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Keywords: Probiotic complexes, piglets, post–weaning diarrhoea, short–chain fatty acids, faecal microbiota
Growth performance and post-weaning diarrhoea in piglets fed a diet supplemented with probiotic complexes

ABSTRACT

Weaning stress can affect the growth performance and intestinal health of piglets. Dietary alternatives to antibiotics, such as dietary probiotics, especially those containing multiple microbial species, are a preventive strategy for effectively controlling post-weaning diarrhoea. In this study, we investigated forty-eight crossbred piglets in three treatment groups for 21 days: the control and experimental group supplemented with Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 plus Saccharomyces cerevisiae ATCC 28338 (EBS) or Lactobacillus paracasei L9 CGMCC No. 9800 (EBL). On day 21, weaned piglets supplemented with two kinds of probiotic complexes increased growth performance of piglets, reduced post-weaning diarrhoea significantly (P < 0.05). The EBS treatment increased acetic acid and propionic acid in the faeces (P < 0.05), and the EBL treatment increased faecal acetic acid, propionic acid, butyrate and valerate (P < 0.05). Moreover, the faecal microbiota of piglets changed markedly in EBL treatment. The addition of EBS and EBL may have similar effects on the prevention of diarrhoea by improving the intestinal morphology and regulating the microbiota during the weaning period.

Keywords: Probiotic complexes; Piglets; Post-weaning diarrhoea; Short-chain fatty acids; Faecal microbiota

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INTRODUCTION

Post-weaning diarrhoea (PWD) is the most common disease in weaned piglets and is a major economic problem worldwide. PWD is mainly caused by conditioned pathogens and enterotoxins, with a high incidence of intestinal microbiota disturbances[1, 2]. Impaired gut microbiota composition after weaning can lead to slower growth, suggesting a greater permissiveness to pathogen colonisation and induction of pro-inflammatory status. A recent study found a relationship between the faecal microbiota of post-weaning pigs and PWD[3]. Improving the gut microbiota to avoid dysbiosis is a potential method of controlling PWD.

Dietary supplementation with antibiotics is the most effective strategy for preventing PWD. However, with increasing public concern regarding antibiotic resistance, banning antibiotics as growth promoters has been proposed in China[4]. Thus, finding acceptable alternatives to antibiotics is important for the livestock industry. As a safe and feasible replacement for antibiotics in commercial animal production, dietary probiotics are a promising preventive strategy for the control of PWD in piglets[5].

Probiotic bacteria have been shown to improve growth performance and health in weaned piglets[2]. In addition, dietary supplementation with probiotics can improve the intestinal barrier function of piglets by altering immune function[6] and intestinal microbiota[7]. These benefits are produced by the combined effects of a number of different probiotics[8].

Many studies have shown that different probiotic complexes have various
probiotic properties and can be used to improve the health status of weaned piglets. For example, the combination of *Lactobacillus casei* and *Enterococcus faecalis* was found to significantly increase average daily gain and decrease the diarrhoea rate and mortality[9]. Supplementation with a bacteria–yeast complex was shown to result in better growth performance and lower incidence of diarrhoea compared with supplementation with lactic acid bacteria alone[10].

*Enterococcus faecalis* can improve the efficiency of feed utilization and can balance the microbial environment in weaned piglets[11]. *Bacillus pumilus* spore suspension can lower the incidence of diarrhoea and improve growth performance in piglets after weaning[12]. *Lactobacillus reuteri* can also promote growth, reduce diarrhoea and regulate the immune system of pigs[8]. Complexes combining these different kinds of probiotics might be more beneficial to growth performance and PWD control than individual microbial species alone during the weaning period in piglets.

The objective of the present study was to evaluate the effects of dietary probiotic complexes containing *Enterococcus faecalis* and *Bacillus subtilis* plus either *Saccharomyces cerevisiae* or *Lactobacillus* on growth performance, PWD, faecal short-chain fatty acid levels and microbiota in weaned piglets.

**MATERIALS AND METHODS**

**Animal care**

All husbandry practices and euthanasia in this experiment were performed with full consideration of animal welfare, and were approved by the Laboratory Animal Welfare
and Animal Experimental Ethical Committee of China Agricultural University (CAU20170603-3).

