Short Communication

THE EFFECT OF ADMINISTRATION OF BIFIDOBACTERIA ON THE INTESTINAL FLORA AND GROWTH OF NEWBORN PIGLETS

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Antibiotics have been widely used as growth-promoting agents for livestock. For newborn calves and piglets, administration of antibiotics is useful to prevent infections caused by pathogenic bacteria. However, the use of antibiotics in livestock can have serious consequences, such as the development of resistant populations of bacteria. Subsequent use of the same antibiotics for therapy may be ineffective. Also, residual antibiotics in dairy foods, meat, eggs, and milk are unacceptable.

Recently, the use of probiotics has attracted the attention of many researchers (1). *Lactobacillus acidophilus*, *Streptococcus faecalis* and other lactic acid bacteria have been tested as probiotics in livestock (3,5,9). Pollmann et al. (13–15) observed that *L. acidophilus* was effective to promote body weight (BW) gain and feed conversion in piglets. Ozawa et al. (12) reported that administration of *S. faecalis* to calves and piglets promoted colonization by beneficial bacteria and decreased the occurrence of detrimental bacteria, such as *Salmonella*, in the intestine.

Bifidobacterium have also been tested as probiotic agents (4,10). *Bifidobacterium*, a prominent species of bacteria in the human intestines, is a beneficial and important species of intestinal bacteria in humans (16). Also, Ochi and Mitsuoka (11) have studied *Bifidobacterium* from animal feces. Subsequently, many species of *Bifidobacterium* have been found in various animals, suggesting that these bacteria are a common component of the intestinal flora of animals as well as humans (6–8,19). The use of *Bifidobacterium* strains as probiotic agents is an attractive possibility that is expected to provide beneficial effects for livestock as previously observed with *L. acidophilus*.

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However, the use of probiotics is too expensive commercially when a farmer administers it everyday to livestock, if probiotic can be expected to be effective on growth or health of livestock. An administration method which is not expensive and is useful for livestock has been sought after. Usually all animals just after birth have not been infected by any bacteria and there is no bacteria in its intestine. It is thought that the administration of useful bacteria to animals just after birth is more effective on the formation of useful intestinal flora. If an administration of a probiotic to livestock only just after birth has some effects on the following livestock, it may be useful for commercial feeding of livestock because the cost for probiotic is expected to decrease.

In this paper, when bifidobacteria was administered to piglets only just after birth, the effect on the intestinal flora and BW gain of the following piglets was investigated.

Bifidobacterium pseudolongum M-602 isolated from chicken feces was used as a probiotic in this study. *B. pseudolongum* M-602 was cultured for 16 h at 37°C in liquid medium containing 2.5% glucose, 2.0% yeast extract, 1.0% casein peptone, 0.4% K₂HPO₄, 0.2% KH₂PO₄, and 0.02% L-cystine. The culture solution was centrifuged at 8,000 × g to collect cells. The cells were freeze-dried at 30°C after they were washed twice with distilled cool water and suspended with distilled cool solution containing 10% skim milk. Dried cells were mixed with dried dextrin to adjust the cell count. This probiotic contained 1.1×10^{11} CFU/g that was measured by plating onto an MGL medium (Modified Garcke's agar with lithium chloride) as described by Teraguchi et al. (18).

Two litters of newborn Randress piglets were used in this study. Each litter consisted of 6 piglets. One litter was used as a control group and the other was fed probiotic. Each litter was kept independently at the same farm in order to prevent cross-contamination between piglets. Each newborn piglet was grown conventionally with the mother piglet until 35 days of age, and fed breast milk only until 10 days of age. After 10 days of age, breast milk and milk replacer without antibiotics was available for ad libitum intake. Data of BW and BW gain for this investigation were analyzed by the unpaired *t*-test to compare pairs of measurements.

