. oligosporus* 1.0×10^6 cfu/g, in a basal diet based on corn–soybean meal for 35 days. Piglets fed diets supplemented with *R. oligosporus* had higher average daily gain during days 15–35 and 1–35 (quadratic, $P < 0.05$). Feed conversion ratio during 1–35 days showed a significant improvement, as dietary *R. oligosporus* supplementation increased from 0.15% to 0.30% (linear, $P < 0.05$). Apparent total tract digestibility (ATTD) of crude protein (CP) was increased at 2 weeks, as dietary *R. oligosporus* supplementation increased significantly (linear and quadratic, $P < 0.05$), and ATTD of dry matter, CP, and energy were linearly increased at 5 weeks ($P < 0.05$). The serum IgG concentration was significantly increased in the *R. oligosporus*-fed groups (quadratic, $P < 0.05$). Fecal *Lactobacillus* counts were significantly increased quadratically and coliform bacteria counts were decreased linearly, as dietary *R. oligosporus* supplementation increased ($P < 0.05$). In conclusion, diets containing *R. oligosporus* may improve the growth performance, nutrient digestibility, IgG concentration, and intestinal environment in weanling pigs.

Key words: Blood profiles, growth performance, nutrition digestibility, *Rhizopus oligosporus*, weanling pig

1. Introduction

Antimicrobial agents have been widely used in swine feed due to their prophylactic and therapeutic effects, specifically for inhibiting diseases, for several decades. However, since the threat of bacterial antimicrobial resistance has been recognized, a number of countries have recently banned the subtherapeutic use of antibiotics. Over less than two decades, probiotics have been used as an effective alternative to antibiotics in farm animals (1). Probiotics are a group of nonpathogenic live microorganisms that are known to have positive effects on animal health, and are derived from various origins for use as feed additives (2). Beneficial effects of many probiotic strains administered orally during each stage of growth on the growth of pigs in terms of growth performance, nutrient retention, gut health, and intestinal microflora have been shown (3).

Some fungi, usually belonging to the genus *Rhizopus*, have been used in the process of food fermentation in Asia, and their products have been generally recognized as safe (4). *Rhizopus oligosporus*, contained in tempeh, can also be considered a reliable source of probiotic by producing antibiotics (5). However, there is limited information available regarding the effects of *R. oligosporus* in pigs. Therefore, the objective of the current study was to evaluate the effect of dietary *R. oligosporus* supplementation on the growth performance, nutrient digestibility, blood profiles, and fecal microbial shedding in weanling pigs.

2. Materials and methods

The protocols used for the current experiment were approved by the Animal Care and Use Committee of Dankook University (DKU-09-015).

2.1. Experimental design, animals, housing, and diets

A total of 75 cross-bred pigs [(Landrace × Yorkshire) × Duroc, weaning at 21 days, body weight (BW) = 7.51 ± 0.73 kg] were allocated to 1 of 3 treatments [5 replicates with 5 pigs per pen (3 barrows and 2 gilts)]. Piglets were fed different levels of 0%, 0.15%, and 0.30% of *R. oligosporus* 1.0×10^6 cfu/g in a basal diet based on corn–soybean meal for 35 days after a 5-day adaptation period. A two-phase feeding program composed of phases one (days 1 to 14) and two (days 15 to 35) was used. Basal diets used in the present study were formulated to meet the nutrient recommendations of the NRC (6) (Table 1). Digestible energy of basal diet samples was determined by an indirect method, using chromium oxide as an indicator in triplicate before initiation of the experiment. Dry matter, crude protein, crude fiber, ether extract, ash, Ca, and P

* Correspondence: inhokim@dankook.ac.kr

Table 1. Compositions of basal weaned pig diets (as-fed basis).

