Note

Effect of dietary supplementation of fermented botanical products (FBPs) on coliform bacteria and *Salmonella* counts in the swine gut

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Abstract This study was a preliminary experiment aimed at observing the general microbial condition in the gut of pigs fed a diet supplemented with fermented botanical products (FBPs). At 3months of age, 12 crossbred pigs were divided into two groups with the same average body weight and a 2:1 male:female sex ratio. The control group was given a commercial formula feed and the experimental group was given the same feed supplemented with 0.125% FBPs. Two months after the start of experimental feeding, fresh feces were collected directly from the rectum to determine aerobic and facultative anaerobic cells, coliform bacteria, and *Salmonella* counts. The mean aerobic and facultative anaerobic cell counts were lower in the experimental group than in the control group, but not significantly. Mean coliform bacteria counts were significantly lower in the experimental group than in the control group ($P < 0.05$). *Salmonella* was not detected in either group. The results suggest the possibility that the FBPs used in this study might affect the composition of the swine gut microbiota.

Key words: fermented botanical product, coliform bacteria, *Salmonella*, gut, swine

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Introduction

It is well known that the structure of the swine gut microbiota fundamentally affects the health and growth performance of pigs [5,8,10]. Maintaining a healthy gut microbiota is fundamental for pigs to digest and absorb dietary nutrients. Diet, including the prebiotics, probiotics, and antibiotics added to feed, contribute to changes in the gut microbiota throughout the life of the pig [7]. Conventional use of in-feed antibiotics is permitted to reduce the number of undesirable microbes. Recently, many studies have been conducted to examine the effects of reduced use of in-feed antibiotics in swine production due to the increased use of natural alternatives. [2,4,6,8]. However, the process by which a healthy microbial ecosystem can be established remains to be clarified. Fermented foods are produced through the enzymatic activity of various microbes. There is strong evidence for the impact of fermented products on general health, and gut microbiota balance. This study examined the use of fermented botanical products (FBPs) produced by Manda Fermentation Co., Ltd. Rats and broiler

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chickens fed a diet including FBPs showed a reduced accumulation of body fat [13] and improved feed efficiency [10], respectively. These results suggested that FBPs might improve the gut environment and consequently the health of the gut microbiota. To our knowledge, no studies have investigated the microbial condition of animals fed a diet including FBPs. Therefore, the purpose of this study was to preliminarily observe the effect of FBPs on aerobic and facultative anaerobic cells as a standard plate count as well as coliform bacteria and *Salmonella* counts as unfavorable microorganism [1,11] in the swine gut.

Materials and Methods

1. Fermented botanical products (FBPs)

The FBPs used in this study were established over 3.25 years through fermentation by Lactobacillus and Saccharomyces at room temperature by Manda Fermentation Co., Ltd. (Onomichi, Japan) using black sugar, rice, apples, oranges, bananas, persimmons, pineapples, soybeans, carrots, seagrass, grapes, honey, garlic, sesame, and bayberries as substrates. This product is a paste and was mixed with rice bran for the present use. The FBPs were developed to regulate the gut environment as a prebiotics.

2. Animals and management

This study was conducted at a farm belonging to Tochigi Prefectural High School and involved 12 crossbred (LWD: Landrace, Large White, and Duroc) pigs born at the farm. At 3 months of age, the pigs were divided into two groups with the same average body weight and a 2:1 male:female sex ratio. The control group was given a commercial formula feed and the experimental group was given the same feed supplemented with 0.125% FBPs. The composition of the formula feed was as fallows: 70% grains (maize, wheat, and wheat flower), 10% defatted rice bran, 8% oil seed meal (soybean meal and rapeseed meal), and 12% others (bakery waste, calcium carbonate, sodium chloride, calcium phosphate, citric acid, and vitamin and /or mineral mixtures). The pigs were housed in 6 pens for group feeding. The pens had a flat concrete floor with an area of 8㎡ and no air conditioning, the temperature ranged from 26 and 37°C. The pigs were given access to feed and water ad libitum. Weekly feed intake amounts were calculated from the values for feed supply and rest, and live body weights were determined each week during the 3 months of experimental feeding.

3. Collection and observation of microbes from fecal samples

Two months after the start of experimental feeding, fresh feces were collected directly from the rectum to determine microorganism counts. Specimens were treated 10 times by step dilution with isotonic saline, from 10^{-1} to 10^{-10}. In this study, aerobic and facultative anaerobic cells, coliform bacteria, and Salmonella counts were determined as a preliminary test. The aerobic and facultative anaerobic cell counts were performed using standard agar medium (Nissui, Co., Ltd., Tokyo, Japan) cultured at 37°C for 24 h. Desoxycholate agar medium and deoxycholate-hydrogen sulfide-lactose agar medium (Nissui, Co., Ltd.) were used to perform coliform bacteria and Salmonella counts, respectively.

4. Statistical analysis

The determined values (mean ± SD of 6 replicates) for each microbial count were evaluated by one-way analysis of variance. $P < 0.05$ was considered to

Table 1. Results of the growth performance of pigs in this study

	Body weight gain/group (n=6)	Feed intake /group (n=3)	Feed conversion (n=3)
	157.8	535.7	3.39
Control group[1]	166.8	516.6	3.10
	147.0	525.4	3.57
Mean±SE	157.2 ± 5.7	525.9 ± 5.5	3.35 ± 0.14
	153.6	523.1	3.40
Experimental group[2]	151.4	512.0	3.38
	165.0	519.5	3.15
Mean±SE	156.7 ± 4.2	518.2 ± 3.3	3.31 ± 0.08

[1] The pigs in the control group were given a commercial formula feed during the experimental period.

