Effects of Fermented Milk Supplements on the Growth Performance and Gut Conditions in Piglets during the Pre-and Post-Weaning Periods

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포유기 및 이유기 동안 발효유 급여가 자돈의 성장 및 장관 환경에 미치는 영향

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Abstract This study investigated the effects of fermented milk (FM) supplementation on the growth performance and gut conditions of weaning piglets. A total of one hundred 10-day-old piglets were assigned to the control and FM treatment groups during the pre-weaning (10-27 day-old) and post-weaning (27-64 day-old) periods. The FM was provided daily in the form of two l/replication/d for the pre-weaning and twenty l/replication/d for the post-weaning periods. There was no difference observed in the growth performance between the two experimental groups during pre-weaning. However, during post-weaning, the daily gain and feed intake were higher in the FM group than in the control, and the feed conversion ratio decreased in the treatment group compared with the control. The blood corpuscles and physiological indexes showed no difference between the control and FM groups. Fecal Escherichia coli was lower in the FM group compared to the control, while Lactobacillus spp. was higher. Emissions of fecal hydrogen sulfide and total mercaptans were reduced by the FM supplementation. The intestinal villi in piglets fed with the FM were morphologically denser than in the control. Therefore, these results suggest that the growth performance, noxious fecal emission, and intestinal environment would be improved by FM supplementation.

요 약 본 연구는 발효유 급여가 포유 및 이유자돈의 성장 및 장관 환경에 미치는 영향을 구명하고자 실시하였다. 공시동 물은 총 100마리의 포유자돈을 준비하였고 대조구 및 발효유 급여구에 임의로 50두씩 배치하여 처리구당 5반복, 반복당 10두씩 할당하였으며 이유 후에는 동일 처리구 내에서 공시돈을 처리구당 3반복, 반복당 16두씩 재배치하였다. 사양시 혐은 포유기(생후 10~27일) 및 이유기(생후 27-64일)동안 실시하였다. 발효유 급여는 포유기에 top-dressing법(2리터 /일)로 공급하였으며 이유기에는 별도의 급여기를 이용하여 20리터/일씩 공급하였다. 포유기간동안 증체량은 처리구간 차이는 없었다. 반면에 이유기간동안 증체량 및 사료섭취량은 대조구 대비 발효유 급여구에서 통계적으로 증가하였고 사료요구율은 대조구보다 발효유 급여구에서 유의한 개선을 나타냈다. 분변 내 Echerichia coli 및 Lactobacillus spp.는 발효유 급여구에서 대조구보다 각각 통계적으로 감소 및 증가하였다. 또한, 분변 유래 황화수소 및 총메르캅탄은 발효유 급여에 따라 유의하게 감소하였다. 게다가 장 융모 발달은 대조구보다 발효유 급여구에서 형태학적으로 조밀한 상태가 확인되었다. 그러나 혈중 혈구세포 및 영양대사지표들은 처리구간 차이를 나타내지 않았다. 이러한 결과는 발효 유의 급여는 성장 및 장 내 환경을 개선시켜 이유 전·후 자돈의 생산성 향상에 기여할 것으로 사료된다.

Keywords : Fermentation, Productivity, Microorganism, Noxious Gas, Weaner Pigs

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1. Introduction

Food waste has become a global issue, due to a steady increase in living standards and population [1]. Animal-based food only accounts for approximately 12 % of the total waste, but it has a greater impact on the economy and environment rather than other food [2]. As for milk, in particular, about 100,000 kilotons were discarded around the world in 2009 alone [3], which is expected to further increase due to the unpredicted outbreak of COVID-19. Since milk contains proteins, fats, lactose, and minerals as well as all types of essential amino acids, it has a high economic value [4]. Recent studies have revealed that ethanol [5], carbon material for supercapacitors [6], and protein [7] can be produced from fresh milk that has expired. Thus, it is necessary to start developing more ways to efficiently use expired milk.

In the swine industry, weaning is a critical period from an economic point because weight at the weaning has a strong positive correlation with market weight [8]. In addition, weight of the weaned piglets is dependent on the intake of sow's milk owing to support bone health and digestive and immune systems by rich nutrients such as protein and vitamins [9,10]. The lactose in milk indicates beneficial effects on the growth of intestinal microorganisms, which contribute to digestion and absorption of dietary nutrients by previous review [11]. However, milk production of sows is highly variable, and a large litter size can lead to malnutrition in suckling piglets [12]. Thus, it is important to supply suckling piglets with additional sources of nutrients in order to optimize growth performance [13,14].

