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Effect of milk flavor supplementation on growth performance, nutrient digestibility, fecal score, and blood profiles in weaning piglets

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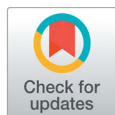
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Abstract

This study explored the effects of milk flavor (MF) supplementation on growth efficiency, nutrient absorption, fecal score, and blood profiles in weaning piglets. A total of 80 (21 days old) crossbred ([Yorkshire × Duroc] × Landrace) healthy weaned piglets with an initial body weight (BW) of 7.05 ± 1.22 kg were randomly allotted to one of two nutritive treatments with 8 repetitions and five pigs (2 female and 3 male) per pen. The experiment was divided into 2 phases (d 0 - 21, and d 21 - 42), and the dietary treatments consisted of TRT1, basal diet, TRT2 and basal diet + $1.0 \text{ g} \cdot \text{kg}^{-1}$ MF. At days 21 - 42 and the overall period, the average daily gain (ADG) and average daily feed intake (ADFI) increased ($p < 0.05$) by receiving the MF added feed. However, MF inclusion did not impact ($p > 0.05$) the feed efficiency (G : F) throughout the entire experiment. Piglets consuming the MF supplemented diet showed that the apparent total tract digestibility of dry matter (DM), nitrogen (N) and energy (E) did not vary significantly ($p > 0.05$) between the treatments. All through the experiment, the fecal score and blood profile of the piglets fed the flavor diet also remained unaffected ($p > 0.05$). In conclusion, MF addition to the diet of the piglets increased their body weight and had no adverse effects on nutrient utilization, fecal score, and blood profile. Thus, MF addition could improve the performance outcomes of weaning piglets.

Key words: growth performance, milk flavor, weaning piglets



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Introduction

In the pork production cycle, weaning is the most dangerous period because of the physiological adaptations and stresses that piglets undergo as a result of the concurrent stressors they are subjected to (Bruininx et al., 2002). To overcome weaning stress, it is important to give the piglets a palatable diet by the addition of sweeteners or pelleting the feed (Lewis et al., 1955). Antibiotics are supplemented to diets as growth promoters (AGP) to enhance feed efficiency, carcass weight, and to modulate gut micro biota (Van Immerseel et al., 2009). Overuse of AGP can lead to antimicrobial resistance in animals and serious health problems in humans through the food chain. As a result, the use of AGP in livestock feed has been strictly prohibited in many countries, including South Korea,

since 2011 (Shanmugam et al., 2021). Since most antibiotics have been prohibited, flavor imprinting in young animals' diets could counteract these problematic consequences (Zhong et al., 2011).

Flavors are feed additives that attempt to enhance the taste and smell of feed to stimulate feed intake. The higher nutrient consumption has acquired from controlled feed intake, strongly influenced by feed taste and flavor (Frederick and van Heugten, 2003). Feed digestion, absorption, and utilization can be promoted by using flavor to stimulate appetite. As a result, palatability is improved and poor ingredients become more appealing, which can ultimately lead to a loss of natural flavors (Rogers et al., 1988). Usage of the chosen flavor of feed attributes includes increasing acceptance of less palatable or altered diets, accumulative intake of palatable feed, and improving dietary acceptance through stressful times, such as piglet weaning (Bradley, 1980). A study found that adding flavor to creep feeds could enrich post-weaning feedback significantly when the same flavor was included in nursery diets. Hence, adding flavors to the creep feed could contribute to an improved feed intake and performance for pre-and post-weaning feed (Langendijk et al., 2007). The milky flavor and the fruit flavor can both increase feed consumption among growing pigs (Lv et al., 2012). In previous studies, participating in prenatal and postnatal flavor learning was shown to reduce stress-associated effects, impact offspring acceptance, and stimulate the consumption of similar-testing foods (Mennella et al., 2001; Oostindjer et al., 2010). Adding anise flavor to growing-finishing pigs' diets had a minor impact on growth performance and nutrient digestibility but had no effect on blood profiles (Ao et al., 2019). The inclusion of flavors during late gestation and lactation can improve reproductive performance by increasing FI and modulating the gut micro biota of sows (Wang et al., 2021). Studies have been unable to demonstrate an outcome of dietary sweeteners or flavors on the performance of pigs in some trials (Munro et al., 2000; Thacker and Haq, 2008).

