ANIMAL

# Effect of saccharin (sweetener) supplementation on growth performance, nutrient digestibility, fecal microbial, and fecal score in weaning piglets

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## Abstract

Sweeteners are beneficial for weaning pigs as they contribute to improved palatability, increased feed intake, energy provision, gut health support, and alleviation of post-weaning stress. This study was conducted to evaluate the effects of saccharin on growth performance, nutrient digestibility, fecal microbial, and fecal score of weaning pigs. A total of 80 (21 days old) crossbred ([Yorkshire  $\times$  Duroc]  $\times$  Landrace) healthy weaned piglets with an initial body weight (BW) of  $6.85 \pm 1.36$  kg were randomly allotted to one of two nutritive treatments with 8 repetitions and five pigs (2 female and 3 male) per pen. The dietary treatments consisted of control (CON): basal diet; treatment (TRT): basal diet + 0.03% saccharin. The result showed that average daily gain (ADG) and average daily feed intake (ADFI) were increased (p < 0.05) in weaning fed saccharin supplemented diets compared with control diet. However, there was no significant difference in nutrient digestibility, fecal microbial, and fecal score among dietary treatments (p > 0.05). In summary, the sweetener supplementation with diet had a significant effect on ADG and ADFI without compromising nutrient digestibility, fecal microbial, and fecal score among dietary score in the weaning pigs.

Keywords: growth performance, nutrient digestibility, saccharin, weaning pigs

## Introduction

Weaning is a critical period in swine production, marked by the transition from a sow's milkbased diet to a solid feed intake (Holman et al., 2021). This transition is often associated with stress, decreased feed intake, and changes in gut microbiota composition, which can adversely affect the growth performance and health of weaning piglets (Arnaud et al., 2023). To mitigate the challenges associated with weaning, various nutritional strategies, including the use of feed additives such as sweeteners, have been explored.

Saccharin, a widely used artificial sweetener, has gained attention as a potential dietary supplement for weaning piglets due to its palatability-enhancing properties and potential benefits on

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of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/ licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. gut health (Zhang et al., 2020). Previous studies have reported that artificial sweeteners, routinely included in piglets' diet, were thought to enhance feed palatability, feed intake and reduces stress (Moran et al., 2010). The study showed that the addition of high intensity sweeteners to diets for weaning pigs can affect feed intake (FI) characteristics to a limited extent (Sterk et al., 2008). However, limited information is available on the effects of saccharin supplementation specifically on the growth performance, nutrient digestibility, fecal microbial populations, and fecal score of weaning piglets.

Understanding the impact of saccharin supplementation on weaning piglets is essential for optimizing nutritional strategies during this critical phase of development. Therefore, this study seeks to examine the impact of saccharin supplementation on the growth performance, nutrient digestibility, fecal microbial populations, and fecal score of weaning piglets. By clarifying the potential advantages or disadvantages of adding saccharin to the diet, this study aims to aid in the creation of efficient nutritional strategies to promote the health and welfare of weaning piglets within swine farming operations.

### **Materials and Methods**

This experimental protocol was reviewed and approved by Dankook University, Cheonan, Korea, Animal Care, and Use Committee (DK-2-2313) to describe the management and care of piglets.

#### Animal husbandry and dietary regimens

In a 42-day trial, 80 crossbred weanling piglets ([Yorkshire × Landrace] × Duroc),  $(6.85 \pm 1.36 \text{ kg})$  were randomly allocated with two nutritional treatments (eight replicate pens, each including two gilts and three barrows) based on the body weight and sex. The nutritional treatment consisted of a basal diet (control), and a basal diet with 0.03% saccharin (TRT). The trial period was allocated into three phases: week 0 - 1 of age (phase 1), week 2 - 3 of age (phase 2), and week 4 - 6 of age (phase 3). The diet formulation in our experiment was followed, according to the guidelines of NRC (2012) (Table 1). Each pig had a 0.26 m × 0.53 m area in an environmentally maintained room with a mechanical aeration system. Throughout the experiment, the piglets had unlimited access to water and feed from pens with a nipple drinker and a feeder. Artificial light was provided 12 h per day. During the first week, the room's ambient temperature was kept at about 30°C; thereafter, it was lowered by 1°C every week.

