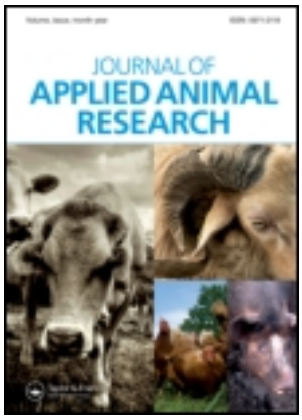

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Effect of probiotics supplementation in diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, faecal microbial population and faecal noxious gas content in growing pigs

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Effect of probiotics supplementation in diets with different nutrient densities on growth performance, nutrient digestibility, blood characteristics, faecal microbial population and faecal noxious gas content in growing pigs

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A total of 144 pigs with initial BW of 29.48 ± 0.58 kg were used in a 6-wk experiment to evaluate the probiotics supplementation (*Enterococcis faecium*, 1.0×10^{10} CFU/g) with different nutrient densities in growing pigs. Pigs were randomly allotted to one of four treatments in a 2×2 factorial arrangement of treatments with two levels of nutrient density and probiotic (0 or 0.1%) according to their sex and BW (nine replicate pens with two gilts and two barrows per pen). Pigs fed the higher nutrient density diets led to a higher ($P < 0.05$) ADG and G:F ratio than those fed low nutrient density diet. The inclusion of probiotic increased ($P < 0.05$) ADG, ADFI, G:F, and the apparent total tract digestibility of DM, N, energy. Dietary high nutrient density led to a higher ($P < 0.05$) faecal H_2S and NH_3 content, whereas the probiotic supplementation led to a reduced ($P < 0.05$) faecal H_2S and NH_3 content. An increased faecal *lactobacillus* concentration was observed with probiotic supplementation. An interactive effect between energy and nutrient density diet and probiotic was observed on the ADG and G:F ratio, faecal *lactobacillus*, apparent total tract digestibility and faecal noxious gas content. In conclusion, both dietary probiotic supplementation and a high nutrient diet could improve growth performance and nutrient digestibility in growing pigs. The beneficial effect of probiotic supplementation in pigs could be enhanced with the high energy and nutrient density diets.

Keywords: nutrient density; probiotics; growing pigs

1. Introduction

Recently, the application of probiotics had received considerable attention in the discussion about developing suitable alternative for antibiotic growth promoters in the pig industry (Chen et al. 2006; Meng et al. 2010; Yan, Meng, et al. 2010; Yan and Kim 2011). However, the effect of probiotics supplementation in practice is highly inconsistent because of the different diet composition, strain differences, dose level, age of the animal, as well as its interactions with environmental factors (Loh et al., 2008; Khan, Yousaf, et al. 2011; Khan, Atif, et al. 2011).

Mountzouris et al. (2010) had suggested that the effect of probiotics could be affected by various factors such as the quality of feed component and anti-nutritional compound. Meng et al. (2010) also suggested that nutrient density could influence the effect of probiotics in growing pigs. It has been suggested that probiotics require some nutrient and energy cost because of its effect on immune cell development and function (Fuller 1989; FAO 2002). Mountzouris et al. (2010) also demonstrated that the beneficial functions of probiotic require a nutrient and energy cost for the host because of the growth and proliferation of live microbials. Thus, a high nutrient density diet would be expected to provide more nutrients for growth and proliferation of live microbials. Therefore, we hypothesised that the effect of probiotics in pigs may be different with diets varying in nutrient density.

Lojanica et al. (2010) and Cernauskiene et al. (2011) had recently suggested that dietary *Enterococcus faecium* DSM7134 increased ADG and feed conversion ratio in weaned pigs and finishing pigs. Therefore, our study was conducted to determine the efficacy of *Enterococcus faecium* with diets varying in nutrient density in growing pigs. We hypothesised that the effect of *Enterococcus faecium* could be affected by the different nutrient density diet in growing pigs.

2. Materials and methods

The Animal Care and Use Committee of Dankook University approved the experimental protocols.

2.1. Source of probiotics

The probiotics used in the current experiment is manufactured by a commercial company (Dutch State Mining). This product is a probiotic preparation of

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Enterococcus faecium contains a minimum of 1.0×10^{10} CFU/g of *Enterococcus faecium* (DSM 7134)

2.2. Experimental design, animals and housing

A total of 144 [(Duroc × Yorkshire) × Landrace]) pigs with an average initial BW of 25.49 ± 0.58 kg were used in this 6-wk growth trial. Pigs were allotted into four dietary treatments based on their initial BW using a 2 × 2 factorial arrangement of treatments with two levels of nutrient density (low or high energy, CP, and Lys) and (0 or 0.1% probiotic). Each dietary treatment consisted of nine replication pens with four pigs per pen (two gilts and two barrows). Diets (Table 1) were formulated to meet or exceed the nutrient requirements recommended by NRC (1998). Pigs were housed in an environmentally controlled, slatted-floor facility in 36 adjacent pens (1.8 × 1.8 m) at the pig farm of Dankook University. Throughout the experiment, all pigs were provided with *ad libitum* access to feed and water through a self-feeder and nipple drinker, respectively. The target room temperature and humidity were 25°C and 60%, respectively.