Supplementing animal feed with probiotics or fermented products have shown profitable effects such as inhibiting harmful bacteria growth in the intestine, reducing odor emission in feces, improving intestinal development, and enhancing nutrient digestibility and growth performance [15-17]. Fermented milk (FM) is a nutritionally superior food product containing lactic acid, peptone, peptides, and other key nutrients [18]. Furthermore, FM fed in early-weaned pigs has been shown to enhance growth performance [19].

Therefore, we produced the FM with expired milk and there are supplied the FM to piglets during the pre- and post-weaning periods in order to observe its effects on performance, blood properties, intestinal microflora and villi development.

2. Materials and methods

2.1 Fermented milk preparation

Expired market milk was obtained from local markets. Fermented milk (FM) was prepared by mixing the 100 g of commercial yogurt with the 20 liter of milk, and then incubating the mixture at room temperature (approximate 25 °C) for three days. We analyzed the microflora content of the resulting product using a selective medium, which revealed a 3.0×10^8 colony-forming (CFU)/g of *Bacillus* and 3.5×10^8 CFU/g of *Lactobacillus*.

2.2 Experimental design and animal management

All animals and protocols used in this study were reviewed and approved by the Animal Care and Use Committee of National Institute of Animal Science (NIAS-2020-459). Ten crossbred sows (Landrace×Yorkshire) were inseminated by Duroc boars, and allocated into farrowing crates during gestation periods. After parturition, a total 100 piglets (born body weight 1.43 ± 0.17 kg) and 10 sows were randomly assigned to two experiment groups (control group and FM treatment group) with five replications for each group. One sow and ten piglets per single pen were placed for pre-weaning periods (born to 27 days). After 10 days, commercial creep feed was

provided to the all experimental groups, and the FM (2 liters/pen/day) was additionally supplied to FM treatment group by top dressing. After 27 days, all the piglets were separated from the sow, and 48 piglets from each experiment group were relocated to the floor pen with three replications per replication). (16 piglets During the post-weaning period (27 to 64 days), all the experiment groups were fed a commercial diet, and the FM (20 liters/pen/day) was additionally supplied to FM treatment group by using an extra feeder. All animals used in this study were allowed water and feed ad libitum. The chemical compositions of commercial feed in pre- and post-weaning period were shown in Table 1.

Table 1. The chemical compositions of the basal diets

Items	Pre-weaning (10-27 day-old)	Post-weaning (27-64 day-old)
ME, Mcal/kg	3.35	3.35
CP, g/kg	180	170
Lysine, g/kg	15.6	13.9
Methionine, g/kg	4.7	4.2
Calcium, g/kg	7.8	6.8
Phosphorus, g/kg	6.4	5.8

Abbreviation: ME, Metabolizable energy; CP, Crude protein.

2.3 Growth performance

Body weight (BW) of each pig was obtained on the initial and final day of each pre- and post-weaning periods. The average daily gain (ADG) of the pre- and post-weaning periods was calculated by dividing weight gain by the number of feeding days, respectively. Average daily feed intake (ADFI) was obtained from the feed intake for each replicate pen per treatment for the post-weaning period. The feed conversion ratio (FCR) was calculated by dividing the ADG by the ADFI.

2.4 Sample collection and analysis

At the termination of the feeding trial, six pigs

from each experimental group were selected based on average BW and sacrificed; samples for analysis were collected and treated as follows. Blood was collected in tubes both with and without ethylenediamine tetraacetic acid (EDTA).