We hypothesized that MF might have a number of positive effects on weaning pig performance. The objective of this study was to identify the impact of MF inclusion on the production efficiency, nutrient absorption, fecal score, and blood profiles in weaning piglets.

Materials and Methods

Ethical endorsement

This experimental protocol was reviewed and approved by Dankook University Cheonan, Korea, Animal Care, and Use Committee (DK-2-2112) to describe the management and care of piglets.

Source of flavors

The flavors used in the current study were manufactured by DadHank Biotechnology Corporation (Chengdu, People's Republic of China) as a non-hygroscopic powder.

Animal husbandry and dietary regimens

All equipment and pen for usage were sterilized prior to testing. A total of 80 crossbred ([Yorkshire \times Duroc] \times Landrace) 3 week old healthy weaned piglets with initial body weight (BW) of 7.05 ± 1.22 kg were purchased commercially from Gene-pig Farm (Gongju, Korea) for 42 days experiment. Pigs were housed based on body weight and sex and randomly

selected to 1 - 2 dietary treatments (8 replicate pens per treatment, 2 gilts, and 3 barrows per pen). Treatments consisted of i) TRT1, basal diet, ii) TRT2, basal diet + 1.0 g·kg⁻¹ MF. The diets were designed to meet or exceed the minimum requirements set by the National Research Council (NRC, 2012) for nutrients (Table 1), and DDK-801 feed mixer was used to mix feed ingredients finely (Daedong Tech, Anyang, Korea). The animals received a feeding program consisting of 2 phases: day 0 - 21 (phase 1), day 21 - 42 (phase 2). All piglets were housed in an environmentally organized room (0.26 m × 0.53 m for each pig) with a mechanically delivered ventilation system. All trial animals were provided the same basal diet during the experiment. Initially, the temperature was kept at 30°C, and then declined by 1°C every week. All over the experiment, every pen was furnished with a stainless steel feeder and a nipple drinker so that pigs were permitted *ad libitum* access to feed and water.

Sample measurement and laboratory procedures

Individual piglets BW were assessed initially and at the end of each phase to determine ADG using a GL-6000S weighing machine (G-Tech Inc., LTD., Seoul, Korea) while the extent of feed intake and rejection (on a pen basis) were documented to regulate gain/feed (G : F) ratio and ADFI.

An indigestible marker (0.2% Cr₂O₃, Samchun Pure Chemical Co., Ltd, Seoul, Korea) was included to the diet of piglets for one week prior to fecal collection to measure the digestibility of nutrients. At day 21 and 42, fresh fecal samples were casually accumulated by direct rectal massage from 5 piglets·treatment⁻¹. The collected fecal specimens were shipped to the laboratory within half an hour and stored at -20°C to prevent changes in the nutrient content. Fecal samples were thawed and dried (57°C for 72 h) afore analysis and grounded well to pass through a 1 mm screen. Nutrient digestibility of DM and N were examined according to the formula outlined by AOAC (2000). Chromium content was determined by ultraviolet absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). Gross energy (GE) was examined by measuring the heat of combustion in the specimens using a Parr 6100 bomb calorimeter (Parr Instrument Co., Moline, IL, USA). The apparent total tract digestibility (ATTD) was evaluated based on the chromium concentrations of diet and fecal samples using the subsequent formula:

$$\text{Digestibility (\%)} = \{1 - [(N_f \times C_d) / (N_d \times C_f)]\} \times 100 \quad (1)$$

Where N_f was the nutrient concentration in feces (% DM), N_d was the nutrient concentration in the diet (% DM), C_d was the chromium concentration in the diet (% DM), and C_f was the chromium concentration in the feces (% DM).

The fecal score was determined at initial, 21st and 42nd day. The following values corresponded to the score: 1 (well-firmed feces); 2 (slightly softer feces); 3 (partially formed and soft feces); 4 (loose and semi-fluid feces); 5 (watery and mucus-like feces). A pen-by-pen analysis of feces and stool consistency was conducted on each pig.

