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Effects of *Enterococcus faecium* SLB 130 probiotic on the performance of weaning pigs

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Abstract: This study aims to analyse the effect of an *Enterococcus faecium* SLB 130 probiotic on the growth performance, nutrient digestibility, and blood profile in weaning pigs. A total of 200 weaning pigs were taken and assigned to 1 of 5 dietary treatments according to their average initial body weight and sex in a complete randomised block design. The experimental diets were as follows: CON – basal diet, and the basal diet supplemented with 2.5×10^5 cfu/g (TRT1), 1.29×10^6 cfu/g (TRT2), 1.15×10^7 cfu/g (TRT3), and 1.1×10^8 cfu/g (TRT4) of *E. faecium* for 6 weeks. Pigs fed a diet containing an *E. faecium* SLB 130 probiotic supplement significantly increased ($P < 0.05$) the body weight, average daily gain, and average daily feed intake at weeks 1, 3, 6, and the overall period. In addition, *the E. faecium* SLB 130 ($P < 0.05$) supplement group pigs showed an increased gain to feed ratio at week 6 and the overall experimental period. Moreover, the dietary inclusion of the *E. faecium* SLB 130 probiotic supplement linearly increased ($P < 0.05$) the nutrient digestibility of the dry matter and nitrogen, however, there were no improvements observed on weanling pigs' blood profile. In summary, the inclusion of an *E. faecium* SLB 130 probiotic additive in the weanling pigs' diet would be beneficial to enhance their growth performance and nutrient digestibility.

Keywords: blood profile; growth performance; nutrient digestibility; weanling pigs

Early weaning is a viable way to achieve better breeding and economic benefits in the modern intensive swine industry (Patil et al. 2015). However, piglets are prone to numerous stressors at the early weaning stage, which include separation from their dam and introduction to new litter mates, changes in their feed from liquid to solid that generate a negative impact on their growth performance. Additionally, post-weaning diarrhoea and post-weaning mortality leads to huge economic losses in the swine production (Xiong et al. 2019). To overcome this escalating problem, antibiotics are widely used as a prophylactic measure and as a growth promoter in livestock feedstuffs (Woods 2011). However, the overuse of certain antibiotics as growth promoters (AGPs) in animal feedstuff has culminated in antibiotic resistance issues and, thus, many countries including those in the European Union and South Korea have declared a moratorium on AGP usage in livestock feed since 2006 and 2011, respectively (Upadhaya et al. 2016). From that, several researchers' have tried to implement various additives to replace AGPs with organic acids, enzymes, and prebiotic and probiotic additives in their studies, among which, probiotics have received more attention and have become a considerable viable alternative in livestock feed due to their beneficial effects (Sampath et al. 2021).

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Probiotics, live microorganisms, have been extensively used in animal feed to enhance their growth and production performances (Balasubramanian et al. 2018). Recently, different probiotics strains have been explored and used with different efficacies; some of them were proven to provide certain benefits to the host while others have not (Mohana Devi and Kim 2014).

The most widely used probiotic strains in monogastric animals are lactic acid bacteria (LAB) including: *Enterococcus*, *Bacillus*, and *Lactobacillus* (Sato and Tanaka 1997). *Enterococcus faecium* (*E. faecium*), a gram-positive bacterium, belonging to the *Enterococcus* genus, is a kind of facultative anaerobic lactobacillus that can colonise the digestive tract of humans and animals (Nolasco-Hipolito et al. 2012). In 2012, the European Food Safety Authority (EFSA) approved the *E. faecium* 11181 probiotic strain (EF) as the best feed additive to improve the growth performance of animals (Pajarillo et al. 2015). Earlier studies addressed the fact that the *E. faecium* DSM 7134 supplement promotes the growth of weanling pigs by forming dominant microorganisms in their gastrointestinal tract. For instance: Pajarillo et al. (2015) reported that probiotics *E. faecium* NCIMB 11181 administration improved the growth performance and reduced the diarrhoea incidence in weaning pigs. Similarly, Zhao et al. (2018) proved that the inclusion of an *E. faecium* supplement enhanced the growth performance of pigs. Likewise, Mallo et al. (2010) stated that a dietary supplementation with 10^6 cfu/g of *E. faecium* increased the daily gain, feed efficacy, and the gut microbiota of weaning pigs. However, the research of Busing and Zeyner (2015) demonstrated that a dietary supplement with *E. faecium* had no effect on the body weight gain and feed intake or feed efficiency in piglets. Also, Broom et al. (2006) discovered that adding *E. faecium* to the post-weaning piglets' diet had no effect on the growth, and gastrointestinal bacterial populations. Ambiguous results from previous studies on pigs using *E. faecium* additives prompted us to initiate this research and we hypothesised that the inclusion of an *E. faecium* SLB 130 probiotic supplement would improve the performance of weaning pigs.