Items	Phase 1 (days 0 to 14)	Phase 2 (days 15 to 35)
Ingredient, g/kg		
Extruded corn	34.42	49.07
Soybean meal, 440 g crude protein/kg	39.42	35.07
Dried whey	10.00	-
Extruded oat	5.00	5.00
Soybean oil	4.58	4.03
Fish meal, 630 g crude protein/kg	3.00	3.00
Monocalcium phosphate	1.01	0.90
Phenyllacitic acid	1.00	1.00
Tricalcium phosphate	0.79	0.98
Zinc oxide (ZnO)	0.30	0.30
Salt	-	0.20
Vitamin premix[1]	0.18	0.18
Mineral premix[2]	0.13	0.13
L-Lysine·HCl, 780 g/kg	0.12	0.12
DL-methionine	0.05	0.02
Analyzed chemical composition, g/kg		
Digestible energy, MJ/kg	16.67	16.65
Dry matter	89.00	88.57
Crude protein	23.86	21.91
Crude fiber	3.55	3.74
Ether extract	5.50	5.41
Ash	4.83	4.54
Calcium	8.93	8.94
Phosphorus	7.08	7.85
Lysine	1.50	1.42
Methionine	0.42	0.39

[1]Provided per kilogram of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.
[2]Provided per kilogram of complete diet: Fe (as $FeSO_4 \cdot 7H_2O$), 80 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

content in the diet were analyzed according to the method of AOAC (7). Lysine and methionine were measured using an amino acid analyzer (Beckman 6300, Beckman Coulter Inc., Fullerton, CA, USA) after acid hydrolysis for 24 h in HCl.

Piglets were housed in an environmentally controlled room with slatted plastic floor facility and room temperature was maintained approximately between 23 °C and 25 °C with 60% humidity. Each pen was equipped with a self-feeder and nipple drinker to allow ad libitum access to feed and water throughout the experimental period.

R. oligosporus probiotic used in the current study was provided by a commercial company (Sunbio Co. Ltd., Cheonan, Korea). The lyophilized *R. oligosporus* probiotic was produced by fermentation of soybean in the presence of *R. oligosporus* NRRL 2710, which was confirmed to contain at least 1.0×10^6 cfu/g of *R. oligosporus*.

2.2. Sampling and measurements

Individual body weight of piglets in each pen was monitored at the beginning of the experimental period, at 2 weeks, and at the end (5 weeks) of the experimental period, and feed intake was recorded weekly on a pen basis during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Chromium oxide (Cr_2O_3, 2 g/kg) was added to the diets as an indigestible marker at the beginning of 2 weeks and 5 weeks to measure digestibility. Fresh fecal grab samples were collected directly via rectal massage from 5 piglets in each pen at the end of 2 weeks and 5 weeks to determine the coefficient of apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), and energy. All the feed and fecal samples were freeze-dried and finely ground to be able to pass through a 1-mm screen, and stored in a refrigerator at –20 °C until analysis. DM and N concentrations by Kjeldahl method were determined according to the AOAC (7). The chromium quantification in fecal samples was determined according to the method described by Williams et al. (8). Fecal samples were ashed in a muffle furnace at 600 °C for 90 min, and then digested in 3 mL of phosphoric acid/manganese sulfate solution with 4.5% (wt/vol) potassium bromate solution until effervescence ceased. Samples were transferred to a 100-mL volumetric flask, and brought to volume with deionized water. Chromium concentration in prepared samples by digestion process was determined by atomic absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Gross energy was determined using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, IL, USA). The equation was adapted in calculating digestibility as follows: digestibility (%) = $(1 - ((Nf \times Cd)/(Nd \times Cf))) \times 100$, where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cf = chromium concentration in feces (% DM), and Cd = chromium concentration in diet (% DM).

At the end (35 days) of the experiment, blood samples from all piglets were collected into vacuum tubes containing no additive and tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain serum and whole blood, respectively. The red blood cell (RBC), white blood cell (WBC), and lymphocyte counts of whole blood samples were determined using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA). The serum was separated by centrifugation for 30 min at 2000 × *g* at 4 °C, and the aliquot was stored at –4 °C (within 24 h) until determination of the serum immunoglobulin G (IgG). The concentration of IgG in serum was determined by ELISA using a porcine polyclonal immunoglobulin-specific kit (Bethyl, Montgomery, TX, USA) according to the manufacturer's recommendations.