Items	Phase 1	Phase 2	Phase 3
Ingredients (%)			
Corn	37.62	52.03	60.85
Soybean meal	18.25	16.68	19.05
Tallow	2.90	2.69	2.25
Fermented soybean meal	5.00	4.00	3.00
Animal protein	5.00	3.00	2.00
Limestone	0.93	0.94	1.00
Salt	0.20	0.10	0.10

#### Table 1. Composition of weaning pig diets (Continued).

Items	Phase 1	Phase 2	Phase 3
Lactose	13.46	7.78	3.18
Sugar	3.00	3.00	3.00
Whey protein	11.00	7.00	3.00
Monodicalcium phosphate	1.10	1.30	1.35
Methionine (99%)	0.22	0.15	0.09
Lysine (78%)	0.51	0.65	0.57
Mineral mix <sup>y</sup>	0.20	0.20	0.20
Vitamin mix <sup>z</sup>	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03
ZnO (80%)	0.38	0.25	0.13
Total	100.00	100.00	100.00
Calculated value			
Crude protein (%)	20.00	18.00	18.00
Metabolizable energy (kcal·kg <sup>-1</sup> )	3,450	3,400	3,350
Calcium	0.80	0.80	0.80
Phosphorus	0.60	0.60	0.60
Lysine (%)	1.60	1.50	1.40
Methionine (%)	0.48	0.40	0.35
Fat (%)	4.56	4.79	4.66
Lactose (%)	20.00	12.00	5.00

Table 1. Composition of weaning pig diets.

<sup>y</sup> Provided per kilogram of diet: vitamin A, 15,000 IU; vitamin D<sub>3</sub>, 3,750 IU; vitamin E, 37.5 mg; vitamin K<sub>3</sub>, 2.55 mg; thiamin, 3 mg; riboflavin, 7.5 mg; vitamin B<sub>6</sub>, 4.5 mg; vitamin B<sub>12</sub>, 24 μg; niacin, 51 mg; folic acid, 1.5 mg; biotin, 0.2 mg; pantothenic acid, 13.5 mg.

<sup>2</sup> Provided per kilogram of diet: Zn, 37.5 mg; Mn, 37.5 mg; Fe, 37.5 mg; Cu, 3.75 mg; I, 0.83 mg; S, 62.5 mg; Se, 0.23 mg.

#### Sample measurement and laboratory procedures

#### Growth performance

Piglets' body weights were recorded on days 1, 7, 21, and 42 to calculate the average daily gain (ADG). To calculate average daily feed intake (ADFI), the consumed feed and remained feed was measured based on the pen. Gain to feed ratio (G : F) was calculated using ADG and ADFI.

#### Nutrient digestibility

Chromium oxide ( $Cr_2O_3$ , 0.20%) was added to the diets and given to pigs seven days before collecting fecal samples. Two pigs (one barrow and one gilt) were chosen from each pen after the experiment (42 days) to be the subjects of a fecal sampling that was collected via rectal palpation. Firstly, samples were merged and pooled based on the pen, and then a sample selected at random was preserved at -20°C in the freezer until it was analyzed. Fecal samples were dried at 60°C for 72 h to conduct chemical analysis. Following this, they were crushed so they were able to move through a 1 mm screen. Dry matter and nitrogen levels in the feed and fecal samples were examined following AOAC (2000) method. To determine energy (E) and excrete samples, specimens were taken and put in a calorimeter (Parr Instrument Company, USA) to assess thermal combustion in the samples and chromium, which was analyzed by atomic absorption spectrophotometer (Shimadzu UV-1201, Shimadzu Corp., Japan). We used the formula for apparent total tract digestibility (ATTD) as follows: Digestibility (%) = [1 - (Nf × Cd) / (Nd × Cf) ] × 100, where Nf stands for the number of nutrients in feces (% DM), Nd for the number of nutrients in the diet (% DM), Cd for the amount of chromium in the diet (% DM), and Cf for the amount of chromium in the feces (% DM) (Ahammad et al., 2023).

#### Fecal microbiome

Two pigs (each pen) were selected to collect fecal samples through rectal palpation and after that; these samples were taken in an ice box to the laboratory for further testing to measure the gastrointestinal microbial flora. Fecal samples were obtained to study the microbiota in the feces. To prepare every sample, 1 g measured fecal sample diluted with 9 mL of 1% peptone water was vortexed for proper mixing. Samples were mixed sequentially from  $10^{-1}$  to  $10^{-6}$  and were inserted by 50 µL in two selective agar medias, MRS Agar (Difco, USA) for *Lactobacillus* and MacConkey agar (Difco, USA) for *E. coli*. Before colony counting, *Lactobacillus* and *E. coli* were incubated at 39°C for 48 h, 37°C for 24 h, respectively. The results of the encompassing of the colonies were then displayed as  $log_{10}$  converted data.

