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Effects of *Enterococcus faecium* DSM 7134 on weanling pigs were influenced by dietary energy and crude protein density

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ABSTRACT

This experiment was conducted to evaluate the effects of *Enterococcus faecium* DSM 7134 supplementation, in different energy and crude protein density diets, on the growth performance, macronutrient digestibility, blood profile, fecal microbiota, and fecal noxious gas content of weanling pigs. A total of 140 pigs, with an average BW of 6.1 ± 0.35 kg, were randomly allocated into four treatments, with two levels of *E. faecium* (0 or 1×10^9 cfu/kg of feed), and two levels of energy and crude protein density. The experiment lasted 28 d. There were seven replicate pens per treatment with five pigs per pen. During d 15 to 28 and overall, average daily gain and gain:feed ratio were increased in pigs fed the high density (HD) and *E. faecium* diet ($P < 0.05$). Pigs fed the diet with *E. faecium* showed greater apparent total tract digestibility (ATTD) of nitrogen on d 14 and 28 than those fed the non-supplemented diet ($P = 0.033$ and 0.030, respectively). The ATTD of energy in pigs fed the HD and *E. faecium* treatment was greater than in pigs fed the low density (LD) and non-*E. faecium* treatment ($P < 0.05$). Supplementation with *E. faecium* in the HD diet increased the serum IgG concentration compared with the LD and non- *E. faecium* treatment ($P = 0.039$). The fecal lactobacilli population was increased ($P = 0.009$ and 0.013, respectively), and fecal NH_3 content was decreased ($P = 0.013$ and 0.032, respectively) on d 14 and 28 in pigs fed the *E. faecium* supplemented diet. Fecal *E. coli* counts were decreased on d 28, with the application of *E. faecium* ($P = 0.024$). In conclusion, a HD diet containing 1×10^9 cfu *E. faecium* DSM 7134/kg of diet led to better growth performance and nutrient digestibility in weanling pigs.

1. Introduction

Weaning stress results in gastrointestinal dysfunction in piglets, thereby causing depressed performance, nutrient malabsorption, and a high incidence of diarrhea (Wang et al., 2012). The recent concern about antibiotic resistance has brought about large numbers of studies on antibiotic alternatives in animals (Simon et al., 2003).

The addition of probiotics, which are health promoting live organisms, to pig diets, has been shown to improve the performance and nutrient digestibility, as well as the intestinal ecosystem (Roselli et al., 2007; Choi et al., 2011). However, the effects of probiotics are genera, species, and strain specific. It has been demonstrated that administration of probiotics in high energy and protein density diets is more effective than in low density diets in growing and finishing pigs (Meng et al., 2010; Yan and Kim, 2013). Therefore, we hypothesize that the efficacy of *Enterococcus faecium* may be enhanced by feeding high density (HD) diet to weanling pigs.

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This study was conducted to evaluate the main and interactive effects of *E. faecium* DSM 7134 in weaning pigs feeding diets containing different energy and crude protein contents.

2. Materials and methods

2.1. Source of probiotic

The probiotic product (Bonvital®) used in our study was provided by a commercial company (Schaumann Agri International GmbH, Pinneberg, Germany). This product is composed of spray-dried spore-forming *E. faecium*, which is guaranteed to contain at least 1.0×10^{10} cfu/g of live *E. faecium* DSM 7134.

2.2. Experimental design, animals, housing and diets

The Animal Welfare Committee of Dankook University approved the animal care protocol used for this experiment.

A total of 140 weanling pigs [Yorkshire × Landrace × Duroc, 21 ± 1 day (d) of age] with an average body weight (BW) of 6.1 ± 0.35 kg were randomly assigned in a 2 × 2 factorial arrangement, with two levels of *E. faecium* (0 or 1×10^9 cfu/kg of feed), and two levels of energy and crude protein. The experiment lasted 28 d. There were seven replicate pens per treatment and five pigs per pen. The diets were fed in two phases, consisting of phase 1 from d 1 to 14, and phase 2 from d 15 to 28. Diets were formulated to provide nutrients to meet or exceed NRC (1998) requirements (Table 1). Feeds in 1 mL of PBS were serially diluted from 10^{-1} to 10^{-7}, and were plated on bile esculin azide agar plates in duplicates for 24 h at 37 °C. The HD and low density (LD) diets with no probiotic added had the following *E. faecium* counts: 5.43×10^4 and 4.96×10^4/kg of diet during d 1 to 14, and 5.17×10^4 and 4.87×10^4/kg of diet during d 15 to 28. The enterococci counts in *E. faecium* treatments were 1.54×10^9 and 1.28×10^9 cfu/kg in the HD diet and 1.37×10^9 and 1.45×10^9 cfu/kg in the LD diet, during d 1 to 14 and d 15 to 28, respectively.