[2] The pigs in the experimental group were given the commercial formula feed supplemented with 0.125% FBPs during the experimental period.

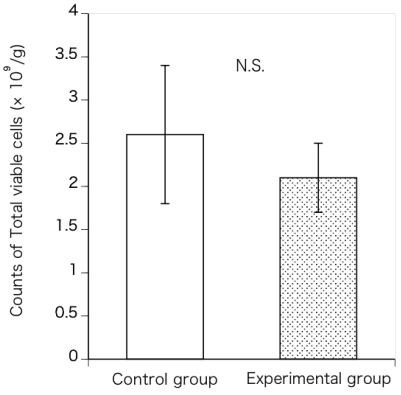

Fig. 1 Aerobic and facultative anaerobic cell counts (n=6)

There was no significant difference between the groups. N.S., not significant.The pigs in the control group were given a commercial formula feed and the pigs in the experimental group were given the commercial formula feed supplemented with 0.125% FBPs during the experimental period.

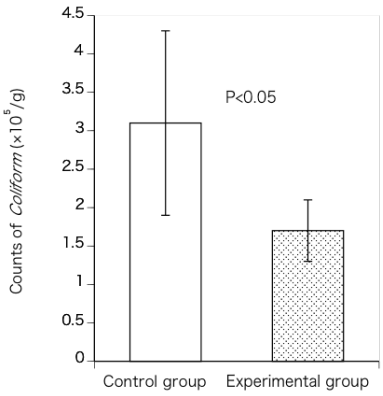

Fig. 2 *Coliform* counts (n=6)

A significant difference was observed between the groups ($P < 0.05$). The pigs in the control group were given a commercial formula feed during the experimental period. The pigs in the experimental group were given the commercial formula feed supplemented with 0.125% FBPs during the experimental period.

indicate statistical significance.

Results

1. Growth performance of pigs fed and not fed FBPs

There were no significant differences in body weight gain and feed intake between the groups (Table 1).

2. Coliform bacteria and Salmonella counts from the feces of pigs fed and not fed FBPs

Mean aerobic and facultative anaerobic cell counts were lower in the experimental group than in the control group ($2.1 \pm 0.4 \times 10^9$ CFU/g vs. $2.6 \pm 0.8 \times 10^9$ CFU/g), but the difference was not significant (Fig. 1). The mean coliform bacteria counts were significantly lower in the experimental group than in the control group ($1.7 \pm 0.3 \times 10^5$ CFU/g vs. $3.1 \pm 1.1 \times 10^5$ CFU/g; $P < 0.05$; Fig. 2). The *Salmonella* count could not be determined in this study because all specimens were below the limit of detection.

Discussion

Previous studies have reported a critical role for FBPs in intestinal morphology and nutrient metabolism, and it has been suggested that FBPs cause changes in the gut microbiota [10,13]. In the present study, no benefit was observed in terms of growth performance in the control group and the experimental group; however, there were also no drawbacks. *Salmonella* was not

detected in feces from either group, suggesting good growth performance and a good feeding environment. Although the difference in aerobic and facultative anaerobic cell counts between the groups was not significant, it tended to decrease in the group fed FBPs. Coliform bacteria counts were significantly decreased in the experimental group, demonstrating that the FBPs used in this study enhanced the diversity of the gut microbiota and conferred secondary health effects, in line with previous reports [10,13]. Demeckova et al. [3] reported that a fermented liquid feed produced by rifampicin-resistant mutant *Lactobacillus platarum* decreased the fecal coliform bacteria population in farrowing sows; the present study showed the same tendency. Many previous studies investigating the effects of fermented foods and other substances on the gut microbiota showed an increased population of *Lactobacillus* and reduced *Salmonella* reproduction in the gut microbiota [2,3,5,8,12]. These reports demonstrated a beneficial change in the gut microbiota as well as effects such as inhibiting colonization by undesirable microorganisms. This change also promoted good growth performance in the pigs and subsequently enhanced their immune response [10]. The present results suggest the possibility of using FBPs as an alternative to antibiotics for preventing diarrhea due to coliform bacteria. The mechanisms of gut microbiota diversification and the effects of fermented products on the swine gut microbiota remain to be clarified, and thus a comprehensive determination of the gut microbiota from pigs fed a diet including FBPs is needed to build on the findings of the present study. In the future, the potential role of using FBPs as an alternative to antibiotics for preventing diarrhea and increasing growth performance in swine production will be examined using weaned piglets.

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研究ノート

植物発酵製品の食餌添加が豚腸内の大腸菌とサルモネラに及ぼす影響

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植物発酵製品を摂取した豚の腸内微生物叢を観察するための予備試験として本試験を行った。 3 ヵ月齢時に 12 頭の交雑種(LWD)豚を平均体重と性比（雄：雌＝2:1）が同じになるように 2 区に配分した。対照区は市販飼料を与え、試験区は植物発酵製品を 0.125%添加した市販飼料を給与した。試験開始 2 ヵ月後に、好気性菌・通性嫌気性菌、大腸菌群数、サルモネラ菌数を調査するべく、肛門から直腸糞を採取した。試験区の平均好気性菌・通性嫌気性菌数は対照区よりも少なかったが、有意差はなかった。試験区の平均大腸菌群数は対照区よりも有意(P<0.05)に少なかった。 サルモネラは両区共に検出されなかった。本成績は供試した植物発酵製品は豚の腸内微生物叢に影響する可能性を示唆した。

キーワード：植物発酵製品，大腸菌群、サルモネラ，腸，豚

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