At the finish of the research, blood was collected from one pig from each of the eight replication pens, and the samples (5 mL) were placed into vacuum tubes containing no additive and tubes containing K₃EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to acquire serum and whole blood individually. The serum was separated from the blood sample by centrifuging (3,000 g) for 15 minutes at 4°C after blood collection. Blood samples were investigated using an automatic blood analyzer (HITACHI 747, Tokyo, Japan) to regulate the number of white blood cell (WBC), red blood cell (RBC), and lymphocyte (LYM).

Table 1. Diet composition (as-fed basis).

Item	Phase 1 ^x	Phase 2 ^x
Ingredient (g·kg ⁻¹)		
Extruded corn	444.9	619.7
Soybean meal, 480 g·CP·kg ⁻¹	162.0	253.0
Fermented soybean meal, 450 g·CP·kg ⁻¹	50.0	25.0
Fish meal, 660 g·CP·kg ⁻¹ Brazil	35.0	-
Soy oil	25.5	10.5
Lactulose	83.0	-
Whey	100.0	50.0
Di calcium phosphate	15.0	15.0
Sugar	30.0	-
Plasma powder, AP 920	30.0	-
L-Lys HCl	3.9	4.6
DL-Met	3.0	2.4
L-Thr	1.9	2.0
Choline chloride	1.0	1.0
Vitamin premix ^y	1.0	1.0
Trace mineral premix ^z	2.0	2.0
Limestone	9.8	11.3
Salt	2.0	2.5
Total	1,000.0	1,000.0
Calculated composition (g·kg ⁻¹)		
ME (kcal·kg ⁻¹)	3,540	3,410
CP	200	190.0
Lys	15.0	13.5
Met	6.2	5.3
Met + Cys	9.7	8.4
Ca	9.5	9.0
Total P	7.5	7.0
Avail P	5.5	4.3
Crude fat	50.2	39.8
Crude fiber	18.7	24.5
Analyzed composition (g·kg ⁻¹)		
CP	199.9	189.7
Lys	14.9	13.4
Ca	9.4	9.0
Total P	7.4	6.9

ME, metabolizable energy; CP, crude protein; Lys, lysine; Met, methionine; Cys, cysteine.

^x Phase 1, provided during d 0 to 14; phase 2, provided during d 15 to 42. Replaced the same amount of corn with lactulose to create dietary treatments.

^y Provided per kilogram of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D₃, 37.5 mg of vitamin E, 2.55 mg of vitamin K₃, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B₆, 24 µg of vitamin B₁₂, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of pantothenic acid.

^z Provided per kilogram of diet: 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, 62.5 mg of S, and 0.23 mg of Se.

Statistical analysis

A SAS general linear model procedure was used to analyze all data in a randomized complete block design (SAS Institute Inc., Cary, NC, USA), with the pen as the trial unit. In cases where substantial differences between treatments were initiated, they were parted using a t-test. The initial body weight was used as a covariate for ADG. A significant difference was determined by a $p < 0.05$, and a trend by a $p < 0.10$.

Results

In Table 2, it is shown that MF inclusion affected weaned piglets' growth performances. The piglets receiving MF supplement enhanced ADG during days 21 - 42 ($p = 0.023$) and overall period ($p = 0.011$), respectively. Moreover, MF led to a significant improvement in ADFI during days 21 - 42 ($p = 0.006$) and overall period ($p = 0.028$). Nevertheless, there was no difference ($p > 0.05$) detected on feed efficiency (G : F) throughout the trial.

Table 3 is shown the effects of MF addition on the ATTD of weaned pigs. By MF addition, the ATTD of DM, N and E did not alter ($p > 0.05$) between treatment groups during the entire experiment.

Table 4 is presented the results of MF inclusion on the fecal score of piglets. A diet supplemented with milk flavor did not affect ($p > 0.05$) the fecal score of piglets all over the experiment.

Table 5 is described the effect of MF supplementation on piglets' blood profile. Piglets that consumed milk flavor with diet showed no significant variation on WBC, RBC, and LYM between treatments throughout the trial.

Table 2. Effect of dietary supplementation of 'milk flavor' additive on growth performance in weaning piglets.