Pigs were housed in a nursery facility with slatted plastic flooring, and pens were separated by wood boards with a space to avoid pigs having physical contact between pens. The temperature of the room was maintained at 30 ± 1 °C for the first wk, after which the temperature was gradually reduced by 1 °C per wk. Pens were provided with a stainless steel feeder and one nipple drinker, which allowed *ad libitum* access to feed and water throughout the experiment. The diets were presented in mash form.

2.3. Growth performance and digestibility

Individual BW was determined at the beginning of the experiment, on d 14 and 28 of the experiment, and feed consumption was recorded on pen basis to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F).

From d 8 to 14 and d 22 to 28, chromium oxide was added to the diets at 2 g/kg, as an indigestible marker for determination of the apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and gross energy (Ball and Aherne, 1987). On d 12, 13, 14 and d 26, 27, 28 of the experiment, fecal samples were collected from all pigs in each pen in the afternoon via rectal massage and pooled within pen. Fecal samples (7 samples per treatment) and feed samples were stored in a freezer at –20 °C until further analysis. For chemical analysis, fecal and feed samples were freeze-dried, and ground to pass through a 1–mm screen, after which they were analyzed for DM, N, Ca, and P (AOAC, 1995, 2000). Individual amino acid composition was measured using an Amino Acids Analyzer (Beckman 6300; Beckman Coulter, Inc., Fullerton, CA) after 24-h hydrolysis in HCl. Nitrogen was measured using a Kjeltec 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Gross energy was determined using a Parr 6100 Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL, US).

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

Ingredients, g/kg	d 0 to 14		d 15 to 28	
	HD	LD	HD	LD
Expanded maize	291.6	348.0	388.3	445.8
Soybean meal, 440 g/kg CP	176.1	152.5	355.8	332.0
Fish meal, 620 g/kg CP	50.0	50.0	40.0	40.0
Soy oil	52.6	20.5	53.2	19.4
Dried whey	166.8	165.0	101.2	100.0
Biscuit meal[b]	130.0	130.0	20.0	20.0
Monocalcium phosphate	11.8	12.5	9.2	10.0
Sucrose	40.0	40.0	20.0	20.0
Plasma powder	65.0	65.0	–	–
L-lysine-HCl, 780 g/kg	1.0	1.2	2.2	2.5
DL-Methionine, 500 g/kg	2.5	2.6	1.4	1.5
L-Threonine, 890 g/kg	7.6	7.7	0.7	0.8
Choline chloride, 250 g/kg	2.0	2.0	1.0	1.0
Vitamin premix[c]	1.0	1.0	1.0	1.0
Mineral premix[d]	2.0	2.0	2.0	2.0
Limestone	–	–	2.0	2.0
Salt	–	–	2.0	2.0
Calculated composition, g/kg				
ME, MJ/kg	14.9	14.4	14.4	13.9
Analyzed composition, g/kg				
GE, MJ/kg	18.9	18.4	18.6	18.1
Crude protein	206.8	196.3	186.2	176.7
Crude fat	95.4	67.3	80.9	49.7
Lysine	15.3	14.4	13.3	12.7
Methionine	6.1	5.5	5.0	4.3
Calcium	9.0	9.1	8.1	7.9
Phosphorus	7.9	7.8	6.9	6.9

[a] Abbreviations: HD=high energy and crude protein density diet; LD=low energy and crude protein density diet. The HD or LD diet was supplemented with 0 or 1×10^9 cfu *E. faecium* DSM 7134/kg of diet.

[b] Biscuit meal contained 210 g/kg crude fat and 220 g/kg CP.

[c] Provided per kg of complete diet: retinyl acetate, 12,500 IU; cholecalciferol, 1,050 IU; DL-a-tocophery acetate, 40 IU; menadione sodium bisulfate, 4 mg; riboflavin, 8 mg; niacin, 46 mg; thiamine, D -calcium pantothenate, 25 mg; choline chloride, 160 mg; and cyanocobalamin, 30 μg.