Diet and feeding

Forty-eight crossbred piglets (Duroc × Landrace × Large Yorkshire) weaned at 28 d of age, with an average initial body weight of 7.75 ± 0.20 kg, were randomly assigned to one of three dietary treatments (four replicates and four pigs per pen). All piglets were housed indoors on hard plastic slatted flooring. The temperature of the nursery room was initially controlled at approximately 27°C and was progressively decreased by 1°C each week. The humidity was approximately 60% ± 5% and the lighting was natural. Feed and water were available throughout the 21-d feeding trial. The basal diet was formulated to meet or exceed the nutrient requirements of 7 to 11 kg pigs. No antibiotics were used in any diet. The ingredients and chemical composition of the basal diet are presented in Table 1.

Grouping design

The control diet was a corn–soybean meal basal diet which included 1 g whey powder/kg basal diet (Control treatment). The probiotic complex was mixed into the diet with whey powder as a carrier. The diets with probiotic complex supplementation were formulated with 1 g probiotic complex/kg basal diet. Supplementation with two experimental probiotic complexes was evaluated. The first probiotic complex (EBS treatment) was a commercial product containing Enterococcus faecium DSM 7134 (1 \times 10^8 CFU/g) + Bacillus subtilis AS1.836 (2 \times 10^{10} CFU/g) + Saccharomyces cerevisiae
ATCC 28338 (1 × 10^{10} CFU/g). The second probiotic complex (EBL treatment) contained *Enterococcus faecium* DSM 7134 (1 × 10^{8} CFU/g) + *Bacillus subtilis* AS1.836 (2 × 10^{10} CFU/g) + *Lactobacillus paracasei* L9 (1 × 10^{10} CFU/g).

The probiotic complex supplements were both purchased from Beijing He Yiyuan Biotechnology Company. *Lactobacillus paracasei* L9 (CGMCC No. 9800) was isolated from human intestine[13].

**Growth performance**

Feed and piglets were weighed individually at the beginning of the study and on day 21. The amount of feed in the feeder was monitored; any wasted feed was also weighed and this amount was subtracted from the amount of feed added to the feeder to determine feed disappearance. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were then calculated.

**Faecal score, moisture content and pH values**

Faecal consistency within each pen was visually assessed during the study by a technician who was blind to dietary treatments, according to a modification of the method described by Pierce et al.[14] as follows: hard faeces = 1; slightly soft faeces in pen = 2; soft, partially formed faeces = 3; loose, semi-liquid faeces = 4; watery, mucoid faeces = 5. The moisture content of faecal samples was measured by vacuum freeze-drying. The pH of faecal samples was measured directly with a combined pH electrode.

**Sampling and measurements**

On day 1 and day 21, fresh faecal samples were collected from each piglet. Faeces
were placed in a container with stabilization solution (RNAlater, a saturated ammonium sulfate solution [pH 5.2] containing 20 mL [0.5 M] EDTA solution and 25 mL [1 M] sodium citrate solution). Fresh faecal samples were also immediately frozen in liquid nitrogen and stored at −80°C.

At the end of the 21-d experiment, three piglets with similar body weight were randomly chosen from each group and euthanized. The abdomen was aseptically opened and the duodenum (about 10 cm distal to the pylorus) and jejunum (the middle portion of the small intestine) were sampled according to the methods described by Yang et al.[15].

**Determination of short-chain fatty acids in faecal samples**

Short-chain fatty acids (acetic acid, propionic acid and butyric acid) were quantified with gas chromatography[16]. The gas chromatography system consisted of a 7890A equipped with a flame ionization detector (Agilent, USA). The chromatographic column was HP-FFAP (25 m, 0.32 mm, 0.5 um) (Agilent, USA).

**DNA extraction and high-throughput sequencing of faecal samples**

Microbial DNA was extracted from 250mg faecal samples according to the methods [6, 16], the V3–V4 region of the 16S ribosomal RNA gene was amplified by polymerase chain reaction (95°C for 2 min, followed by 20 cycles at 94°C for 30 s, 48°C for 30 s, and 72°C for 2 min) using primers 338F5′-(ACTCCTACGGAGGCAGCAG)-3′ and 806R 5′-(GGACTACHVGGGTWTCTAAT)-3′, by thermocycler PCR system (GeneAmp 9700, ABI, USA). High-throughput sequencing of the community
structure of faecal samples was performed on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Operational Taxonomic Units (OTUs) were clustered with 97% similarity cut off using UPARSE (version 7.1, http://drive5.com/uparse/). The relative abundance at the family and genus levels were compared between the two treatments, the top 10 most abundant families were defined as predominant, and sorted for the comparison. One-way analysis of variance (ANOVA) and other calculations were performed with the SPSS software, version 20.0. The principal coordinate analyses (PCoA) based on unweighted UniFrac distances presents discrepancy was conducted using the SAS 9.1 software to evaluate differences in the faecal microbiota between sample groups.