Item	TRT1	TRT2	SEM	p-value
Body weight (kg)				
Initial	7.05	7.05	0.003	0.999
Day 21	13.91	13.65	0.12	0.662
Day 42	24.32	25.06	0.27	0.100
Day 0 - 21				
ADG (g)	326	314	6	0.134
ADFI (g)	445	437	8	0.412
G : F	0.734	0.720	0.008	0.136
Day 21 - 42				
ADG (g)	477	553	12	0.023
ADFI (g)	708	800	17	0.006
G : F	0.675	0.691	0.014	0.426
Overall				
ADG (g)	402	434	6	0.011
ADFI (g)	576	619	10	0.028
G : F	0.698	0.701	0.009	0.776

TRT1, basal diet; TRT2, basal diet + 1.0 g·kg⁻¹ milk flavor; ADG, average daily gain; ADFI, average daily feed intake; G : F, feed efficiency; SEM, standard error of mean.

Table 3. Effect of dietary supplementation of ‘milk flavor’ additive on nutrient digestibility in weaning piglets.

Item	TRT1	TRT2	SEM	p-value
Day 21				
Dry matter (%)	81.86	82.71	0.75	0.438
Nitrogen (%)	81.80	83.55	0.69	0.182
Energy (%)	83.34	84.31	0.61	0.206
Day 42				
Dry matter (%)	81.03	82.58	0.76	0.301
Nitrogen (%)	79.49	80.80	1.08	0.414
Energy (%)	80.99	81.98	0.54	0.386

TRT1, basal diet; TRT2, basal diet + 1.0 g·kg⁻¹ milk flavor; SEM, standard error of mean.

Table 4. Effect of dietary supplementation of ‘milk flavor’ additive on fecal score in weaning piglets.

Item	TRT1	TRT2	SEM	p-value
Initial	3.16	3.17	0.046	0.322
Day 21	3.10	3.13	0.048	0.503
Day 42	3.13	3.16	0.040	0.672

TRT1, basal diet; TRT2, basal diet + 1.0 g·kg⁻¹ milk flavor; SEM, standard error of mean.

Table 5. Effect of dietary supplementation of ‘milk flavor’ additive on blood profile in weaning piglets.

Item	TRT1	TRT2	SEM	p-value
Initial				
WBC	15.92	14.71	2.34	0.614
RBC	6.61	4.87	0.57	0.072
LYM	43.98	46.25	0.64	0.205
Final				
WBC	15.98	17.70	1.40	0.672
RBC	5.72	4.45	0.51	0.263
LYM	38.58	40.20	1.10	0.654
Difference				
WBC	0.06	2.99	0.94	0.058
RBC	-0.89	-0.42	0.06	0.191
LYM	-5.40	-6.05	0.46	0.449

TRT1, basal diet; TRT2, basal diet + 1.0 g·kg⁻¹ milk flavor; WBC, white blood cell; RBC, red blood cell; LYM, lymphocyte; SEM, standard error of mean.

Discussion

In our present study, we tried to investigate the effects of flavor addition on the performance of piglets' health. It is widely well-known that the performance of piglets impacts the productivity of the entire swine industry. Flavor supplementation was particularly effective in the early weaning piglets, as it reduced weaning stress (Jacela et al., 2010).

In line with our study, dietary supplementation of flavor enhanced growth performance (GP) and FI for sows and ADG and ADFI for piglets (Sun et al., 2019). Wang et al. (2021) pointed out that, at a level of 0.1% flavor inclusion in the maternal diet through late gestation and lactation periods improved ADG of piglets, and ADFI of sows exhibited an increasing trend.

Wang et al. (2014) assumed that fruit-milk-anise flavor improved ADG and ADFI of nursing pigs. The addition of a volatile flavored herbal extract mixture to the diet of weaned piglets improved GP, ADG, and feed conversion in weaned piglets (Dang et al., 2021). Similarly, feed flavors examined to improve FI and performance in lactating sows and weanling pigs (Torrallardona et al., 2000; Johnston et al., 2003). In addition, Long et al. (2017) demonstrated that sows fed 0.05% or 0.10% milky flavors, had a higher ADFI of sows and ADG of piglets. Prenatal exposure to oregano essential oil flavor through maternal intake greatly influenced feeding preferences in lambs till adulthood, but the ADFI did not affect (Simitzis et al., 2008). Ao et al. (2019) demonstrated that pigs fed the ANF (anise flavor) diets had a higher ADG and ADFI than pigs fed the CON and APF (apple flavor) diets. In addition, the use of star anise flavor proved to be a useful tool for improving FI, and GP in piglets (Langendijk et al., 2007; Wang et al., 2015). Similarly, by using milk flavor in the diet we found significant GP and FI in piglets. Previous works indicated that feed flavorings are often included in the diets of weaning pigs to increase diet acceptance and energize FI (van Heugten et al., 2002; Sulabo et al., 2010). In contradictory, feed flavor (0.05%) did not affect the growth performance of growing-finishing pigs (Thacker and Haq, 2008). Therefore, the main reason for the increase in body weight, a daily gain of weaned piglets in this study was mainly fed preference and palatability of the milk-flavored supplement.