[d] Provided per kg of complete diet: Fe (as $FeSO_4 \bullet 7H_2O$), 75 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 11 mg; Zn (as $ZnSO_4 \cdot H_2O$), 100 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.25 mg; Se (as $NaSeO_3 \cdot 5H_2O$), 0.15 mg.

2.4. Blood profiles

On d 1 of the experiment, blood samples were collected from two pigs per pen by jugular venipuncture using a sterilized syringe and non-heparinized tubes and K3EDTA

vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, US). On d 28 of the experiment, blood samples were collected from the same pigs. The concentration of white blood cells (WBC), red blood cells (RBC) and lymphocytes in the whole blood samples were determined using an Automatic Blood Analyzer (ADVIA 120, Bayer, Tarrytown, NY, US). Whole blood samples were subsequently centrifuged for 15 min at 3000 × g at 4 °C to separate the serum, and then to determine serum IgG concentration. Serum IgG was determined using a Nephelometry (Dade Behring, Marburg, Germany).

2.5. Fecal microbiota

On d 28 of the experiment, fecal samples were collected by rectal massage from all pigs from each pen, then pooled, and transported to the lab for immediate analysis. One gram of the composite fecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, US), and then homogenized. Viable counts of bacteria in fecal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates and lactobacilli spp. medium III agar plates to isolate the *Escherichia coli* and lactobacilli, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39 °C under anaerobic conditions. MacConkey agar plates were incubated for 24 h at 37 °C. *E. coli* and lactobacilli colonies were counted immediately after removal from the incubator.

2.6. Fecal noxious gas contents

On d 28 of the experiment, fecal NH_3 and H_2S concentrations were determined using the methods described by Zhang and Kim (2013). A total of 300 g fresh fecal samples were collected from at least two pigs in each pen, then transferred to a 2.6-L sealed plastic box and fermented for 48 h at 32 °C. After the fermentation period, a Gas Detector (GV-100S; Gastec Corp., Kanagawa, Japan) was utilized for gas detection. In these measurements, the plastic boxes were punctured, and headspace air was sampled approximately 2 cm above the samples at a rate of 100 mL/min. Concentrations of NH_3 and H_2S were measured within the scope of 5 to 100 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2 to 20 ppm (No. 4LL, detector tube; Gastec Corp. Kanagawa, Japan).

2.7. Statistical analyses

All data were analyzed by ANOVA (SAS Inst. Inc., Cary, NC, US) using a 2 × 2 factorial arrangement of treatments with the pen being considered as the experimental unit. When the ANOVA revealed significances, Tukey's test was performed to establish where means differed. The final model included the main effects of probiotic and dietary energy and nutrient density, as well as their interactive effects. Data variability was expressed as standard error of the means (SEM). Alpha level for determination of significance was 0.05.

3. Results

In the present study, no diarrhea episode was found, and no pig died during the experiment (data not shown). During d 15 to 28 and overall, pigs fed the HD and *E. faecium* supplemented diet had greater ADG and G:F than pigs fed any other diet ($P < 0.05$, Table 2). The daily dose of *E. faecium* product per pig was calculated as follows ($\times 10^9$ cfu/pig): 0.81, 1.20, and 1.01 in the HD, and 0.70, 1.30, and 1.00 in the LD during d 1 to 14, d 15 to 28, and overall. There was no difference in ADFI throughout the experiment.

No difference was observed in the ATTD of DM among treatments during the experiment (Table 3). Pigs fed the diet supplemented with *E. faecium* had greater ATTD of N than pigs fed the non-supplemental diet on d 14 and 28 ($P=0.033$ and 0.030, respectively). The ATTD of energy was increased on d 28 in pigs fed the HD and *E. faecium* diet than in pigs fed any other diet ($P < 0.05$).

Supplementation of *E. faecium* in the HD diet led to a greater serum IgG concentration than pigs fed the LD and non-supplemental diet (232 ± 11 vs. 216 ± 8 mg/dL, $P=0.039$). No difference was observed in WBC, RBC and lymphocyte levels among dietary treatments (data not shown).