Hence, the focal aim of the study is to examine the effect of *E. faecium* SLB 130 on the growth performance, nutrient digestibility, and blood profile of weaning pigs.

MATERIAL AND METHODS

The experimental protocol describing weaning care and management was well reviewed and approved by the institutional ethics committee of Dankook University, Cheonan, the Republic of Korea (Approval No.: DK-2-2038).

One sterile loop *Enterococcus faecium* SLB 130 stored in liquid nitrogen was inoculated on a de Man, Rogosa and Sharpe (MRS) agar (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) medium and was cultured for 24 h at 37 °C in an incubator (JS Research, Gongju-si, Republic of Korea), later, the culture was inoculated into 200 ml of sterilised MRS broth for 24 h of incubation at 37 °C. For each step, the number of bacteria and contamination were checked in the MRS agar medium and a Nutrient Agar (MB-cell, Kisan Bio Co., Ltd., Seocho-gu, Seoul, Republic of Korea) medium. The final number of cultured bacteria was 5.0×10^8 cfu/ml. After incubation, the pH was 5.4 ± 0.2 and the final cultured 20-l MRS culture solution were incubated at 121 °C for 15 min, 8-l of soybean meal (Sajo; Seodaemun-gu, Seoul, Republic of Korea; 46% CP – crude protein) was mixed and dried in a dryer at 70 °C. Two kilograms (2 kg) of the first dried sample (1 000 ppm) was used as the 2.5×10^5 cfu/g (TRT1) sample. 6-l of the sterile culture solution was mixed with the remaining 6 kg of the 1 000-ppm sample and dried at 70 °C in a dryer. Two kilograms (2 kg) of the secondary dried sample was used as the 1.29×10^6 cfu/g (TRT2) sample. 4-l of the sterilised culture solution was mixed with the remaining 4 kg of the 2 000-ppm sample and dried in a dryer at 70 °C. Two kilograms (2 kg) of the 3rd dried sample was used as the 1.15×10^7 cfu/g (TRT3) samples. 2-l of the sterile culture solution was mixed with the remaining 2 kg of the 3 000-ppm sample and dried in a dryer at 70 °C. Two kilograms (2 kg) of the 4th dried sample (4 000 ppm) was used as the 1.1×10^8 cfu/g (TRT4) sample.

This study was carried out for six weeks at Dankook University in the experimental swine farm located at Jeonui, the province of Sejong (Republic of Korea). A total of two hundred weaning piglets [(Yorkshire × Landrace) × Duroc], 21 days old were assigned to 1 of 5 dietary groups according to their initial average body weight (6.48 ± 0.01 kg) and sex in a complete randomised block design. Each treatment groups contained 8 replicates with

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5 pigs per pen (2 females and 3 castrated males). The experimental diets were: corn-soybean meal based basal diet as the control (CON), and the basal diet supplemented with 2.5×10^5 cfu/g (TRT1), 1.29×10^6 cfu/g (TRT2), 1.15×10^7 cfu/g (TRT3), and 1.1×10^8 cfu/g (TRT4) of *E. faecium* were included in the pigs' diet during phase 1 (week 0–1), phase 2 (week 1–3), and phase 3 (week 3–6). The basal diets (Table 1) were formulated to meet the nutritional demand of weaning pigs as recommended by the National Research Council (NRC 2012). The piglets were offered a mash diet and water *ad libitum* through a feeder and nipple drinker, respectively. The piggery temperature was maintained at 32 °C during first week and lowered 1 °C each week until reaching 27 °C, which was maintained thereafter along with 60% humidity.

The individual body weight (b.w.) of the piglets were measured at the initial point and at the end of weeks 1, 3, and 6. During the experiment, the feed consumption and residue were weighed and recorded on a pen basis to monitor the average daily gain (ADG), average daily feed intake (ADFI) and gain feed ratio (G : F).