Just after birth, all piglets in the test group were inoculated 1.0 ml of probiotic solution orally by syringes. Probiotic solution (1.0 ml) contained 2×10^{10} CFU of *B. pseudolongum* M-602 dissolved in water. After 24 h, the same treatments were carried out to the same piglets. Actually, probiotic contained 2×10^{10} CFU of *B. pseudolongum* M-602 was inoculated twice to newborn piglets of tested group orally at 0 and 1 day of age. After 2 days of age, the piglets were no longer treated with probiotics and the piglets in the group fed probiotic and control group were bred similarly.

The fecal samples were obtained from all piglets of 10 days of age in both groups. The feces were put into the transfer medium as soon as possible after collection, and kept at 4°C until the measurement of flora in the feces. Each bacterial number in feces was determined by the method of Mitsuoka and Kaneuchi

(8). Data of the number of bacteria in feces were analyzed by the unpaired *t*-test to determine the difference between probiotic treatment and the untreated control. A probability level of $p < 0.05$ was considered to be statistically significant.

The bacterial flora in the fecal samples of all piglets when aged 10 days in the control and probiotic groups (each $n = 6$) were measured, and the mean number and the detected frequency of each bacteria were calculated (Table 1). Bifidobacteria were observed in the feces of 4 out of 6 piglets in the control group, but were detected in the feces of all 6 piglets in the group fed probiotic, and the number of bifidobacteria cells in the feces of piglets fed probiotic tended to be higher than that of the control piglets. However, it could not be determined whether the bifidobacteria isolated from the feces of piglets in the group fed probiotic was the *B. pseudolongum* M-602 administered. However, even if *B. pseudolongum* M-602 was not isolated from the feces of test piglets, it appeared that administration of *B. pseudolongum* M-602 to newborn piglets caused an increase in the number of latent bifidobacteria cells in the intestine because *B. pseudolongum* M-602 administered appeared to create an intestinal environment that was conducive for the growth of bifidobacteria. In the other bacteria, the frequency of occurrence of Staphylococci, Corynebacteria and Bacilli in feces of control piglets tended to be higher than that of piglets fed probiotic, and the mean number of bacterial counts of Bacilli in feces of control piglets were significantly higher than that of piglets fed probiotic. It was observed that the number of Enterobacteriaceae and *Clostridium perfringens* tended to be slightly low following the administration of *B. pseudolongum* M-602, but there was no effect on either of the detected frequencies. The number of Lactobacilli in the feces of piglets fed probiotic was not significantly different from that in control

Table 1. Effect of administration of *Bifidobacterium pseudolongum* M-602 on the fecal score of piglets.^a

Organism	Control ($n = 6$)	Probiotic ($n = 6$)
Enterobacteriaceae	9.70 ± 0.26 (6/6)	9.46 ± 0.84 (6/6)
Streptococci	8.72 ± 1.46 (6/6)	9.07 ± 0.58 (6/6)
Staphylococci	6.11 ± 1.64 (6/6)	5.89 ± 1.10 (2/6)
Corynebacteria	8.62 ± 1.31 (4/6)	9.11 ± 0.98 (2/6)
Bacilli	8.48 ± 1.39 (5/6)	3.91 ^b ± 0.58 (4/6)
Lactobacilli	9.67 ± 1.00 (6/6)	9.89 ± 0.83 (6/6)
Bifidobacteria	7.34 ± 1.18 (4/6)	8.54 ± 1.96 (6/6)
Eubacteria	N.D. ^c (0/6)	7.49 ± 1.82 (2/6)
Bacteroidaceae	8.26 ± 1.65 (6/6)	9.32 ± 0.33 (6/6)
<i>Clostridium perfringens</i>	9.39 ± 0.70 (4/6)	8.60 ± 0.70 (5/6)
Peptococcaceae	7.68 ± 1.40 (3/6)	7.87 ± 0.70 (3/6)
Others	4.82 (1/6)	N.D. (0/6)
Total	10.38 ± 0.70 (6/6)	10.36 ± 0.63 (6/6)

^a Data are indicated as mean ± SD of log 10 per gram and each detected frequency is shown in parentheses.