Fecal microbial shedding of lactobacilli was increased in the HD and *E. faecium* treatment compared with pigs fed any other diets on d 14 and 28 ($P < 0.05$, Table 4). Fecal *E. coli* counts were decreased by *E. faecium* administration on d 28 ($P=0.024$). Pigs fed the diets supplemented with *E. faecium* had lower fecal NH_3 content compared with pigs fed the non-*E. faecium* diet on d 14 and 28 ($P=0.013$ and 0.032, respectively). Fecal H_2S concentration was not affected by dietary energy and crude protein density and *E. faecium*.

4. Discussion

In the present study, we increased the dietary metabolizable energy (0.5 MJ/kg of diet) and crude protein level by substitution of expanded maize mainly with soybean meal and soy oil, but maintain the calorie to Lys ratio. Dietary addition of fat and increasing nutrient concentration has been reported to improve ADG and feed efficiency (Jones et al., 1992; Song et al., 2003; Hastad et al., 2005). We found that pigs fed the HD diet with *E. faecium* had greater ADG and G:F than those fed other diets. The result was in agreement with Lojanica et al. (2010), who reported that dietary *E. faecium* DSM 7134 increased ADG, and decreased the feed conversion ratio compared with control treatment in weaned pigs. Similar results were also reported by Guerra et al. (2007) and Mallo et al. (2010). In this study, the digestibility of N and energy was improved in pigs fed the *E. faecium* supplemented and HD diet, which was consistent with the results reported by Lee et al. (2009). In a study conducted by Yan et al. (2009) who reported that growing-finishing pigs fed the high energy density diet had greater ADG and G:F associated with improved feed intake and digestibility compared with low energy density diet. We also found that the ADFI in pigs fed the HD and *E. faecium* diet was numerically higher than those fed the LD and non-*E. faecium* diet. Therefore, the increased digestibility and tendency towards higher feed intake in pigs

Table 2
The effect of supplementation of *E. faecium* DSM 7134 in different energy and crude protein density diets on growth performance in weanling pigs[a].

Item	HD		LD		SEM[b]	P-values		
	−Pro	+Pro	−Pro	+Pro		Density	Pro	Density × Pro
d 1 to 14								
ADG, g	355	386	349	360	13	0.254	0.139	0.423
ADFI, g	503	528	501	512	17	0.734	0.305	0.691
G:F	0.71	0.73	0.70	0.70	0.02	0.243	0.376	0.501
d 15 to 28								
ADG, g	491[y]	570[x]	474[y]	481[y]	13	0.004	0.007	0.015
ADFI, g	898	939	875	897	23	0.174	0.221	0.663
G:F	0.55[y]	0.61[x]	0.54[y]	0.54[y]	0.02	0.045	0.127	0.044
d 1 to 28								
ADG, g	423[y]	478[x]	412[y]	421[y]	10	0.012	0.009	0.039
ADFI, g	701	734	688	705	16	0.191	0.125	0.619
G:F	0.60[y]	0.65[x]	0.60[y]	0.60[y]	0.01	0.021	0.047	0.031

[a]Abbreviations: HD=high energy and nutrient density diet; LD=low energy and nutrient density diet. The HD or LD diet was supplemented with 0 (−) or 1×10^9 (+) cfu *E. faecium* DSM 7134/kg of diet.
[b] Standard error of the means ($n=7$).
[x,y] Means in the same row with different superscripts differ ($P < 0.05$).

Table 3
The effect of supplementation of *E. faecium* DSM 7134 in different energy and crude protein density diets on nutrient digestibility in weanling pigs[a].

Item	HD		LD		SEM[b]	P-values		
	−Pro	+Pro	−Pro	+Pro		Density	Pro	Density × Pro
d 14								
DM, %	80.8	82.7	79.8	81.9	1.3	0.454	0.121	0.931
N, %	77.2	80.5	77.3	78.5	1.0	0.401	0.033	0.403
Energy, %	77.8	82.1	77.6	78.0	1.5	0.211	0.140	0.232
d 28								
DM, %	81.0	83.0	80.1	81.9	1.6	0.542	0.245	0.939
N, %	78.0	81.5	77.7	79.7	1.2	0.386	0.030	0.526
Energy, %	78.0[y]	83.2[x]	78.0[y]	78.4[y]	1.1	0.039	0.028	0.047

[a] Abbreviations: HD=high energy and nutrient density diet; LD=low energy and nutrient density diet. The HD or LD diet was supplemented with 0 (−) or 1×10^9 (+) cfu *E. faecium* DSM 7134/kg of diet.
[b] Standard error of the means ($n=7$).
[x,y] Means in the same row with different superscripts differ ($P < 0.05$).

fed the HD and *E. faecium* diet may explain the improved growth performance in this study (van Heugten et al., 1996). In contrast, Broom et al. (2006) reported that 1.4×10^9 cfu *E. faecium* SF68/kg of diet did not affect growth performance in piglets aged from d 26 to 46. The inconsistency may be caused by the different age of animals, strain of bacteria, and supplementation level.