2.3. Sampling and measurements

Pig weights were measured at the beginning and the end of the experiment period, feed consumption were also recorded on a pen basis during the experiment to calculate ADG, ADFI, and G:F. Chromium oxide was added to the diet as an indigestible marker at 0.20% of the diet for 7 days prior to faecal collection at the sixth week for calculation of DM, N, and energy digestibility. Faecal grab samples were collected at random from at least two pigs in each pen (one gilt and one barrow; 18 pigs per treatment). All feed and feces samples were stored immediately at −20° until analysis. All feed and faecal samples were freeze-dried and finely ground to be able to pass through a 1 mm screen. The determination of DM, N, and energy digestibility were conducted in accordance with the methods established by the Association of Official Analytical Chemists (1995). Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the apparent total tract digestibility (ATTD) of DM, N, and energy were calculated using indirect methods described by Williams et al. (1962).

Two pigs were randomly selected from each pen (one gilt and one barrow) and bled via jugular venipuncture at the beginning of the experiment (18 pigs per treatment at 0 d). The same pigs were bled at the end (18 pigs per treatment at 35 d) of the experiment. Blood samples 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 cells

Table 1. Composition of basal diets (as-fed basis).[a]

	High nutrient diet		Low nutrient diet	
Item	+Probiotics	−Probiotics	+Probiotics	−Probiotics
Ingredient (%)				
Corn	56.80	56.90	62.43	62.53
Soybean meal	35.39	35.39	32.18	32.18
Fish oil	5.63	5.63	3.12	3.12
Limestone	0.84	0.84	0.84	0.84
Salt	0.20	0.20	0.20	0.20
TCP	0.70	0.70	0.72	0.72
L-Lys·HCl (78%)	0.14	0.14	0.11	0.11
Probiotics	0.10		0.10	
Vitamin premix[b]	0.20	0.20	0.20	0.20
Mineral premix[c]	0.10	0.10	0.10	0.10
Analysed composition				
GE, kcal/kg	4283	4283	4127	4127
CP,%	19.41	19.41	18.34	18.34
Lys,%	1.29	1.29	1.30	1.30
Ca,%	0.64	0.64	0.65	0.65
Total P,%	0.53	0.53	0.54	0.54

[a]High and low: high or low in energy, CP, and Lys; High or low diet was supplemented with 0 or 0.2% probiotics.
[b]Provided per kg diet: 20,000 IU of vitamin A; 4000 IU of vitamin D_3; 80 IU of vitamin E; 16 mg of vitamin K_3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B_{12}; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid; and 0.08 mg of biotin.
[c]Provided per kg diet: 140 mg of Cu (as copper sulfate); 179 mg of Zn (as zinc oxide); 12.5 mg of Mn (as manganese oxide); 0.5 mg of I; 0.25 mg of Co (as $Co_2O_3 \cdot 7H_2O$); and 0.4 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

(RBC), white blood cells (WBC) and lymphocyte counts of whole blood samples were determined using an automatic blood analyser (ADVIA 120, Bayer, Tarrytown, NY, USA).

At the end of the experiment, faecal samples were collected via massaging the rectum from two pigs randomly selected from each pen (one gilt and one barrow; 18 pigs per treatment) and pooled and placed on ice for transportation to the laboratory where analysis was immediately carried out. The composite faecal sample (1 g) from each pen was diluted with 9 mL of 10 g/L peptone broth (Becton, Dickinson and Co., Rutherford, NJ, USA) and homogenised. Viable counts of bacteria in the faecal 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 *E. coli* and *Lactobacillus,* respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C under anaerobic conditions. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

For analysis of the faecal NH_3 H_2S concentration, fresh faecal samples were collected from at least two pigs in each pen (18 pigs per treatment) at the end of experiment. The NH_3 concentration was then determined using the method described by Chaney and Marbach (1962). To determine the faecal H_2S concentration, 300 g of fresh faecal samples were transferred to a sealed box and fermented for 30 h in an incubator (35°C). The fermented samples were then analysed using a gas search probe (Gastec Corp., Kanagawa, Japan).