RESULTS

Growth performance

Compared with the control treatment, piglets fed a basal diet supplemented with either EBS or EBL probiotic complexes had higher body weight (BW) and ADG (P < 0.05) and lower ADFI and FCR (P < 0.05) at day 21 after weaning (Table 2). However, there were no differences in BW, ADG, ADFI or FCR between the two probiotic groups (P > 0.05) (Table 2). Both probiotic complexes improved the growth performance of piglets during the 21 d after weaning.

Faecal score, moisture content and pH values

Faecal scores, moisture content and pH values correlated with the severity of
diarrhoea, as summarized in Table 3. A high faecal score (3.69 ± 0.87) was observed in the control treatment. The faecal scores of weaned piglets in the EBS and EBL treatments were significantly lower than those in the control treatment (P < 0.05). Compared with the control treatment, both EBS and EBL treatments had significantly lower faecal moisture content (P < 0.05). Feeding the probiotic EBL resulted in lower pH values than control or EBS treatment (P < 0.05). These findings suggest that the probiotic complexes EBS and EBL were powerful agents for controlling PWD.

**Short-chain fatty acids**

Short-chain fatty acids (SCFAs) are produced by microbial fermentation and play an important role in digestion and absorption. The content of SCFAs in the faeces of weaned piglets supplemented for 21 days was determined. Our results (Table 4) showed that levels of acetic acid and propionic acid were significantly higher in the faecal samples of weaning pigs that received EBS and EBL treatments than in controls (P < 0.05). The EBL treatment also significantly increased butyrate and valerate in faecal samples compared with both control and EBS treatments. The results show that supplementation with EBS or EBL probiotics had effects on the production of SCFAs in faecal samples.

**High-throughput sequencing**

The effect of different probiotic treatments on faecal microbiota of weaned piglets on day 1 and day 21 was evaluated with 16S rRNA gene amplicon sequencing (Table 5). The results of the analyses of the OTUs, Chao1 and Shannon diversity indices are
presented in Table 5. Overall faeces samples' coverage was about 98%, indicating that the majority of microbial phylotypes were detected. Based on the sequencing results, a total of 4059 OTUs were found in all of the faeces samples. Compared with the initial microbial community on day 1, the Sobs, Shannon diversity indices and Chao1 of the microbial communities the all three treatments on day 21 were decreased significantly (P < 0.05). The results indicated that neither EBS nor EBL had effect on microbiota diversity of the faeces samples.

Fig. 1. Effects of probiotics on faecal bacterial communities on family and genus levels in the faeces of weaned piglets. Values are means; n = 16. Families and genus with proportions less than 1% are not listed.

day1_control, the day before weaning; day21_control, basal diet supplemented with whey powder (no bacteria) on day 21 after weaning; day21_EBS, basal diet supplemented with EBS complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Saccharomyces cerevisiae ATCC 28338) on day 21 after weaning; day21_EBL, basal diet supplemented with EBL complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Lactobacillus paracasei L9) on day 21 after weaning.

Fig. 2. One-way ANOVA bar plots showing predominant bacterial families and genuses in the faeces of weaned piglets. * significant differences (P < 0.05), ** significant differences (P < 0.01), *** very significant differences (P < 0.001).

day1_control, the day before weaning; day21_control, basal diet supplemented with whey powder (no bacteria) on day 21 after weaning; day21_EBS, basal diet supplemented with EBS complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Saccharomyces cerevisiae ATCC 28338) on day 21 after weaning; day21_EBL, basal diet supplemented with EBL complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Lactobacillus paracasei L9) on day 21 after weaning.

Relative read abundance of different bacterial families and genuses within different communities was found in the faeces of different treatment groups. The bacteria whose relative abundance was > 1% at the family and genus levels for all treatment
groups are shown in Fig. 1. The one-way ANOVA bar plot of family and genus of the main faecal bacteria in weaned piglets are shown in Fig. 2. The relative abundance of *Prevotellaceae, Acidaminococcaceae* and *Veillonellaceae* in the faeces of weaned piglets in the control treatment was significantly higher on day 21 than on day 1 (P < 0.001). The relative abundance of *Lactobacillaceae* in the control treatment was likewise significantly higher on day 21 than on day 1 (P < 0.05). However, the relative abundance of *Ruminococaceae, Bacteroidales S24-7, Christensenellaceae* and *Rikenellaceae* in the faeces of control treatment piglets on day 21 were significantly lower than on day 1 (P < 0.001). On day 21, piglets in the EBS treatment had a significantly higher relative abundance of *Acidaminococcaceae* (P < 0.01) and *Streptococcaceae* (P < 0.05) in their faeces than piglets in the control treatment.