At the end of week 5, the experimental diets were mixed with 0.20% chromium oxide-III (Cr_2O_3) as an indigestible marker to determine the nutrient digestibility of the DM (dry matter), gross energy (GE), and nitrogen (N). At the end of week 6, fresh faecal samples were collected randomly (one barrow and one gilt) from each pen, and stored at –20 °C for further analysis. Prior to the chemical analysis, the feed and faecal samples were placed in a digital hot air convection drying oven (Model Rxh-27-C, Shanghai, P.R. China) for 36 h at 105 °C. Later, the dried samples were ground well (Knife Mill Blender Pulverisette 11; Laval Lab Equipment; Laval, Quebec, Canada) and sieved using a 1-mm screen sieve. The nutrient digestibility of the DM (method 930.15), N (method 984.13), and GE analysis were carried out according to the guidelines of the Association of Official Agricultural Chemists (AOAC 2003). The crude protein (Nx6.25) was analysed using a FOSS Tecator™ Kjeltec 8400 Nitrogen Analyser (Hoeganaes, Skåne, Sweden). The GE was determined using an adiabatic bomb calorimeter (Model 6400; Parr Instruments, Moline, IL, USA), which was calibrated using benzoic acid as a standard. The chromium content in the samples was determined using a UV-1201 Spectrophotometer (Shimadzu, Kyoto, Japan) and the results were recorded for the statistical analyses. The nutrient digestibility (ND) was calculated as:

Table 1. Composition of the weaning pig diets (as fed-basis)

Ingredients (%)	Phase 1 (week 0–1)	Phase 2 (week 1–3)	Phase 3 (week 3–6)
Corn	39.22	52.48	59.29
Soybean meal	16.42	16.60	22.46
Fermented soybean meal	5.00	4.00	3.00
Spray dried plasma protein	6.00	3.00	–
Tallow	2.49	2.53	2.49
Lactose	13.46	7.78	3.18
Sugar	3.00	3.00	3.00
Whey protein	11.00	7.00	3.00
Monocalcium phosphate	0.90	1.08	1.14
Limestone	1.17	1.20	1.22
Salt	0.20	0.10	0.10
Methionine (99%)	0.22	0.15	0.08
Lysine	0.49	0.65	0.61
Mineral mix[a]	0.20	0.20	0.20
Vitamin mix[b]	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03
Total	100.00	100.00	100.00
Calculated value			
Crude protein (%)	20.00	18.00	18.00
Ca (%)	0.80	0.80	0.80
P (%)	0.60	0.60	0.60
Lysine (%)	1.60	1.50	1.40
Methionine (%)	0.48	0.40	0.35
Metabolizable energy (kcal/kg)	3 450	3 400	3 350
Fatty acid transferase (%)	4.18	4.65	4.89
Lactose (%)	20.00	12.00	5.00

[a]Provided per kg of diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite; [b]Provided per kilogram of diet: vitamin A, 10 800 IU; vitamin D3, 4 000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg

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$$ND = \{1 - [(Nf \times Cd)/(Nd \times Cf)]\} \times 100 \quad (1)$$

where:

Nf – nutrient concentration in the faeces (% DM);
Cd – chromium concentration in the diet (% DM);
Nd – nutrient concentration in the diet (% DM);
Cf – chromium oxide concentration in the faeces (% DM).

At the end of the experiment, 2 pigs from each pen were subjected to the collection of blood samples. A half a millilitre (0.5 ml) blood sample was collected from the anterior vena cava of the pigs using a sterile syringe. Two-tenths of a millilitre (0.2 ml) of the sample was stored in K_3EDTA (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA) tubes to estimate the haematological evaluations. The second aliquot was used to separate the serum after centrifugation at 3 000 × *g* at 4 °C for 15 minutes. An automatic RA-1000 (Bayer Corp., Tarrytown, NY, USA) biochemical analyser was used to assess the white blood cell (WBC), red blood cell (RBC), lymphocyte, and glucose concentrations in the whole blood samples.

Statistical analysis

The general linear model procedure of SAS v9.2 (SAS Institute Inc., Cary, NC, USA) was used to analyse the experimental data in a complete randomised block design. Linear and quadratic effects were used to investigate the polynomial contrast of the dietary *E. faecium* SLB 130 supplementation on the growth performance, nutrient digestibility, and blood profile to compare the means for each treatment. Significant results were defined as less than $P < 0.05$ and the results less than $P < 0.001$ were defined as statistically highly significant.