For the measurement of the nutritional value of animal fed, digestibility is an essential factor. The finding of our current study reported that the weaned pig's feed supplementation of MF showed no significant difference in nutrient digestibility (ND). Dietary flavors inclusion in diets decreased intestinal permeability and increased the digestibility of DM and E (Long et al., 2017). Sun et al. (2019) demonstrated that feeding sows and their weaned piglets with a diet containing anise oil flavor enhanced ND in weaned piglets, inconsistency with this study. Ao et al. (2019) reported an improvement in ATTD of N by consuming a flavor included diet compared to the CON diet in growing pigs. A study conducted by Lei et al. (2017) found that DM, N, and GE digestibility were all increased upon combined flavor ($500 \text{ mg} \cdot \text{kg}^{-1}$) and sweetener ($150 \text{ mg} \cdot \text{kg}^{-1}$) addition. In agreement with this study, addition of flavor to growing-finishing swine-fed diets containing lucerne did not affect ND (Thacker and Haq, 2008). It is widely believed that the digestive capacity of the small intestine matures with age; therefore, the inconsistent outcomes may be due to the young age (Graham et al., 1986) and reduction of enzyme activity of piglets (Kelly et al., 2007).

Gut micro biota configuration is influenced by the development animal's stage, physiological state, and various environmental factors such as nutritional content, pathogen infection, antibiotic use, etc (Ji et al., 2017). In recent studies, it has been discovered that the gut microbiota of sows plays a crucial role in the performance of mother and offspring (Wang et al., 2018; Li et al., 2019; Xiong et al., 2019). The effect of dietary flavor addition on sow fertility was accompanied by a reduction in potentially pathogenic bacteria as well as an increase in beneficial bacteria (Wang et al., 2021). In a study conducted by Lee and Kim (2018), there was no statistically significant difference between treatments regarding the efficacy of creep feeding on piglets' fecal scores. According to the study by Sun et al. (2019), through the administration of flavor, feed intake was enhanced to a certain extent, helping to promote intestinal development and, ultimately, lowering the fecal score. There was no significant difference found in diarrhea scores when feed flavor supplementation was used pre- and post-weaning periods (Yan et al., 2011). In addition, Oostindjer et al. (2010) revealed that prenatal exposure to anise the flavor resulted in diarrheal problems in piglets compared with non-exposed animals. However, in the current study, the fecal score in pigs receiving milk flavor showed no significant difference. This may be due to the quality and quantity of supplements used, and weaning age because Sun et al. (2019) showed that fecal score has an interactive relationship between weaning age and flavor supplementation.

Very few studies have been performed to estimate the outcome of flavor on the immune response of piglets. Yan et al. (2011) presented that feed flavor supplementation on pre-and post-weaning piglet and sow showed no differences in blood characteristics among treatments. In agreement with our study, Ao et al. (2019) stated that no effect on blood characteristics (WBC, RBC and LYM) was observed in their study by flavor inclusion, which could be attributed to a more developed digestive system, improved immunity, and increased resistance to intestinal disease. In our present study, there was no effect observed on WBC, RBC and LYM in weaning piglets. The controversial results on blood profile may be due to the presence of indigenous micro flora, the difference in the types and age of animals.

Conclusion

In brief, the data from this study pointed out that the application of MF inclusion had a significant effect on growth performance, without a significant change in nutrient digestibility, fecal score, and blood profile in weanling piglets. According to these improvements in piglets' health, milk flavor can be economically useful to the swine production industry.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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