2.4. Statistical analyses

Data were analysed as a randomised complete block design, with a 2×2 factorial arrangement using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The final model included the main effects included dietary nutrient density and probiotic administration, as well as the interaction between probiotic and dietary nutrient density. For all response criteria, the pen served as the experimental unit. Variability in the data was expressed as the pooled SE and a $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Growth performance

In the current study, pigs fed the high nutrient density diets increased ($P < 0.05$) ADG and G:F ratio rather than those fed low energy and nutrient density diet (Table 2). Likewise, the inclusion of probiotics led to a higher ADG, ADFI, and G:F ratio than those fed no-probiotics treatments. Moreover, with probiotic supplementation, pigs fed high energy and nutrient density increased ADG, G:F was much better than

Table 2. Effects of supplementation of high and low energy and nutrient density diets with probiotics on growth performance, nutrient digestibility and blood characteristics in growing pigs.

	High		Low			*P*-value		
Items	+P	−P	+P	−P	SE[c]	D	P	D × P
Growth performance[a]								
Initial BW	24.8	25.4	25.5	26.2	0.58	0.313	0.254	0.216
Final BW	54.1	51.9	52.7	51.5	1.01	0.034	0.012	0.007
ADG, g	697	632	647	602	21	<0.05	<0.001	0.004
ADFI, g	1556	1510	1535	1545	37	0.281	0.243	0.188
G:F	0.449	0.419	0.422	0.389	0.014	<0.001	<0.05	0.012
Nutrient digestibility[b]								
DM	79.3	74.9	78.1	73.7	1.98	0.462	0.002	0.552
N	82.8	75.3	78.9	74.4	2.01	0.241	<0.001	0.010
Energy	81.8	74.2	77.6	73.5	1.84	0.548	0.003	0.015
Blood characteristics[2]								
WBC, 10^3/μl	14.7	13.6	14.5	13.2	0.79	0.254	0.347	0.292
RBC, 10^6/μl	7.9	7.5	7.4	7.2	0.54	0.357	0.628	0.185
Lymphocyte,%	59.9	56.8	57.6	54.5	3.18	0.486	0.684	0.241

[a]Each mean represents six pens with two gilts and two barrows per pen. High: high energy and nutrient diet ±0.2% probiotics (P); Low: low energy and nutrient diet ±0.2% probiotics (P).
[b]Each mean represents nine pens with two gilts and two barrows per pen. High: high energy and nutrient diet; ±0.1% probiotics (P); Low: low energy and nutrient diet ±0.1% P.
[c]Pooled standard error.

those fed low energy and nutrient density diets (density × probiotics).

3.2. Nutrient digestibility and blood characteristics

Dietary probiotics supplementation led to a higher DM, N and energy digestibility than those fed non-probiotics diet. No difference was observed in the nutrient digestibility between different nutrient density levels. An interactive ($P < 0.05$) effect between probiotics and nutrient density level was observed on the energy levels in the current study ($P < 0.05$; Table 2). No difference ($P > 0.05$) was observed on the other blood profiles (WBC, RBC, Lymphocyte count) in the current study (Table 2).

3.3. Faecal microbial population and noxious gas content

An increased ($P < 0.05$) faecal *lactobacillus* concentration was observed with probiotics supplementation in the current study (Table 3), and it increased ($P < 0.05$) more dramatically with the high nutrient density diet. Pigs fed a high nutrient diet led to a higher faecal H_2S and NH_3 content than those fed low nutrient diets (Table 3). A probiotics supplemented diet reduced faecal H_2S and NH_3 content compared with non-probiotics supplemented diet.

4. Discussion

4.1. Effect of probiotics

In the current study, the inclusion of *Enterococcus faecium* DSM7134 (*E. faecium DSM 7134*) led to a greater ADG and G:F ratio compared with those fed a non-supplemented diet, which is in agreement with Lojanica et al. (2010) and Cernauskiene et al. (2011), who suggested that dietary *E. faecium DSM 713* increased ADG and feed conversion ratio in weaned pigs and finishing pigs. Similarly, nutrient digestibility was also increased by dietary *E. faecium DSM 713*. Therefore, we suggest that the reason for the improved growth performance and feed efficiency is likely to be the increased nutrient digestibility. Previously, it has been well suggested that *E. faecium* possesses an inhibitory substance and function as a probiotics bacterial inhibitor of *Streptococcal mutans* (Kumada et al. 2009). Cernauskiene et al. (2011) also suggested that *E. faecium* are normal components of the swine intestinal microbiota, which could produce lactic acid to reduce the pH value of the intestinal content and inhibit the development of invasive pathogens. In our study, the faecal *lactobacillus* was increased by the dietary probiotics supplementation, which is in accordance with Maruta et al. (1996), who suggested that the *lactbacillus* supplementation could increase the faecal *lactobacillus* in pigs. Kaper et al. (2004) had previously suggested that the presence of *Lactobacillus* in the gastrointestinal tract is positively related to gut health. Therefore, our study confirmed that the *E. faecium DSM 713* could be used as a good probiotics in growing pigs. Moreover, the faecal noxious gas content was also reduced with the probiotics supplementation, which could also be explained by the increased nutrient digestibility and intestinal microbiotia ecosystem. This hypothesis is strength by Ferket et al. (2002), who suggested that probiotics could indirectly reduce environmental pollutants from animal manure by improving feed efficiency, nutrient retention and the intestinal microbiotia ecosystem. However, no difference was observed on the blood characteristic in the current study, which is in agreement with our previous study (Chen et al. 2006), which suggested that no difference was observed with probiotic supplementation in pigs. In contrast, Cho et al. (2005) suggested that red blood cell was improved with probiotics supplementation. The reason for the difference is unknown; therefore, further study is still warrant to make a conclusion