Piglets in the EBL treatment had higher relative abundance of *Erysipelotrichaceae* (P < 0.05) and lower relative abundance of *Bacteroidales S24-7* (P < 0.05) than piglets in control treatment.

The relative abundance of *Prevotella* 9 in the faeces of weaned piglets in the control treatment (P < 0.001). The relative abundance of *Prevotella* 1 and *Lactobacillus* on day 21 (P < 0.05) was also significantly higher than on day 1 (P < 0.05). However, the relative abundance of *Clostridium sensu 1* in the control treatment on day 21 was significantly lower than on day 1 (P < 0.01), the relative abundance of *Norank_f Bacteroidales S24-7_group, Christensenellaceae R-7_group* and *Ruminococcaceae UGG-005* in the faeces of control treatment piglets on day 21.
were significantly lower than on day 1 (P < 0.001). On day 21, piglets in the EBL treatment had higher relative abundance of Sharpea than piglets in control treatment (P < 0.05). And piglets in the EBS treatment had a significantly higher relative abundance of Streptococcus in their faeces than piglets in the control treatment (P < 0.05).

Fig. 3. PCoA of faecal bacterial communities at genus level.

day1_control, the day before weaning; day21_control, basal diet supplemented with whey powder (no bacteria) on day 21 after weaning; day21_EBS, basal diet supplemented with EBS complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Saccharomyces cerevisiae ATCC 28338) on day 21 after weaning; day21_EBL, basal diet supplemented with EBL complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Lactobacillus paracasei L9) on day 21 after weaning.

The PCoA based on unweighted UniFrac distances presents discrepancy among samples, the microbial communities were clustered into the three groups by time and feed addition at genus (Fig. 3). It is obvious that the faecal samples on day 1 and day 21 were separated (Fig. 3). The microbial communities of day21_EBS was clustered with day21_control, and day21_EBL treatment were clustered into different groups from them at the genus level.

DISCUSSION

Weaning stress in piglets may cause changes in the gut environment and morphology that can result in low feed intake, high diarrhoea incidence and imbalanced intestinal microbiota[17]. In the current study, supplementing the diet of post-weaning piglets with probiotic complexes increased ADG and improved ADFI and FCR compared with these values in control piglets fed an unsupplemented diet. These improvements could
have resulted from the higher nutrient digestibility in EBS-fed and EBL-fed piglets. These findings are supported by earlier studies that showed positive effects on feed intake and growth performance in piglets fed diets supplemented with a complex of lactic acid bacteria during a 21-day post-weaning period[18]. The enhanced growth performance observed in our study is consistent with previous published reports[9, 19, 20].

Increased SCFAs of the piglets could maintain normal gut function by serving among variety of regulatory features in the host's metabolism[21], butyric acid serves as an important energy source for gut epithelia and proliferation and differentiation of epithelial cells[22]. In the present study, levels of acetic acid and propionic acid of faecal samples in EBS and EBL treatments are significantly increased(P<0.05). Besides, the EBL treatment also significantly increased butyrate content of faecal samples as compared with both control and EBS treatments. This result is in agreement with Mori K et al.[23], who reported that daily probiotic intake could increase the SCFAs levels in fecal samples of pigs. We could conclude that the EBS and EBL treatments had more SCFAs observed are probable caused by the probiotic complexes which could improve intestinal microbial balance.