The whole blood in the EDTA tubes was immediately transferred to the laboratory, and corpuscle compositions were evaluated using an automatic blood corpuscle analyzer (Hemavet 950FS, Drew Scientific, Oxford, UK). Serum was isolated by centrifuging at 2,000 ×g and 4 °C for 15 min. and stored at -75 °C before analysis. Glucose, total cholesterol (T-CHO), total protein (T-PRO), triglyceride (TG), blood urea nitrogen (BUN), and non-esterified fatty acid (NEFA) in the serum were measured using an automatic biochemical analyzer (Hitachi 7180, Hitachi, Tokyo, The Japan). concentration of immunoglobulin G (IgG, E101-104, Bethyl Laboratories Inc., USA), tumor necrosis factor-alpha (TNF- α , PTA00, R&D Systems, USA) and cortisol (CSB-E06811p, Cusabio Biotech Co. Ltd., China) were analyzed using enzyme-linked immunosorbent assay kits. All analyses were conducted according to the protocols recommended by the manufacturers.

Feces were collected from the large intestine, and transferred into the laboratory for analysis of fecal microflora contents and noxious gas emissions. For the fecal microbial analysis, 1 g of feces sample was serially diluted to 10^{-9} with a sterile 0.85 % NaCl solution. The dilutions were inoculated onto two selective medium plates for Escherichia coli (E. coli, MacConkey agar, BD, Difco, USA) and Lactobacillus spp. (MRS agar, BD, Difco, USA) and then incubated at 37 °C for 24 h under aerobic conditions. At the end of the incubation, microbe colonies were counted. Emission of fecal noxious gas was measured according to the method of a previous study [23]. Briefly, a 200 g of feces sample was incubated in 2 liter of airtight container at 32 °C for 30 h. A gas sampling pump (Gastec, GV-110S, UK) with

detector tube was carefully inserted 2 cm above feces and the hydrogen sulfide (tube No. 4LK) and total mercaptans (tube No. 70) were measured.

Ileal tissue was collected from 20 cm ileum at 30 cm from the cecal junction, and carefully flushed with cold saline solution by syringe. The tissue was immediately immersed in 10 % neutral buffered formalin. The tissue was submerged thrice in 0.1 M phosphate buffered saline (PBS, pH 7.4), and then cut into 0.5 cm segments and placed in 1 % osmium tetroxide (in the 0.1 M PBS) for 1 h at 4 °C under a light block. Following fixation, the segment was rinsed thrice by immersing it in 0.1 M PBS for 15 min and then dehydrated via serial submersion using 50, 70, 90, and 100 % ethanol. The dehydrated segment was freeze-dried at -50 °C and the lyophilized segment was placed on a specimen stub by carbon sealing. Ileal villi morphology was observed using a TM-1000 scanning electron microscope (Hitachi, Tokyo, Japan).

2.5 Statistical analysis

All data were analyzed using the Student's t-test from SPSS version 17.0, and differences between treatment groups were determined to be significant if the p value was less than 0.05.

3. Results

The growth performance of pre- and post-weaning piglets fed FM is shown in Table 2. No significant differences were found in the BW and ADG between the control and FM treatment groups during the pre-weaning period. However, during the post-weaning period, the piglets fed FM showed higher ADG than in the control group; whereas their ADFI was lower ($p\langle 0.05 \rangle$). Similarly, the FCR was lower in the FM treatment group compared with the control group ($p\langle 0.001 \rangle$).

Table 2.	Effect of fermented milk supplements on the	e
	growth performance in piglets during the	į
	pre-and post-weaning periods	

Items	Со	ntrol	Trea	itment
Pre-weaning (10 to 27	d)			
Born live BW, kg	1.42	± 0.06	1.43	± 0.20
Weaning BW, kg	7.69	± 0.53	8.19	$\pm 0.20^{t}$
ADG, g	231.93	± 17.77	250.37	$\pm 3.15^{t}$
Post-weaning (27 to 64 d)				
Initial BW, kg	7.62	± 1.61	8.15	± 1.09
Final BW, kg	23.20	± 2.60	25.79	± 1.28
ADG, g	421.00	± 29.06	476.73	± 13.25 [*]
ADFI, g	758.00	± 30.00	696.00	± 20.00 [*]
FCR	1.80	± 0.06	1.46	± 0.01***

Control: basal diet, Treatment: basal diet + fermented milk. All traits in this table were analyzed with pen as the experimental unit (Pre-weaning, n= 5: Post-weaning, n= 3). Values are mean \pm standard deviation. Abbreviation: BW, body weight: ADG, average daily gain: ADFI, average daily feed intake: FCR, feed conversion ratio. , $\rho(0.05)$; ***, $\rho(0.001; {}^{t}, \rho(0.1)$.