For the measurement of fecal microbiota, at days 35, fecal samples were collected from 5 piglets per pen after massaging the rectum. Approximately 1 g of fecal sample was diluted with 9 mL of 10 g/L peptone broth (Becton, Dickinson and Co., Rutherford, NJ, USA) and homogenized. Viable counts of bacteria in the fecal samples were then conducted by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI, USA) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the coliform bacteria and *Lactobacillus,* respectively. The lactobacilli medium III agar plates were then incubated for 24 h at 37 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C under aerobic conditions. The colonies on each agar plate were counted using a colony counter and the results were presented as logarithm of colony-forming units per gram ($\log_{10}$ CFU/g) according to the method of White et al. (9).

Fecal scores were determined twice daily (at 0800 and 2000) on days 7, 14, 21, 28, and 35 using the following fecal scoring system by Sherman et al. (10): 1, hard (dry pellet); 2, firm (formed stool); 3, soft (moist stool that retains shape); 4, soft (unformed stool that assumes shape of container); 5, watery (liquid that can be poured).

2.3. Statistical analysis

All data were analyzed using the general linear model (GLM) procedure of the SAS program. The pen was the experimental unit for productivity measurement and in the nutrient digestibility, blood profiles, and fecal microbial analysis, individual piglet was used as the experimental unit. Polynomial contrasts were used to determine linear and quadratic effects of increasing *R. oligosporus* levels on all measurements. Data are reported as means with standard error and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Growth performance

Piglets fed diets supplemented with 0.15% and 0.30% *R. oligosporus* showed significantly higher ADG than those in the control group during days 15–35 and 1–35 (quadratic, $P < 0.05$) (Table 2). Average daily feed intake was not influenced by feeding *R. oligosporus* throughout the entire experimental period, but FCR during days 1–35 was improved linearly ($P < 0.05$).

3.2. Nutrient digestibility

At 2 weeks, ATTD of nitrogen was increased in piglet fed diets supplemented with *R. oligosporus* (linear and quadratic, $P < 0.05$) (Table 3). In addition, at 5 weeks, there were linear effects on ATTD of DM, nitrogen, and energy as dietary levels of *R. oligosporus* increased ($P < 0.05$).

3.3. Blood characteristics

The concentration of serum IgG was increased quadratically in piglets fed diets supplemented with *R. oligosporus* at 5 weeks ($P < 0.05$) (Table 4). No effect was observed on the RBC, WBC, or lymphocyte count among the treatments ($P > 0.05$).

3.4. Fecal microbial shedding and fecal score

There was no significant difference in fecal scores of piglets fed with different levels of *R. oligosporus* in the diet. However, the fecal *Lactobacillus* counts were significantly increased quadratically and coliform bacteria counts were decreased linearly as dietary *R. oligosporus* supplementation increased ($P < 0.05$) (Table 5).

4. Discussion

The purpose of this study was to investigate the effects of *R. oligosporus* strain as a feed additive and it was found to exert beneficial effect on performance in weanling pigs. Probiotics have been tested in numerous studies in various pig models with varying responses, depending upon the probiotic strains or induced disease challenges, and different environmental conditions. The results also varied depending on the strains used, or stress types (11,12). The period after weaning is an extremely important time in the life of pigs as piglets face many stress factors including disease-causing factors and environmental and dietary changes in a short span of time. It is thought that piglets are more susceptible to stress, thereby resulting in poor productivity generally, under a combination of these circumstances. Our study using weanling pigs concluded that the ADG during days 15–35 and 1–35 and FCR during days 1–35 was found to increase significantly in the *R. oligosporus* treated groups. According to the results of our experiment, improvement in ADG and FCR following the addition of *R. oligosporus* to piglet diets is thought to be induced by the effects of probiotics including the maintenance of beneficial microbial population and improvement in digestion. In addition, Pereira et al. (13) and Suo et al. (14) observed that the villi height in ileum, jejunum, or duodenum was greater and the density was thicker after probiotic (yeast or *L. plantarum*) treatment, thereby leading to improvement in feed utilization of pigs. Currently, there is a lack of useful data regarding *R.*

Table 2. Effect of dietary *Rhizopus oligosporus* supplementation on growth performance in weanling pigs.

