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Short communication

Effects of *Enterococcus faecium* DSM 7134 supplementation in different energy and crude protein density diets on ileal amino acid digestibility and intestinal shedding of lactobacilli and *Escherichia coli* in finishing pigs

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ABSTRACT

This experiment was conducted to determine the efficacy of *Enterococcus faecium* DSM 7134, in different energy and crude protein density diets on ileal amino acid digestibility and intestinal microbial shedding in finishing pigs. Twelve finishing barrows (initial body weight = 75.1 ± 3.44 kg) were surgically equipped with a T-cannula in the distal ileum and arranged in a triplicate 4 × 4 Latin square design with four diets and four periods. Diets were formulated with two levels of nutrient density (high energy: 14.6 MJ/kg ME or low energy: 14.2 MJ/kg ME) and two levels of probiotic (0 or 1.0×10^9 cfu/kg diet). The coefficient of apparent ileal digestibility (CAID) of crude protein (CP), gross energy (GE), and most of the indispensable amino acids (AA) except for His-Phe and Val were increased ($P<0.05$) by 5.3%, 6.0%, and 5.2–7.2% in pigs fed the *E. faecium* compared with non-supplemented groups. The CAID of Cys and Gly was 6.2% and 7.0% greater ($P<0.05$) in *E. faecium* groups than non-supplemental groups. Ileal and fecal *Lactobacilli* counts were increased ($P<0.05$) by 19.9% and 16.6%, and *Escherichia coli* counts were decreased ($P<0.05$) by 8.9% and 9.5% in pigs fed the diets supplemented with probiotic, respectively. The CAID of CP, Lys-Met-Thr-Leu-Ile-Cys-Gly as well as the lactobacilli counts were increased in both *E. faecium* treatments, but values were increased higher in the high density diet. In conclusion, dietary supplementation with 1.0×10^9 cfu/kg of *E. faecium* DSM 7134 exerted beneficial effects on the CAID of CP and AA and intestinal lactobacilli shedding, especially was administrated in high energy and crude protein density diets.

1. Introduction

The decreased use of antibiotics as growth promoters but also as curative and the necessity to have efficient pig production, probiotics have been suggested as desirable alternatives to benefit the pigs by improving the gut health (Fuller, 1989).

It has been reported that dietary supplementation with lactic acid bacteria based probiotics could improve the growth performance, nutrient digestibility and intestinal microbial balance (Guerra et al., 2007; Giang et al., 2010; Choi et al., 2011).

Abbreviation: AA, amino acids; CAID, coefficient of apparent ileal digestibility; CP, crude protein; GE, gross energy.

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Table 1
Ingredient composition and chemical analysis of the experimental diets (g/kg as-fed basis unless stated otherwise).[a]

Item	High density diet	Low density diet
Ingredients		
Corn	643.5	700.7
Soybean meal	252.7	220.7
Corn gluten	30.0	30.0
Soybean oil	52.7	27.6
Limestone	7.1	7.0
Salt	2.0	2.0
Dicalcium phosphate	6.2	6.5
L-Lys–HCl, 780 g/kg	0.8	0.5
Vitamin premix[a]	1.5	1.5
Mineral premix[b]	1.5	1.5
Chromic oxide	2.0	2.0
Calculated composition		
ME (MJ/kg)	14.6	14.2
Analyzed composition		
GE (MJ/kg)	17.7	17.3
CP	162.1	150.1
Lys	10.3	9.2
Ca	5.6	5.5
Total P	5.1	4.8

[a] Provided per kilogram of diet: 4000 IU of vitamin A; 900 IU of vitamin D_3; 19 IU of vitamin E; 3 mg of vitamin K_3; 20 mg of vitamin B_2; 6 mg of vitamin B_6; 16 µg of vitamin B_{12}; 20 mg of niacin; 15 mg of pantothenic acid, and 0.04 mg of biotin.
[b] Provided per kilogram diet: 15 mg of Cu; 90 mg of Fe; 80 mg of Zn; 35 mg of Mn; 0.3 mg of I; 0.25 mg of Co, and 0.2 mg of Se.

However, different results were reported because of different probiotic species, viability, dosage, application method and feed composition (Ghadban, 2002).