The blood corpuscle compositions and serum metabolites of piglets fed FM during the pre- and post-weaning periods are presented in Tables 3 and 4, respectively. Leukocyte, erythrocyte, and thrombocyte counts did not differ between the control and FM treatment groups (Table 3). Similarly, serum glucose, T-CHO, TG, T-PRO, BUN, NEFA, IgG, TNF- α , and cortisol levels were not influenced by FM supplementation (Table 4).

Table 3. Effect of fermented milk supplements on the complete blood cell count in weaning piglets

Items	Control	Treatment
Leukocytes		
WBC, $\times 10^3/\mu L$	17.06 ± 1.27	18.98 ± 3.04
NEU, $\times 10^3/\mu L$	5.31 ± 0.27	5.98 ± 1.37
LYM, $\times 10^3/\mu L$	10.02 ± 1.15	11.21 ± 1.74
MONO, $\times 10^3/\mu L$	0.56 ± 0.14	0.68 ± 0.19
EOS, $\times 10^3/\mu L$	0.99 ± 0.24	0.89 ± 0.19
BASO, ×10 ³ /µL	0.18 ± 0.04	0.22 ± 0.08
Erythrocytes		
RBC, ×106/µL	6.70 ± 1.06	6.22 ± 0.84
HGB, g/dL	11.63 ± 1.25	11.08 ± 0.97
Thrombocytes		
PLT, $\times 10^3/\mu L$	469.25 ± 49.98	421.00 ± 97.19

Control: basal diet, Treatment: basal diet + fermented milk. Data represent means based on 6 replicate per experimental group. Values are mean ± standard deviation. Abbreviations: WBC, White blood cell: NEU, Neutrophil; LYM, Lymphocyte; MONO, Monocyte; EOS, Eosinophil; BASO, Basophils; RBC, Red blood cell; HGB, Hemoglobin; C; PLT, Platelet.

Items	Control	Treatment
GLU, mg/dL	107.83 ± 21.22	101.67 ± 11.98
T-CHO, mg/dL	84.67 ± 11.08	90.50 ± 11.33
T-PRO, g/dL	8.57 ± 0.75	8.77 ± 0.40
TG, IU/L	69.83 ± 16.23	76.67 ± 16.23
BUN, mg/dL	13.15 ± 3.45	12.07 ± 1.68
NEFA, µmol/L	28.17 ± 5.71	23.83 ± 2.56
IgG, mg/mL	44.40 ± 25.55	46.09 ± 20.21
TNF-a, pg/mL	66.43 ± 23.76	70.02 ± 15.38
Cortisol, ng/mL	88.18 ± 28.30	79.75 ± 15.95

Table 4.	Effect of fermented milk supplements or	1
	the serum characteristic in weaning pigle	ts

Control: basal diet, Treatment: basal diet + fermented milk. Data represent means based on 6 replicate per experimental group. Values are mean \pm standard deviation. Abbreviation: Glucose, GLU: T-CHO, total cholesterol: T-PRO, total protein: TG, triglyceride: BUN, blood urea nitrogen: NEFA, non-esterified fatty acid: IgG, immunoglobulin G: TNF- α , tumor necrosis factor-alpha.

The microflora composition and noxious gas emission from the feces of piglets fed FM during the pre- and post-weaning periods are shown in Table 5 and Fig. 1, respectively. Counts of *E. coli* were lower in the FM treatment group than in the control group (p<0.05; Table 5). The piglets from the FM treatment group showed higher counts of *Lactobacillus* spp. than in the control group (p<0.05; Table 5). The emissions of fecal hydrogen sulfide and total mercaptans were also significantly lower in the FM treatment group than in the control group (p<0.05; Fig. 1).

Table 5. Effect of fermented milk supplements on the fecal microbial flora composition in weaning piglets

Items	Control	Treatment
<i>E-coil</i> , log ₁₀ CFU/g	7.36 ± 0.16	$7.07 \pm 0.28^{*}$
LAB, log ₁₀ CFU/g	5.89 ± 0.31	$7.63 \pm 0.15^{***}$

Control: basal diet, Treatment: basal diet + fermented milk. Data represent means based on 6 replicate per experimental group. Values are mean ± standard deviation. Abbreviation: *E-coil, Escherichia coli:* LAB, *Lactobacillus* spp.; CFU, colony forming units. *, p(0.05); ***, p(0.001).