	Rhizopus oligosporus 1.0×10^6 cfu			SEM[1]	P-value	
	0	0.15%	0.30%		Linear	Quadratic
Phase 1 (days 1–14)						
ADG, g	309	332	311	15.35	0.904	0.238
ADFI, g	388	402	361	14.44	0.084	0.060
FCR	1.256	1.211	1.161	0.03	0.253	0.961
Phase 2 (days 15–35)						
ADG, g	619	655	631	8.41	0.070	0.047
ADFI, g	957	947	927	26.73	0.535	0.891
FCR	1.546	1.446	1.469	0.01	0.092	0.122
Overall (days 1–35)						
ADG, g	495	526	503	8.04	0.065	0.039
ADFI, g	729	729	700	19.45	0.322	0.563
FCR	1.473	1.386	1.392	0.01	0.023	0.110

Each mean is represented by 5 replications. SEM, Standard error of the mean; ADG, average daily gain; ADFI, average daily feed intake; FCR; feed conversion ratio.

Table 3. Effect of dietary *Rhizopus oligosporus* supplementation on nutrient digestibility in weanling pigs.

	Rhizopus oligosporus 1.0×10^6 cfu			SEM	P-value	
	0	0.15%	0.30%		Linear	Quadratic
At 14 days						
Dry matter	80.3	82.7	82.1	1.05	0.291	0.314
Crude protein	82.2	86.9	85.9	1.01	0.013	0.025
Digestible energy	81.5	83.0	83.1	0.97	0.294	0.567
At 35 days (at the end of study)						
Dry matter	79.7	82.6	82.6	0.93	0.044	0.222
Crude protein	80.8	85.3	85.0	1.07	0.013	0.090
Digestible energy	78.9	82.7	82.1	0.93	0.016	0.052

Each mean is represented by 25 replications. SEM, Standard error of the mean.

Table 4. Effect of dietary *Rhizopus oligosporus* supplementation on blood profiles in weanling pigs.

	Rhizopus oligosporus 1.0×10^6 cfu			SEM	P-value	
	0%	0.15%	0.30%		Linear	Quadratic
At 35 days (at the end of study)						
RBC, 10^6/μL	6.2	6.4	6.3	0.18	0.758	0.301
WBC, 10^3/μL	16.9	16.9	17.0	0.72	0.926	0.967
Lymphocyte, %	57.0	58.5	57.3	3.15	0.940	0.595
IgG, mg/dL	301.0	325.8	309.8	6.84	0.199	0.005

Each mean is represented by 25 replications. RBC, Red blood cells; WBC, White blood cells; IgG, Immunoglobulin G; SEM, Standard error of the mean.

Table 5. Effect of dietary *Rhizopus oligosporus* supplementation on fecal microbiota and fecal score in weanling pigs.

	Rhizopus oligosporus 1.0×10^6 cfu			SEM	P-value	
	0%	0.15%	0.30%		Linear	Quadratic
At 35 days (at the end of study)						
Lactobacillus ($\log_{10}$ cfu/g)	7.04	7.75	7.59	0.06	0.087	0.047
Coliform bacteria ($\log_{10}$ cfu/g)	6.51	5.75	5.69	0.08	0.037	0.216
Fecal score	3.21	3.17	3.16	0.02	0.433	0.777

Each mean is represented by 25 replications. SEM, Standard error of the mean.

oligosporus in pigs; however, the findings of the current study support those of a previous report that determined that probiotics including fungi, *Saccharomyces cerevisiae*, and *Aspergillus oryzae* improved growth performance of growing pigs (15). Therefore, in this study, it might be expected that *R. oligosporus* probiotic had a beneficial effect on growth performance in piglets during the weanling period.