^b Treatment effect ($p < 0.05$).

^c N.D., not detected.

piglets. Also, the total number of viable cells in feces showed no difference between the two groups. These results suggested that administration of *B. pseudolongum* M-602 soon after the piglets were born was effective in promoting good intestinal flora of the following piglets. Gilliland et al. (2) reported that calves fed pasteurized whole milk containing *L. acidophilus* exhibited greater increases in the numbers of lactobacilli and greater reductions in the numbers of coliforms in the feces. In our studies, although the numbers of Enterobacteriaceae in feces tended to decrease slightly, the numbers of Bacilli decreased significantly and the detected frequency of Staphylococci decreased in feces of piglets administered *B. pseudolongum* M-602. These results suggested that the administration of *L. acidophilus* or *B. pseudolongum* to livestock was effective in improving the intestinal flora although these effects on intestinal flora were different between the kinds of bacteria of probiotics administered.

The change of the mean BW and the mean BW gain of piglets in both groups in the study period were observed. These results are shown in Table 2. Regarding the initial mean BW, there were no significant differences between the groups although BW tended to be higher in the probiotic group. The piglets fed probiotic grew better than piglets in the control group, and the mean BW gain of piglets in probiotic group was significantly higher than that of control piglets. When all piglets were aged 5 weeks, there was a significant difference between the mean BW gain of piglets fed probiotic and that of control piglets ($p < 0.05$), indicating that the administration of *B. pseudolongum* M-602 just after birth was effective in stimulating the subsequent growth of piglets. This effect appeared to result from the improvement of the intestinal environment that was represented the repression of abnormal fermentation in the intestine and the inhibition of production of toxic substances. Furthermore, the administration just after birth may be important for the effects of probiotics on livestock. Pollmann et al. (13) reported that a greater effects was observed with starting than with growing-finishing pigs in the trial of administration of *L. acidophilus*. Sarra et al. (17) also reported that Lactobacilli

Table 2. The effect of administration of bifidobacteria on BW gain of piglets.

	0	7	14	21	28	35 (days)
Control group						
BW (kg)	1.37	2.27	3.57	4.62	5.74	6.94
BW gain (kg)	—	0.90	2.20	3.25	4.37	5.57
Test group						
BW (kg)	1.48	2.98 ^a	4.78 ^a	6.38 ^a	8.23 ^b	9.58 ^a
BW gain (kg)	—	1.50 ^a	3.30 ^a	4.90 ^a	6.75 ^a	8.10 ^a
SE ^c						
BW (kg)	0.04	0.13	0.23	0.33	0.50	0.55
BW gain (kg)	—	0.11	0.20	0.31	0.48	0.53

^a Treatment effect ($p < 0.01$).

^b Treatment effect ($p < 0.05$).

^c Standard error.

administered to newborn piglets was survived in the gastrointestinal tracts of the following piglets. Usually all animals just after birth have not been infected by any bacteria and there is no bacteria in its intestine. The administration of beneficial bacteria like bifidobacteria to animals just after birth appears to be highly effective in promoting the formation of beneficial intestinal flora. In our investigation, the effect of administration of *B. pseudolongum* on piglets seemed to be observed for intestinal flora and BW gain of the following piglets although piglets tested were not administered bifidobacteria after 2 days of age. These results suggest that the administration of *B. pseudolongum* M-602 to piglets just after birth promotes the subsequent growth of the piglets. It was thought that the other toxic bacteria for piglets were not able to colonize the intestine because the administered bifidobacteria initially formed a flora in the intestine.

In this investigation, it was observed that administration of *B. pseudolongum* M-602 to piglets shortly after birth was useful for the improvement of intestinal flora and the growth of the following piglets. These results may be observed because tested piglets were fed under the condition without antibiotics. In further studies, we are going to study the effect of antibiotics contained in milk replacer on the intestinal flora of administered bifidobacteria.

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