RESULTS AND DISCUSSION

Previously, researchers pointed out that *E. faecium* probiotics provide various health benefits to animals (Siepert et al. 2014). For example, Wang et al. (2016) reported that a dietary supplement with *E. faecium* showed a positive effect on the growth performance of piglets. Similarly, Yan and Kim (2013) stated that the inclusion of probiotic supplementation in the growing pigs' diet improves their growth performance and nutrient digestibility. Hence, in this study, weanling pigs were selected as the model to evaluate the efficacy of *E. faecium.* The dietary inclusion of an *E. faecium* probiotic diet showed a linearly increased b.w. at week 1 ($P = 0.009$) and at week 6 ($P = 0.000\ 1$). During weeks 1, 3, 6, and the overall period, the inclusion of the *E. faecium* probiotic supplementation in the pigs' diet linearly increased the daily feed intake ($P = 0.006$, 0.034, 0.064, and 0.001, respectively) and ADG ($P = 0.011$, 0.212, 0.013, and 0.000 1, respectively) compared to those fed the CON diet. However, the pigs fed the *E. faecium* probiotic showed a linearly increased ($P = 0.028$) gain to the feed ratio only at the end of week 6 (Table 2). In an earlier study, Pajarillo et al. (2015) stated that feeding pigs an *E. faecium* strain positively enhanced their feed utilisation, daily weight gain, and gut health. Similarly, Bassiony et al. (2021) noted a higher body weight in rabbits with the addition of an *E. faecium* supplement. Moreover, Mohana Devi and Kim (2014) noted that a dietary supplement with 0.01% of an *E. faecium* probiotic significantly increased the daily gain and feed efficacy of weanling pigs. Also, Lojanica et al. (2010) and Cernauskiene et al. (2011) stated that the dietary *E. faecium* DSM 7134 increased the average daily gain and feed conversion ratio of weanling and finishing pigs, respectively. The administration of probiotics in an animal's diet is still inconsistent due to the different diet compositions, differences in the strains, dose levels, age of the animals, and interactions with environmental factors (Loh et al. 2011). In an earlier study, Zhang et al. (2014) stated that the addition of *E. faecium* to weaning pigs caused better nutrient utilisation. Following this, Lan and Kim (2020) reported that a dietary supplement with *E. faecium* to gnotobiotic piglets challenged with *E. coli* showed increased body weight, thereby reducing the diarrhoea incidence. Also, Mallo et al. (2010) stated that the administration of *E. faecium* to weaning pigs caused better nutrient digestibility. Moreover, Mohana Devi and Kim (2014) reported that the inclusion of a 0.01% *E. faecium* probiotic increased the nutrient digestibility of the dry matter and nitrogen, which agrees with the current outcome in the pigs fed a diet supplement with a graded level of *E. faecium* which showed that it linearly increased the nitrogen and dry matter digestibility. Furthermore, Yan and Kim (2013) reported that *E. faecium* supplementation increased the apparent total track digestibility of the

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Table 2. The effect of the dietary *E. faecium* supplementation on the growth performance of the weanling pigs

Items	CON	TRT1	TRT2	TRT3	TRT4	SEM[1]	*P*-value	
							linear	quadratic
Body weight (kg)								
Initial	6.38	6.37	6.38	6.38	6.38	0.00	0.740	0.779
Week 1	8.01	8.27	8.21	8.15	8.11	0.06	0.009	0.754
Week 3	13.63	13.86	14.04	14.80	14.96	0.19	0.274	0.318
Week 6	25.73	26.38	26.67	26.71	27.93	0.19	< 0.000 1	0.123
Week 1								
ADG (g)	234	246	254	261	270	9	0.011	0.777
ADFI (g)	315	333	342	350	354	9	0.006	0.408
G : F	0.739	0.738	0.741	0.749	0.761	0.010	0.468	0.288
Week 3								
ADG (g)	401	412	419	428	435	11	0.212	0.861
ADFI (g)	567	595	614	626	639	13	0.034	0.355
G : F	0.708	0.692	0.682	0.689	0.689	0.009	0.144	0.148
Week 6								
ADG (g)	576	588	600	612	614	12	0.013	0.666
ADFI (g)	923	938	951	952	964	16	0.064	0.739
G : F	0.624	0.626	0.631	0.637	0.643	0.006	0.028	0.664
Overall								
ADG (g)	461	476	483	484	489	4	< 0.000 1	0.127
ADFI (g)	703	726	739	730	743	7	0.001	0.125
G : F	0.656	0.656	0.654	0.663	0.659	0.004	0.285	0.928

[1]Standard error of means – significant results were defined as less than $P < 0.05$ and statistically highly significant as $P < 0.001$

ADG = average daily gain; ADFI = average daily feed intake; CON = basal diet; G : F = gain : feed; TRT1 = basal diet + 2.5 × 10^5 cfu/g *E. faecium*; TRT2 = basal diet + 1.29 × 10^6 cfu/g; TRT3 = basal diet + 1.15 × 10^7 cfu/g; TRT4 = basal diet + 1.1 × 10^8 cfu/g

dry matter (DM), nitrogen (N) and gross energy (GE) in growing pigs. *E. faecium* is a normal bacterium in swine intestines which produces lactic acid to reduce the intestinal pH and inhibits the load of invasive pathogens (Canibe and Jensen 2003); we thought that this might be one of the probable reasons in which the pigs exhibited an improvement ($P > 0.05$) in the nutrient digestibility of DM and N in the current study (Table 3).