Consistent with the results of Hu et al.[6], the most dominant phyla of all faecal samples of weaned piglets were Firmicutes and Bacteroidetes, and the higher bacterial diversity of piglets were observed in the initial microbial community on day 1 compared to all three treatments day 21(P < 0.05). Besides, the faecal microbiota changed
obviously after weaned, which might because of the changes of food intake and
intestinal morphology of piglets. At the family level, the relative abundance of
*Prevotellaceae, Lactobacillaceae* and *Veillonellaceae* in faeces of weaned piglets of
control treatment on day 21 were enriched than day 1 significantly in this study.
However, the relative abundance of other families in faeces of weaned piglets were
lower. This result was in line with previous studies[6, 24, 25]. This is for the reason that
a dramatic change in microbial populations of pigs usually happen when it comes to
weaning or the withdrawal of milk[26]. Populations of saccharolytic microbes like
*Prevotellaceae* and *Lactobacillaceae* increased mostly because that the diet changed
after weaning. The relative abundance of other families like opportunistic pathogens
including *Enterococcus* in faeces reduced, this might because that the abundance of
them usually decrease as the gut microbiota of weaned piglet gut maturing.
In present study, the relative abundance of *Lactobacillaceae* and *Erysipelotrichaceae*
in faeces of EBL treatment was more than the control treatment on day 21. The elevated
concentrations of SCFAs in faeces of piglets in EBL treatment could be the result of the
higher numbers of *Lactobacillus*, and the major fermentation products of *Lactobacillus*
like lactate produced by them can be further metabolized to other SCFAs by bacteria in
gut[28]. It has been reported that a greater abundance of *Sharpea* within
*Erysipelotrichia* which is a family of bacteria of the phylum *Firmicutes* may related to
the diet and surroundings[29], higher relative abundance *Erysipelotrichaceae* may help
mice gain weight faster[28]. As most of members of *Firmicutes* could degrade
polysaccharide, the absorbing ability weaned piglets in EBL treatment might be better than control treatment. On the contrary, the relative abundance of *Bacteroidales* within *Bacteroidetes* in EBL treatment is lower than control treatment, the relative abundance of the phylum *Bacteroidetes* was significantly elevated in diarrheic piglets[30]. Therefore, the lower abundance of *Bacteroidetes* may allow the piglets in EBL treatment to better adapt to weaning. These showed that dietary probiotics complexes additives could be efficient to decreased PWD by adjusting the gut microbiota of piglets. Compared to day21_control, feeding of probiotics EBS could increase the relative abundance of *Streptococcus*, it is placed within the order *Lactobacillales* which usually increased the SCFAs and might be beneficial to gut health[6]. The results depicted that improvement of the growth and nutrition absorb of weaned piglets than control treatment during the weaning period, might be due to the higher concentrations of SCFAs in EBS treatment produced by beneficial bacteria. The PCoA plot indicated that the microbial communities of day21_EBS was clustered with day21_control at genus level, for the reason that the community diversity microbes in faeces of EBS treatment was similar with the control treatment. Since that *Saccharomyces cerevisiae* only have a little effect on the gut microbiota of piglets[27], because that it is not regulate the gut microbiota by colonization. The EBL with *Lactobacillus paracasei* L9 had a more favourable effect on faecal microbial communities than the use of EBS, and the day21_EBL treatment was clustered into different groups from the control treatment. This result is in the line with the findings
that *Lactobacilli* significantly modify intestinal microbiota in piglets, and reduce the PWD of piglets[9].

Our results suggest that the complexes tested had probiotic properties in weaned piglets and regulated the composition of the intestinal microbiota. Moreover, supplementing the diet of weaned piglets with a probiotic complex improved ADG, ADFI and FCR, reduced PWD after weaning for 21 days. The addition of a probiotic complex improved post-weaning performance, most likely by altering the bacterial community and some metabolites, such as SCFAs, in the faeces of weaned piglets. In conclusion, the results of this study suggest that the addition of EBS and EBL may play an important role in the porcine digestive system and the prevention of diarrhoea, especially during the weaning period.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.
Table 1 Ingredients and nutrient content of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Nutrient content</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extruded maize</td>
<td>24.38</td>
<td>Digestible energy, kcal/g</td>
<td>3.45</td>
</tr>
<tr>
<td>Extruded broken rice</td>
<td>25.00</td>
<td>Crude protein</td>
<td>19.50</td>
</tr>
<tr>
<td>Soybean meal (46% CP)</td>
<td>22.20</td>
<td>Crude fat</td>
<td>7.50</td>
</tr>
<tr>
<td>Soybean protein concentrate (53% CP) 5.30</td>
<td>3.30</td>
<td>Lysine</td>
<td>1.54</td>
</tr>
<tr>
<td>Milk replacer (42% CP)</td>
<td>10.00</td>
<td>Methionine</td>
<td>0.45</td>
</tr>
<tr>
<td>Sweet whey (11% CP)</td>
<td>10.00</td>
<td>Methionine + cystine</td>
<td>0.97</td>
</tr>
<tr>
<td>Vitamin–mineral premix</td>
<td>0.23</td>
<td>Threonine</td>
<td>0.96</td>
</tr>
<tr>
<td>Lysine HCl</td>
<td>0.10</td>
<td>Tryptophan</td>
<td>0.27</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.05</td>
<td>Calcium</td>
<td>0.98</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.11</td>
<td>Available phosphorusa</td>
<td>0.49</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Providing the following amounts of vitamins and minerals per kilogram on an as-fed basis: Zn (ZnO), 50 mg; Cu (CuSO4), 20 mg; Mn (MnO), 55 mg; Fe (FeSO4), 100 mg; I (KI), 1 mg; Co (CoSO4), 2 mg; Se (Na2SeO), 0.3 mg; vitamin A, 8255 IU; vitamin D3, 2000 IU; vitamin E, 40 IU; vitamin B1, 2 mg; vitamin B2, 4 mg; pantothenic acid, 15 mg; vitamin B6, 10 mg; vitamin B12, 0.05 mg; vitamin PP, 30 mg; folic acid, 2 mg; vitamin K3, 1.5 mg; biotin, 0.2 mg; choline chloride, 800 mg; and vitamin C, 100 mg.