Table 3. Effects of supplementation of high and low energy and nutrient density diets with probiotics on faecal microbial shedding and faecal noxious gas content in growing-finishing pigs.[a]

	High		Low			*P*-value		
Items	+P	−P	+P	−P	SE[b]	D	P	D × P
Faecal microbial shedding								
Lactobacillus	8.96	7.45	7.96	7.14	0.278	0.542	<0.001	0.021
E. coli	6.48	6.53	6.64	6.84	0.354	0.613	0.241	0.354
Faecal noxious gas content								
NH_3	702	827	658	705	21.6	0.021	0.016	0.024
H_2S	31.8	39.2	24.9	30.1	2.54	0.001	0.005	0.031

[a]A total of 144 pigs with an initial BW of 29.48 ± 0.58 kg. Each mean represents nine pens with two gilts and two barrows per pen. High: high energy and nutrient diet; ±0.1% probiotics (P); Low: low energy and nutrient diet ±0.1% P.
[b]Pooled standard error.

about the effect of probiotics on the blood characteristics.

4.2. Effect of nutrient density

In the present study, although the difference in energy, crude protein, and lysine level between high and low nutrient density diet was only 100 kal/kg, 1%, and 0.1%, respectively, dietary high nutrient density led to a higher ADG and G:F ratio than the low energy and nutrient density diet. In agreement with this study, Yan, Wang, et al. (2010) also reported that increased nutrient density improved ADG in the growing-finishing pigs. However, in that study, pigs fed a high energy and nutrient density diet had a reduced feed intake compared with the low nutrient density diet, which was not the case in the current study. Thus, we hypothesised that the higher ADG with the nutrient density diet could be attributed to the increased nutrient intake in the current study. Supportably, Beaulieu et al. (2009) and Stahly et al. (1981) also demonstrated that pigs were able to increase energy intake and consequently the growth performance when high energy diets were provided. Moreover, the inclusion of high nutrient density increased faecal noxious gas content such as ammonia nitrogen and hydrogen sulfide concentration, which is in agreement with Carter et al. (1996) and Yan, Wang, et al. (2010), who suggested that dietary high nutrient density diet could increase the faecal noxious gas emission in finishing pigs. In our study, no difference was observed on the feed intake and the nutrient digestibility. Therefore, we hypothesised that the reason for the high faecal noxious gas content is likely to be the high nutrient passed to the large intestine, which provide more substrate for faecal microbial fermentation.

It is evident that an interactive effect of probiotics supplementation and dietary nutrient density was observed on the growth performance and nutrient digestibility. Similarly, our previous study (Meng et al. 2010) also reported that the effect of probiotics on nutrient digestibility (N and energy) could be enhanced with higher nutrient density diet, and suggested that the reason is likely to be the positive effect of probiotics on the microflora balance in the gut. In this study, the interactive effects of probiotic and nutrient density diet were not only observed in G:F ratio and nutrient digestibility, but also the *lactobacillus* concentration. Therefore, our study confirmed that the reason for the interactive effect could be the increased microflora balance, which led to a better metabolism and transformation of feed into body mass. Moreover, the higher faecal noxious gas content resulted from high nutrient density decreased dramatically with the probiotics supplementation. Ferket et al. (2002) had suggested that faecal noxious gas content of animal is related to intestinal microflora in the gastrointestinal tract of pigs. Our previous study also suggested that the increased nutrient digestibility could be considered as a reason for the reduced faecal noxious gas content (Yan, Wang, et al. 2010; Yan et al. 2011). Therefore, the reason for the interactive effect on the faecal noxious gas content is likely to be the results of other interactive effects (nutrient digestibility and faecal *lactobacillus*) observed in the current study.

5. Conclusion

In conclusion, both dietary probiotics supplementation and a high nutrient diet could improve growth performance and nutrient digestibility in growing pigs. The beneficial effect of probiotics supplementation in pigs could be enhanced with the high energy and nutrient density diets.

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