Meng et al. (2010) reported that the addition of *Bacillus* based probiotics in high energy and nutrient density diets (HD) was more favorable than in low energy and nutrient density diets (LD). Yan and Kim (2013) demonstrated that dietary administration of *Enterococcus faecium* DSM 7134 improved total tract nutrient digestibility and intestinal microbial balance of growing-finishing pigs, and the value was increased greater in the HD treatment. Zhang and Kim (2013) also reported that the use of *E. faecium* in the HD was more favorable than the LD in laying hens. Therefore, we hypothesize that the effect of *E. faecium* on ileal amino acids (AA) digestibility may be influenced by feeding finishing pigs with different energy and nutrient density diets.

This study was conducted to determine the effects of *E. faecium* DSM 7134 in different energy and crude protein density diets on ileal digestibility and intestinal microbiota in finishing pigs.

2. Materials and methods

2.1. Source of *E. faecium*

The probiotic product (Bonvital, Schaumann Agri International GmbH, Pinneberg, Germany) used was guaranteed to compose of a minimum of 1.0×10^{10} cfu/g spray-dried spore-forming *E. faecium* DSM 7134. In *E. faecium* treatments, the product was supplemented in a powder form (0.1 g/kg of feed). One gram of each feed was serially diluted from 10^{-1} to 10^{-7} with 1 mL of PBS and then mixed with stomacher (Seward Stomacher® 400 Circulator, West Sussex, UK). Dilutions were plated on bile esculin azide agar in duplicates for 24 h at 37 °C. The enterococci counts were 1.24×10^9 and 1.16×10^9 cfu/g in HD and LD, with the application of the *E. faecium* product, respectively.

2.2. Animal and experimental design

The Animal Care and Use Committee of Dankook University (Cheonan, South Korea) reviewed and approved the experiment. Twelve finishing barrows (Landrace × Yorkshire × Duroc) with the same age and an average initial body weight (BW) of 75.1 ± 3.44 kg were surgically equipped with a T-cannula in the distal ileum using procedures as described by Stein et al. (2007). After the surgery, pigs were raised individually in metabolic cages in an environmentally controlled house for 14 days before the experiment started. Room temperature and humidity were maintained at 21 °C and 60%, respectively. Pigs were allotted to a triplicate 4 × 4 Latin square design with four diets and four periods. Each experimental period lasted 14 days. Diets were fed at 08:00 and 20:00 h at a level of three times the estimated energy requirement for maintenance (*i.e.*, 443.50 KJ ME/kg of $BW^{0.75}$; NRC, 1998) in two equal meals. Water was provided *ad libitum* from nipple drinkers.

2.3. Experimental diets and sample collection

Four diets (corn-soybean meal-based) were prepared (Table 1) and formulated with two levels of energy and nutrient density (14.6 or 14.2 MJ ME/kg) and two levels of *E. faecium* (0 or 1.0×10^9 cfu/kg diet). These diets were fed in a meal form. Vitamins and minerals were included at concentrations to meet or exceed the levels recommended by NRC (1998) for finishing pigs, and 2 g/kg chromic oxide was included in all diets as an indigestible marker.

Individual pig BW was recorded at the beginning of each experimental period, and the daily feed allowance was adjusted at the beginning of each new feeding period according to the recorded BW. After a 12-day adaptation to the diet, ileal digesta samples were collected from 08:00 to 18:00 h on Day 13 and 14 (Stein et al., 1999). A plastic bag (225 mL) was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected (Wang et al., 2013). Bags were removed as filled with digesta or at least every 30 min, and immediately stored at −20 °C to prevent bacterial degradation of AA in the digesta.

2.4. Chemical analyses

At the end of the experiment, ileal samples were pooled within pig and period for chemical analysis. Ileal digesta samples were lyophilized. All samples were finely ground before chemical analysis. Individual AA composition was measured using an AA Analyzer (Beckman 6300, Beckman Coulter, Inc., Fullerton, California, USA) after 24-h 6 N-HCl hydrolysis at 110 °C (AOAC, 2005). Performic acid was used before hydrolysis to oxidize methionine and cystine to methionine sulfone and cysteic acid. Nitrogen was determined by a Kjectec 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as nitrogen × 6.25. Gross energy (GE) was determined by using a Parr 6100 Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, Illinois, USA). Chromium was analyzed according to Williams et al. (1962). The coefficients of apparent ileal digestibility (CAID) were calculated as described by Stein et al. (2007).