#### Fecal score

Feces were measured and recorded on a pen source at 08:00 and 20:00 h on days 7, 14, 21, 28, 30, and 42. The fecal score was determined as the average of the five pigs in each pen using the fecal scoring system described below 1, firm, formed stools; 2, hard, dry pellets; 3, soft, moist stools that retain their shape; 4, unformed, soft stools that conform to the shape of the vessel; and 5, watery, pourable liquids (Ahammad et al., 2023).

#### Statistical analysis

The data were analyzed using a completely randomized design and a t-test conducted with SAS software (SAS Institute Inc., USA). Data variability was indicated by the pooled standard error (SE), a significant difference was determined by a p < 0.05, and a trend by a p < 0.10.

### Results

Sweetener supplementation in weaning diets led to a significant increase in ADG and ADFI compared to the control diet (p < 0.05), as shown in Table 2. However, there were no significant effects on the digestibility of dry matter, nitrogen, and energy (p > 0.05), as indicated in Table 3. Additionally, sweetener supplementation did not result in significant changes in the levels of *lactobacillus* and *E. coli* (p > 0.05), as observed in Table 4. Moreover, the inclusion of sweetener at up to 0.03% in the weaning pig diet did not lead to any alterations in fecal score during the feeding trial (p > 0.05), as depicted in Table 5.

Items	CON	TRT	SEM	p-value
Week 0 - 1				
ADG (g)	269	277	6	0.5698
ADFI (g)	343	356	7	0.4697
G : F	0.78	0.77	0.008	0.9795
Week 2 - 3				
ADG (g)	397	395	9	0.7767
ADFI (g)	533b	575a	12	0.0468
G : F	0.74	0.68	0.009	0.7777
Week 4 - 6				
ADG (g)	579	575	14	0.3749
ADFI (g)	1,084	1,085	23	0.2945
G : F	0.53	0.52	0.017	0.5677
Overall				
ADG (g)	444b	447a	9	0.0389
ADFI (g)	714b	732a	14	0.0455
G : F	0.62	0.61	0.007	0.7308

Table 2. The effect of saccharin (sweetener) supplementation on growth performance in weaning pigs.

Number of replicates, eight.

CON (control), basal diet; TRT (treatment), basal diet + 0.03% saccharin; SEM, standard error of the mean; ADG, average daily gain; ADFI, average daily feed intake; G : F, gain to feed ratio.

a, b: Means in the same row with different superscripts differ (p < 0.05).

Items (%)	CON	TRT	SEM	p-value
Dry matter	84.58	84.85	0.56	0.6309
Nitrogen	78.07	78.22	0.38	0.5987
Energy	83.31	83.56	0.69	0.4987

Table 3. The effect of saccharin (sweetener) supplementation on nutrient digestibility in weaning pigs.

Number of replicates, eight.

CON (control), basal diet; TRT (treatment), basal diet + 0.03% saccharin; SEM, standard error of the mean.

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Items $(\log_{10} \text{CFU} \cdot \text{g}^{-1})$	CON	TRT	SEM	p-value
Lactobacillus	7.32	7.30	0.04	0.1198
E. coli	6.27	6.22	0.06	0.2987

Number of replicates, eight.

CON (control), basal diet; TRT (treatment), basal diet + 0.03% saccharin; SEM, standard error of the mean.

Items	CON	TRT	SEM	p-value
Fecal score <sup>z</sup>				
Initial	3.52	3.50	0.05	0.3298
Week 1	3.51	3.42	0.06	0.1187
Week 2	3.42	3.46	0.04	0.2984
Week 3	3.25	3.23	0.04	0.7433
Week 4	3.17	3.13	0.03	0.3467
Week 5	3.22	3.24	0.03	0.4729
Week 6	3.17	3.18	0.03	0.3098

Table 5. The effect of saccharin (sweetener) supplementation on fecal score in weaning pigs.

Number of replicates, eight.

CON (control), basal diet; TRT (treatment), basal diet + 0.03% saccharin; SEM, standard error of the mean.

<sup>z</sup> 1 hard, dry pellet; 2 firm, formed stool; 3 soft, moist stool that retains shape; 4 soft, unformed stool that assumes shape of container; 5 watery, liquid that can be poured.