Fig. 1. Noxious gas emission in feces of weaned piglets supplemented with without or fermented milk during preand post-weaning period. Data represent means based on 6 replicate per experimental group. Values are mean ± standard deviation. Black and white bar are the control and treatment groups, respectively, with error bars as stand deviation. ppm, part per million; **, $p\langle 0.01$.

The morphology of the ileum in piglets fed FM during the pre- and post-weaning periods is shown in Fig. 2 The tissue of the FM treatment group had denser ileal villi than that in the control group.



Fig. 2. Morphological characteristics of ileum villi of weaned piglets supplemented with or without fermented milk during pre- and post-weaning period. (A) and (B) are the ileal villi of control, and (C) and (D) are the ileal villi of treated pigs. Scale bar, 500 µm.

4. Discussion

The ADG and BW during pre-weaning period in this study tended to be higher (250 vs. 232 g; 8.19 vs. 7.68 kg, respectively; p(0.1) in the FM treatment group compared with the control group. Subsequently, growth performance during the post-weaning period in this study was significantly improved in the FM treatment group than in the control group. These results are similar to those of a previous study, which showed enhanced growth and intestinal environments in pigs fed fermented liquid feed [20]. Another study demonstrated that supplementation of microbial additive including 6.0 × 108 CFU/g L. reuteri and 3.0 × 106 CFU/g L. reuteri improved body weight and stress in weaning piglets [21]. Generally, fermentation products or probiotics stimulate proteolysis and protein digestion in the stomach by increasing organic acid production, thereby lowering pH and increasing hydrolase activity [17,18,20]. Furthermore, fermented feed fed to pigs ameliorates digestibility of fat, energy, mineral and protein [22]. Therefore, improvement of growth performance would be resulted from increasing nutrient utilization due to by the FM supplementation fed during the pre- and post-weaning periods, although we did not determine the nutrient digestibility in this study.

The complete blood cell count was investigated using six pigs from each treatment group; however, two samples of the control group were excluded from statistical analysis because they indicated values below the detection limit of the analyzer used in this study. No differences were found in any blood corpuscle parameters between the control and FM treatment groups. Similarly, previous studies report no changes in blood corpuscle parameters in gilts fed probiotics [23]. Blood corpuscles are known to be influenced by both interior and exterior factors. Under exposure to stress, the number of lymphocytes and neutrophils increase and decrease respectively [24]. Erythrocytes are also reported to be affected by stress, health condition, and high oxygen demand [25]. However, all blood cell counts of both treatment and control pigs in this study were within the range of previously reported reference values [26]. Additionally, cortisol levels as a stress indicator did not differ between the control and FM treatment groups in present study. These results indicate that the piglets are not exposed to stress through the ingestion of FM.

Serum biochemical parameters including glucose, T-PRO, BUN and NEFA in this study were not statistically different between the control and FM treatment groups and were within the range of previously reported values [26]. These results are also similar to a previous study by Frimpong et al. [23], who reported that supplementation of dietary probiotics products did not affect biochemical parameters as serum globulin, albumin and T-PRO in pigs. However, in this study, NEFA slightly decreased in the FM treatment relative to the control group (23.83 vs. 28.17 μ mol/L). The NEFA, an index of lipid mobilization, is widely distributed throughout the body via release from adipose tissue under the condition of insufficient nutrient intake [27]. Therefore, high level of NEFA in the blood indicates an imbalance in energy metabolism. Consequently, the FM ingestion may help to ameliorate metabolic disorders resulting from low nutrient intake in weaning pigs under hyperprolific sows. Thus, further studies are required to determine the effects of fermented products under conditions of decreasing energy availability and intake.