For lactobacilli and *Escherichia coli* counts analysis, ileal digesta were sampled, and fresh feces were taken directly via massaging the rectum from each pig at Day 14 of each period, and placed on ice for transportation to the laboratory, where the analysis was immediately carried out. The composite sample (1 g) from each pig was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in the samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *E. coli* and lactobacilli, respectively. The lactobacilli medium III agar plates were incubated for 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C. The *E. coli* and lactobacilli colonies were counted immediately after removal from the incubator.

2.5. Statistical analysis

All data were analyzed for a triplicate 4 × 4 Latin square design using the GLM procedure (SAS Inst., Inc., Cary, NC). The main effects of diets ($n = 4$), pigs ($n = 12$), and experimental periods ($n = 4$) were included in the model. The effects of dietary energy and nutrient density, *E. faecium*, and interaction were analyzed by orthogonal contrasts (Hicks, 1973). Variability in the data is expressed as the standard error means and alpha level used for determination of significance was 0.05.

3. Results

During the experiment, all pigs were healthy, no feed refusal was observed, and all the diets were consumed in 20 min. The CAID of CP, GE, and most of the indispensable AA except for His-Phe and Val were increased ($P<0.05$) in pigs fed the *E. faecium* diets compared with non-supplemented groups (Table 2). The CAID of Cys and Gly were greater ($P<0.05$) in *E. faecium* groups than non-supplemented treatments. Ileal and fecal lactobacilli counts were increased ($P<0.05$), and *E. coli* numbers were decreased ($P<0.05$) in pigs fed the diets supplemented with *E. faecium* compared with those fed the non-supplemental diets (Table 3). Moreover, interactive effects between density and *E. faecium* were observed on the CAID of CP, Lys-Met-Thr-Leu-Ile-Cys-Gly and lactobacilli counts. Dietary *E. faecium* increased the values greater in the HD treatment compared with the LD treatment.

4. Discussion

In our study, dietary *E. faecium* supplementation increased the CAID of CP and energy by 5.3–6.0%, which was in agreement with Yan and Kim (2013) who reported that the total tract apparent digestibility was increased by administration of the same probiotic (*E. faecium* DSM 7134, 2.0×10^9 cfu/kg diet) in finishing pig. Chen et al. (2006) also found that dietary *E. faecium* SF68 (0, 0.175, and 0.350×10^9 cfu/kg diet) linearly increased the apparent total tract digestibility of dry matter and nitrogen in finishing pigs. In the current study, the CAID of Cys-Gly and most essential AA were increased by 5.2–7.2% in *E. faecium* groups. No study was conducted to determine the effect of probiotics on the ileal AA digestibility in finishing pigs. However, Li et al. (2008) reported that dietary supplementation with 0.2–0.6% probiotics increased the CAID of most AA by 6–11% in broilers. *E. faecium* could produce lactic acid to reduce the intestinal pH value, thus improving animal health and nutrient

Table 2
Effects of supplementation of *Enterococcus faecium* DSM 7134 in different energy density diets on coefficient of apparent ileal digestibility of crude protein, energy and amino acids in finishing pigs.[a]

Item	HD		LD		SEM[b]	P-value		
	−Pro	+Pro	−Pro	+Pro		Density	Pro	Density × Pro
CP	0.763	0.812	0.754	0.785	0.019	0.178	<0.001	0.014
GE	0.761	0.809	0.762	0.806	0.018	0.699	0.009	0.757
Indispensable AA								
Arg	0.862	0.924	0.854	0.916	0.019	0.682	0.013	0.725
His	0.823	0.865	0.819	0.838	0.019	0.721	0.417	0.825
Ile	0.786	0.839	0.765	0.813	0.017	0.883	0.018	0.037
Leu	0.802	0.862	0.797	0.839	0.017	0.322	0.024	0.042
Lys	0.783	0.835	0.777	0.806	0.015	0.485	0.015	0.028
Met	0.762	0.813	0.753	0.784	0.015	0.559	0.038	0.036
Phe	0.792	0.829	0.788	0.804	0.021	0.283	0.187	0.355
Thr	0.688	0.745	0.679	0.703	0.013	0.995	0.027	0.047
Val	0.754	0.789	0.745	0.764	0.019	0.457	0.523	0.781
Dispensable AA								
Ala	0.771	0.789	0.767	0.779	0.022	0.568	0.785	0.874
Asp	0.765	0.782	0.758	0.772	0.018	0.622	0.705	0.478
Cys	0.710	0.768	0.703	0.732	0.019	0.775	0.026	0.021
Glu	0.844	0.859	0.837	0.848	0.015	0.685	0.791	0.725
Gly	0.605	0.678	0.634	0.648	0.016	0.996	0.022	0.045
Pro	0.823	0.846	0.812	0.839	0.021	0.623	0.632	0.541
Ser	0.778	0.801	0.768	0.783	0.017	0.358	0.254	0.325
Tyr	0.793	0.825	0.784	0.813	0.024	0.257	0.189	0.478