### Discussion

In our current study, we aimed to explore the impact of sweetener supplementation on the health and performance of piglets, recognizing that piglet performance significantly influences the productivity of the entire swine industry. Supplementing sweeteners was notably beneficial, especially for early weaned piglets, as it mitigated the stress associated with weaning (Jacela et al., 2010). In line with our study, throughout the study period, piglets fed a diet supplemented with 150 mg·kg<sup>-1</sup> of sucralose demonstrated increased ADG and ADFI compared to the control group. (Zhang et al., 2020). Moreover, the inclusion of 150 mg of the sweetener (sucram) in the diet significantly affected FI and pig performance in weaning pigs (Sterk et al., 2008). In addition, diets supplemented with increasing concentrations of neotame at 10, 20, 30, 40, or 50 mg·kg<sup>-1</sup> demonstrated a linear increase in ADFI during phase I (days 1 - 22) and throughout the entire experimental period (days 1 - 35) (Zhu et al., 2016). Additionally, 0.2% saccharin supplementation improved ADFI in lactating sows (Liang et al., 2017). However, supplementing with 0.05% saccharin, 0.03% saccharin-neotame mix, 0.02% neotame, and 0.02% saccharin-neotame mix did not result in any significant impact on growth performance in weaning pigs (Lee et al., 2019). In our study, the improvement in ADG was attributed to an increase in ADFI.

The addition of dietary sweeteners reduced intestinal permeability and enhanced the digestibility of DM and E (Posta et al., 2023). Supplementing with 150 mg·kg<sup>-1</sup> of sweetener improved the digestibility of DM, N, and gross energy in weanling pigs (Lei et al., 2017). However, there were no significant differences observed in the digestibility of DM and crude protein (CP) among diets containing 0.05% saccharin (50% Saccharin-sodium), 0.03% saccharin-neotame mix (50% Saccharin-sodium + 2% Neotame), 0.02% neotame (10% Neotame), and 0.02% saccharin-neotame mix (10% Saccharin-sodium + 10% Neotame) in weaning pigs (Lee et al., 2019). The finding of our current study reported that the weaned pig's feed supplementation of sweetener showed no significant difference in nutrient digestibility. The digestive system of weaning pigs is a complex and dynamic system that governs the digestion, absorption, and utilization of nutrients from the diet (Tang et al., 2022). Our study observed that sweetener supplementation did not result in alterations in fecal consistency or fecal microbiota in weaning pigs. These findings suggest that the inclusion of sweeteners in the diet did not disrupt digestive function or intestinal integrity. That's why no difference was

observed in nutrient digestibility among the dietary treatments.

The composition of gut microbiota is shaped by an animal's growth stage, physiological condition, and environmental variables like dietary composition, pathogen exposure, and antibiotic administration (Ji et al., 2017). The weaning feed containing 0.05% saccharin (50% Saccharin-sodium) led to an improvement in *Lactobacillus* levels, but it did not result in significant changes in *Escherichia coli* levels (Lee et al., 2019). Moreover, the supplementation of 0.015% sucram (saccharin) resulted in a significant increase in the population of *Lactobacillaceae* (Daly et al., 2016). The addition of lactose or saccharin/neohesperidin dihydrochalcone to pig feed led to a significant increase in the cecal populations of *Lactobacillus* (Daly et al., 2014). Suez et al. (2014) reported that noncaloric artificial sweeteners may affect intestinal microorganisms. Low-caloric sweetener could affect intestinal bacterial mass, volatile fatty acid production, and had prebiotic effects in rats (Ruiz-Ojeda et al., 2019). It has been reported that xylitol ingestion in mice has a positive effect on the metabolism of various intestinal microbial populations (Tamura et al., 2013). However, our study showed that there was no significant effect on fecal microbiota. Further research is needed due to inconsistency result.

Our study revealed no significant effect on fecal score in weaning pigs. Our study findings indicated that including 150 mg of sweetener (Sucram C-150) / kg or 150 mg of sweetener (Sucram 3D) / kg in the diet led to only numerical effects on postweaning pig fecal consistency (Sterk et al., 2008). Similarly, incorporating feed sweetener at a concentration of 150 mg  $\cdot$ kg<sup>-1</sup> did not produce a significant impact on the fecal scores of pigs (Espinosa et al., 2020). Moreover, the fecal consistency score remained unaffected by the dietary treatment of 150 mg  $\cdot$ kg<sup>-1</sup> sweetener (3D; SUCRAM 3D) (Sterk et al., 2008). However, the fecal score was improved with the inclusion of 150 mg  $\cdot$ kg<sup>-1</sup> sweetener in weaning pigs (Lei et al., 2017). This variation in results may be attributed to factors such as the quality and quantity of supplements used, as well as the age at weaning. This is supported by the findings of Callesen et al. (2007), who demonstrated an interactive relationship between weaning age and supplementation of the diet on fecal score.

### Conclusion

In conclusion, adding 0.03% sweetener showed a notable positive influence on the growth performance of weaning pigs without any detrimental effects on nutrient digestibility or fecal score. Therefore, the inclusion of 0.03% sweetener may be beneficial for enhancing the growth performance of weaning pigs.

## **Conflict of Interests**

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

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