The blood characteristics, such as the white blood cells, red blood cells, lymphocytes and glucose were analysed on weanling pigs at the end

Table 3. The effect of the dietary *E. faecium* supplementation on the nutrient digestibility in the weanling pigs

Items (%)	CON	TRT1	TRT2	TRT3	TRT4	SEM[1]	*P*-value	
							linear	quadratic
Dry matter	81.08	82.34	82.55	83.90	84.10	0.71	0.019	0.835
Nitrogen	79.64	80.91	81.76	82.60	83.78	0.87	0.001	0.716
Gross energy	81.10	81.48	83.05	80.06	81.78	0.73	0.978	0.504

[1]Standard error of means – significant results were defined as less than $P < 0.05$ and statistically highly significant as $P < 0.001$

CON = basal diet; TRT1 = basal diet + 2.5 × 10^5 cfu/g *E. faecium*; TRT2 = basal diet + 1.29 × 10^6 cfu/g; TRT3 = basal diet + 1.15 × 10^7 cfu/g; TRT4 = basal diet + 1.1 × 10^8 cfu/g

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Table 4. The effect of the dietary *E. faecium* supplementation on the blood profile in the weanling pigs

Items (week 6)	CON	TRT1	TRT2	TRT3	TRT4	SEM[1]	*P*-value	
							linear	quadratic
WBC (× 10^3/µl)	21.59	20.51	21.07	20.53	21.64	1.97	0.985	0.665
RBC (× 10^6/µl)	5.95	5.72	6.14	6.10	6.01	0.13	0.229	0.719
Lymphocyte (%)	68.05	68.73	63.98	64.25	66.95	5.94	0.728	0.690
Glucose (mmol/l)	4.68	4.56	4.98	4.91	5.00	0.31	0.812	0.272

CON = basal diet; RBC = red blood cells; TRT1 = basal diet + 2.5 × 10^5 cfu/g *E. faecium*; TRT2 = basal diet + 1.29 × 10^6 cfu/g; TRT3 = basal diet + 1.15 × 10^7 cfu/g; TRT4 = basal diet + 1.1 × 10^8 cfu/g; WBC = white blood cells

[1]Standard error of means – significant results were defined as less than $P < 0.05$ and statistically highly significant as $P < 0.001$

of week 6 (Table 4). However, there were no differences observed either in the treatment groups or the control group. The current findings agreed with Chen et al. (2006) who noted a similar result in pigs fed an *E. faecium* SF68 probiotic. In contrast to the present findings, Mohana Devi and Kim (2014) demonstrated that 0.01% of *E. faecium* supplementation increased the glucose concentration in the weaning pigs' blood. The reason for the discrepancies in the results among the studies were probably a consequence of many factors including the probiotic strain, individual health status of the pigs and some environmental factors. Previously, Szabo et al. (2009) reported that *E. faecium* supplementation to piglets challenged with *Salmonella typhimurium* showed a higher serum IgM and IgA concentration; however, it was not sure whether the increased IgM and IgA concentrations were a result of the *E. faecium* supplementation or a result of the elevated *Salmonella* loads. To date, very few studies have been undertaken in evaluating the effects of *E. faecium* on the haematological profile of the white blood cells, red blood cells, lymphocytes and glucose in weanling pigs, thus, further studies are needed to evaluate the ideal mechanism of *E. faecium* on the immune response.

In conclusion, it could be stated that the outcome of the present experiment suggests that the dietary inclusion of *E. faecium* SLB 130 probiotic additives in weanling pigs' diet would be beneficial to enhance their growth performance and nutrient digestion. The exact cause for the lack of results on the blood analysis is presently unknown at this time, thus, our research team plans to conduct further research to find the optimum level of the *E. faecium* SLB 130 probiotic that could improve the blood metabolites of weaning pigs.

Conflict of interest

The authors declare no conflict of interest.

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