According to one meta-analysis, dietary probiotics supplementation led to decreased T-CHO and low-density lipoprotein levels in the blood, whereas TG and high-density lipoprotein levels did not change [28]. Supplementation of probiotic milk product the FM have also been shown to reduce blood T-CHO and low-density lipoprotein levels [29]. Supplementation of FM with single probiotic results in no alteration in the T-CHO and TG levels, whereas multi probiotic-FM increases T-CHO levels [30]. Another study indicated no effects on T-CHO under either single or combined probiotic treatment [31]. In this study, the T-CHO and TG levels did not significantly differ between the control and FM treatment groups. These variations in lipid profiles may be due to different bacterial strains varying lipid-lowering with activity [32]. Furthermore, the biochemical composition is affected by various factors including diet, feeding environment, temperature and the general state as health, pregnancy, age, sex, and stress, however, the mechanisms affecting these biochemical markers are not yet clearly identified [33]. Thus, further study is needed to understand the roles of various metabolic lipid indicators in FM-fed piglets.

Our results showed that blood immunoglobulin, inflammatory and stress indicators were not influenced by FM supplementation. These results are in agreement with previous studies. Frimpong et al. [23] reported no changes of IgA, IgM, and cluster of differentiation 3 and 4 in gilt fed probiotic products. Similarly, other study showed no alteration on TNF- α and IgG in weaning piglets fed microbial *Clostridium butvricum* [34]. However, other previous studies have reported improvement of immune and inflammation responses as results of supplying probiotics to weaned [35] and growing [36] pigs. These contradictory reports may be attributed to the health state of the animals [33]. Mishra et al. [15] reported that immune intensification by probiotics is unexpressed in healthy animals but is detectable under challenging conditions such as acute stress. The pigs used in the present study were not exposed to any challenges or stress and remained healthy during the feeding trial. Thus, pigs in this study were not expected to show alterations in their immune indexes.

The FM-treated piglets in the present study showed higher fecal levels of *Lactobacillus* spp. in the control group (7.63 *vs.* 5.89 log10 CFU/g),

whereas their E. coli levels were lower (7.07 vs. 7.36 log10 CFU/g). Dietary supplementation with fermented products or probiotics have known to decrease harmful microorganisms. Similarly, supplying fermented probiotic products to pigs is shown to increase the abundance of several lactic acid bacterial genera, while the decrease of E. coli counts [22]. This suggested that the intestinal microflora composition is changed by the ingestion of fermented products or probiotics. Our results, which are similar to the reports cited above, can be attributed to the low pH in the digestive tract owing to an increase in organic acid synthesis following consumption of the fermented product, which then stimulates the proliferation of beneficial bacterial species and inhibits harmful ones in the intestine [21-23].

The level of noxious gas emitted from feces is directly associated with intestinal microorganism composition and nutrient availability [37]. Dietary addition of probiotics increased the activity of digestive enzymes improving the digestion of carbohydrate and fat [13]. Improvement of nutrient digestion can suppress the generation of odor-causing substances in the feces by reducing the material available for fermentation in the large intestine [37]. In this study, the FCR and fecal microflora of pigs fed FM was positively altered, and emissions of hydrogen sulfide and total mercaptans were significantly lower in the FM treatment group than in the control group (4 vs. 6; 1.3 vs. 3 ppm, respectively). These gas emission patterns are in agreement with previous study [17].

Nutrients are mainly absorbed via the epithelial cells on villi of the small intestine. Villus height and crypt depth are regarded as indexes of absorption capacity [34]. Thus, development of small intestine can be important for enhancing nutrient availability. Previous reports have indicated the positive influence of probiotic supplementation on the development of intestinal villi. In particular, the villi height and crypt depth were increased in pigs that had been treated with probiotics [35]. Similarly, other studies has reported that small intestine morphology is more developed by probiotics fed to growing-finishing pigs [15], fermented feed fed to growing pigs [36] and liquid feed fed to early weaning pigs [13]. These previous results are similar to those from the present study, which show a higher number of villi per unit area and better villi integrity under FM supplementation. This results suggest that the FM has a positive impact on intestinal villi development in piglets.

5. Conclusion

In conclusion, piglets fed FM showed improvements performance, growth fecal microflora in composition, and small intestine development. No negative effects on blood corpuscle, or serum biochemical and physiological profiles were noted in FM-treated piglets. These results indicate that the supplementation of FM can positively influence the productivity of pre- and post-weaning pigs without any negative effects. Furthermore, fermentation would be a useful application of milk that is past its expiration date.

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