In the present study, nutrient digestibility was found to be significantly improved by *R. oligosporus* supplementation. We did not find any studies assessing the influence of *R. oligosporus* on nutrient digestibility in pigs. However, previous studies have suggested that dietary probiotics supplementation positively affect nutrient digestibility in pigs. Kil et al. (16) observed significant improvements in digestibility of DM, nitrogen, and ash when pigs were fed a diet supplemented with probiotics (*Saccharomyces* + *Enterococcus* + *Phaffia rhodozyma* + *Rhodopseudomonas* + *Bacillus* species) containing different bacterial strains continuously for 20 weeks. Meng et al. (17) demonstrated that pigs fed with probiotics (complex of *B. subtilis* and *C. butyricum*) showed greater digestibility of crude protein and energy compared with that in the control group of growing pigs. Giang et al. (18) also demonstrated that addition of lactic acid bacteria complexes (*E. faecium* + *L. acidophilus* + *P. pentosaceus* + *L. plantarum*) in diets improved the total tract apparent digestibility and the ileal apparent digestibility of crude protein, crude fiber, and organic matter in weanling pigs. An increased fecal *Lactobacillus* count (Table 5) after *R. oligosporus* supplementation could be considered beneficial to the host in terms of metabolic processes of digestion and utilization of nutrition as it would potentially increase the activity of the useful enzymes (19). Thus, the findings of the current study are consistent with previous studies, which suggest that positive effects due to improvement in nutrient digestibility could be expected after including *R. oligosporus* in the weanling pig diet.

In terms of blood profiles, a beneficial effect was observed only in the IgG level with the inclusion of *R. oligosporus*. Proper development of the immune system is absolutely necessary for optimum growth performance of piglets (20), and several probiotic strains have been proven to have immunostimulatory effects by increasing macrophage activation (21), enhancing the local antibody responses (22), activating natural killer cells (23), and mediating immune regulation, particularly through the balance between proinflammatory and antiinflammatory cytokines (24). *Saccharomyces boulardii* has been found to be effective in controlling various pathogens due to its antagonistic action or stimulation of immune systems (25). In addition, live yeast supplementation decreased levels of potential pathogens in the ileum of weaned piglets (26). Molist et al. (27) suggested that yeast supplementation increased serum IgG and IgM responses to sheep red blood cells in weaning pigs. Serum IgG is the major component of blood immunoglobulins, and this antibody plays a major role in defending against antigens. Therefore, our study confirmed that the inclusion of *R. oligosporus* could improve the immunity of weanling pigs.

In our study, *Lactobacillus* population in feces was found to be significantly increased and coliform bacteria population tended to decrease (linear; $P = 0.037$) after *R. oligosporus* supplementation. Previous studies have reported that probiotics act by competitive exclusion of harmful bacteria in pigs (19). This action has been well documented in *Lactobacillus* strains, and some evidence exists that other probiotic strains may have similar mechanisms of action. Probiotics modulate the intestinal microflora, and selectively favor the growth of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*, due to lowering of the pH of the gastrointestinal tract (28). Therefore, the environment of the gastrointestinal tract becomes unsuitable for the activity and proliferation of harmful bacteria in piglets. Furthermore, such inhibition of harmful bacteria is also thought to be associated with secretion of various antimicrobial compounds produced by probiotics, such as organic acids, hydrogen peroxide, and bacteriocin (29). The findings of the present study are in agreement with that of another study (30), which reported a significant increase in fecal *Lactobacillus* and a decrease in coliform bacteria, following the addition of fungus, *Saccharomyces cerevisiae*, to pig diets. Thus, the results of our study imply that *R. oligosporus* may have a modulating effect on the intestinal microflora population, by exerting a stimulating effect on *Lactobacillus* and an inhibitory effect on coliform bacteria.

5. Conclusions

The present study indicates that the dietary inclusion of *R. oligosporus* can be a good alternative feed additive to antibiotics for piglets, particularly in terms of growth performance, nutrient digestibility, blood IgG concentration, and fecal microbial shedding. However, large-scale field studies assessing the effect of *R. oligosporus* on growth performance of pigs are needed.

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