Table 2. Effects of probiotics on BW, ADG, ADFI and FCR of piglets on day 21 after weaning

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>EBS</th>
<th>EBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>13.8±2.19a</td>
<td>14.5±1.24b</td>
<td>14.9±1.31b</td>
</tr>
<tr>
<td>ADG, g</td>
<td>383±16.77a</td>
<td>398±18.62b</td>
<td>417±18.55b</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>557±20.02a</td>
<td>538±18.62b</td>
<td>520±17.98b</td>
</tr>
<tr>
<td>FCR, g/g</td>
<td>1.45±0.08a</td>
<td>1.31±0.12b</td>
<td>1.27±0.13b</td>
</tr>
</tbody>
</table>

abcMeans with different superscripts differ significantly (P < 0.05).

Control, basal diet supplemented with whey powder and no bacteria; EBS, basal diet supplemented with EBS complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Saccharomyces cerevisiae ATCC 28338); EBL, basal diet supplemented with EBL complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Lactobacillus paracasei L9).
Table 3. Effect of probiotics on faecal score, faecal moisture content and faecal pH values of weaned piglets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>EBS</th>
<th>EBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal score</td>
<td>3.69±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.56±1.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Faecal moisture content (%)</td>
<td>73.93±3.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.44±2.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.54±3.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Faecal pH</td>
<td>6.93±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.57±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.32±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means with different superscripts differ significantly (P < 0.05). The method to faecal score: hard faeces = 1; slightly soft faeces in pen = 2; soft, partially formed faeces = 3; loose, semi-liquid faeces = 4; watery, mucoid faeces = 5. The moisture content of faecal samples was measured by vacuum freeze-drying.

Table 4. Effects of probiotics on short-chain fatty acids in faecal samples of weaned piglets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>EBS</th>
<th>EBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate, mM/g</td>
<td>70.74±19.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.78±15.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.21±19.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butyrate, mM/g</td>
<td>3.59±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.16±1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.97±1.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propionate, mM/g</td>
<td>29.65±9.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.18±9.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.46±9.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Valerate, mM/g</td>
<td>15.91±5.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.54±7.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.29±4.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means with different superscripts differ significantly (P < 0.05).

Table 5. Effects of probiotics on the diversity of microbial communities in faecal samples of weaned piglets.

<table>
<thead>
<tr>
<th>Items</th>
<th>day1_control</th>
<th>day21_control</th>
<th>day21_EBS</th>
<th>day21_EBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTUs</td>
<td>1172&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1062&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sobs</td>
<td>599.44±64.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>474.92±76.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>401.07±120.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>417.25±124.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shannon</td>
<td>4.77±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.13±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.92±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.11±0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chao1</td>
<td>693.92±67.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>486.37±169.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>586.96±171.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>550.99±117.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means with different superscripts differ significantly (P < 0.05).

day1_control, the day before weaning; day21_control, basal diet supplemented with whey powder (no bacteria) on day 21 after weaning; day21_EBS, basal diet supplemented with EBS complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Saccharomyces cerevisiae ATCC 28338) on day 21 after weaning; day21_EBL, basal diet supplemented with EBL complex (Enterococcus faecium DSM 7134, Bacillus subtilis AS1.836 and Lactobacillus paracasei L9) on day 21 after weaning.


= *Nihon chikusan Gakkaiho*.


Fig. 1. Effects of probiotics on faecal bacterial communities on family and genus levels in the faeces of weaned piglets
Fig. 2. One-way ANOVA bar plots showing predominant bacterial families and genera in the faeces of weaned piglets.
Fig. 3. PCoA of faecal bacterial communities at genus level.