[a] HD, high energy and nutrient density diet; LD, low energy and nutrient density diet; –Pro, diets without *E. faecium*; +Pro, diet with 1×10^9 cfu *E. faecium*/kg of diet; CP, crude protein; GE, gross energy; AA, amino acids.
[b] Standard error of the mean. Each mean represents 12 observations per treatment.

Table 3
Effects of supplementation of *Enterococcus faecium* DSM 7134 in different energy density diets on ileal and fecal microbiota in finishing pigs.[a]

Item, $\log_{10}$cfu/g	HD		LD		SEM[b]	P-value		
	−Pro	+Pro	−Pro	+Pro		Density	Pro	Density × Pro
Lactobacillus								
Ileal	6.42	7.86	6.05	7.09	0.234	0.785	0.006	0.025
Fecal	7.34	8.94	7.25	8.07	0.192	0.673	0.002	0.013
E. coli								
Ileal	4.86	4.43	4.74	4.32	0.125	0.923	0.021	0.256
Fecal	6.13	5.77	6.25	5.43	0.141	0.523	0.013	0.473

[a] HD, high energy and nutrient density diet; LD, low energy and nutrient density diet; –Pro, diets without *E. faecium*; +Pro, diet with 1×10^9 cfu *E. faecium*/kg of diet; CP, crude protein; GE, gross energy; AA, amino acids.
[b] Standard error of the mean. Each mean represents 12 observations per treatment.

digestibility (Cernauskiene et al., 2011). Djouzi et al. (1997) reported that the intestinal microbial balance of animals was improved by lactobacilli based probiotic. In agreement with their results, the ileal and fecal lactobacilli counts of pigs fed the *E. faecium* diet were increased by 19.9% and 16.6%, and *E. coli* counts were decreased by 8.9% and 9.5%, respectively. Similar results were also reported by other studies (Maruta et al., 1996; Yan and Kim, 2013). The increased lactobacilli may help to restore or maintain a beneficial intestinal community (Broom et al., 2006). Therefore, the improved CAID of energy, CP and AA in our study was probably due to the increased intestinal lactobacilli number and decreased *E. coli* population.

Meng et al. (2010) reported that the effects of probiotics on nitrogen and energy digestibility could be enhanced in a high nutrient density diet than in a low density diet in growing-finishing pigs. In the present study, the administration of *E. faecium* increased the CAID of CP and AA in both treatments, but values were increased higher in the HD compared with the LD. This may be due to the positive effect of *E. faecium* on the intestinal microbial balance was enhanced in the HD, which led to an improved utilization of nutrients compared with the LD (Meng et al., 2010). In agreement with this study, Yan and Kim (2013) reported that feeding HD to growing-finishing pigs enhanced positive effects of *E. faecium* on nutrient digestibility and fecal microbial balance. They also suggested that the improved microbial balance could increase the conversion of feed to body mass, thus increasing the total metabolism of energy and nutrients. Therefore, the current study confirmed that the interactive effect could be the results of improved nutrient digestibility and increased intestinal lactobacilli counts.

5. Conclusions

In conclusion, dietary supplementation with 1.0×10^9 cfu *E. faecium* DSM 7134/kg of diet exerted beneficial effects on the CAID of CP and AA and intestinal health, especially was administrated in a high energy and crude protein density diet.

Conflict of interests

The authors declared that they have no conflict of interest.

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