Černauskienė et al. (2011) suggested that *E. faecium* is a normal microorganism in swine intestine, which could produce lactic acid to reduce the pH value of the intestinal content, and inhibit the development of invasive pathogens. In this study, a higher energy digestibility and greater lactobacilli count were observed in the *E. faecium* supplemented treatments with the HD diet. In agreement with our study, Mallo et al. (2010) reported that 10^6 cfu *E. faecium* CECT4515/g of diet increased the lactobacilli in the ileum, caecum and feces, and reduced the coliforms. Meng et al. (2010) also reported that the inclusion of probiotics in a HD diet was more effective than in a LD diet. They suggested that the improved intestinal microbial balance may increase the total metabolism of energy and nutrients, thus improving the conversion of feed to body mass. Verse and Marteau (2007) reported that microflora in the gastrointestinal tract plays a crucial role in anatomical, physiological and immunological organ development of the host animals. Therefore, the improved ecosystem (increased lactobacilli and decreased *E. coli*) might be another reason for the better growth performance and nutrient digestibility. Moreover, in the present study,

Table 4
The effect of supplementation of *E. faecium* DSM 7134 in different energy and crude protein density diet on fecal microbiota and noxious gas contents in weanling pigs[a].

Item	HD		LD		SEM[b]	P-values		
	−Pro	+Pro	−Pro	+Pro		Density	Pro	Density × Pro
lactobacilli, $\log_{10}$ cfu/g								
d 14	7.2[y]	7.8[x]	7.3[y]	7.4[y]	0.1	0.377	0.009	0.038
d 28	7.4[y]	7.9[x]	7.4[y]	7.5[y]	0.1	0.124	0.013	0.044
E. coli, $\log_{10}$ cfu/g								
d 14	6.2	6.1	6.2	6.2	0.1	0.667	0.530	0.768
d 28	6.2	6.0	6.3	6.0	0.1	0.301	0.024	0.512
NH_3, ppm								
d 14	28.8	18.8	32.0	19.0	3.5	0.673	0.013	0.689
d 28	27.2	20.4	34.0	22.8	3.3	0.195	0.032	0.587
H_2S, ppm								
d 14	7.8	5.6	7.0	5.8	1.8	0.832	0.336	0.801
d 28	3.4	4.2	5.8	4.8	1.0	0.181	0.976	0.402

[a]Abbreviations: HD=high energy and nutrient density diet; LD=low energy and nutrient density diet. The HD or LD diet was supplemented with 0 (−) or 1×10^9 (+) cfu *E. faecium* DSM 7134/kg of diet.
[b]Standard error of the means ($n=7$).
[x,y]Means in the same row with different superscripts differ ($P < 0.05$).

the serum IgG concentration was increased by *E. faecium* supplementation, which might suggest that *E. faecium* had an immune-stimulating effect in weanling pigs. In contrast, Scharek et al. (2005) observed a reduction in total serum IgG of the *E. faecium* SF68-treated piglets aged from 5 to 8 wk. The reason for the inconsistency is not clear.

In addition, in this study, fecal NH_3 contents were reduced by *E. faecium* administration. Similarly, Lee et al. (2009) found that fecal NH_3 was decreased in weanling pigs fed 0.2% probiotics compared with those fed 0.15 % antibiotics. It is well documented that the fecal noxious gas from animal manure is closely related to the feed efficiency, nutrient utilization and intestinal microbiota (Ferket et al., 2002). Therefore, the decreased fecal NH_3 content was probably due to the increased N digestibility and lactobacilli counts.

5. Conclusions

In conclusion, the use of 1×10^9 cfu/kg of *E. faecium* DSM 7134 in the HD diet improved growth performance, nutrient digestibility, increased fecal lactobacilli number, and decreased fecal NH_3 content in weanling pigs.

Conflict of interest

There was no conflict of interest. Thank you for your hard work and wish you always happy.

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