

Postprint of Application Effects of Novel Sweeteners and Umami Flavor Enhancers in Diets for Weaned Piglets

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Abstract

This experiment aimed to investigate the effects of novel sweeteners and umami agents on growth performance, serum gastrointestinal peptide indices, nutrient digestibility, and fecal microbial counts in weaned piglets. A total of 128 Large White weaned piglets with good body condition, weighing (7.81 ± 0.82) kg and aged (28 ± 2) days, were randomly allocated into 4 groups, with 4 replicates per group and 8 piglets per replicate. The control group (Group A) was fed a basal diet without flavor or sweetener additives, while the experimental groups received the basal diet supplemented with 0.2 g/kg sodium saccharin (Group B), 0.2 g/kg umami compound (Group C), and 0.2 g/kg plant sweet extract (Group D), respectively. The experimental period lasted 28 days. The results showed: 1) Compared with the control group, the feed-to-gain ratio of weaned piglets in Groups B, C, and D during days 1-14 and days 1-28 was significantly reduced ($P < 0.01$); 2) Compared with the control group, the serum glucagon-like peptide-1 content in Group C was significantly decreased ($P < 0.05$). 3) No significant differences were observed among groups in the digestibility of organic matter, crude protein, crude fat, calcium, and phosphorus ($P > 0.05$). 4) No significant differences were found among groups in fecal counts of *Escherichia coli*, *Lactobacillus*, and *Bifidobacterium* ($P > 0.05$). These results indicate that dietary supplementation with sodium saccharin, umami compound, and plant sweet extract can improve growth performance and regulate serum gastrointestinal peptide secretion in weaned piglets.

Full Text

Application Effects of Novel Sweetener and Umami Enhancer in Weaned Piglet Diets¹

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Abstract

This study investigated the effects of novel sweetener and umami enhancer on growth performance, serum gastrointestinal peptide indexes, nutrient digestibility, and fecal microbe numbers in weaned piglets. One hundred twenty-eight healthy Large White piglets at (28 ± 2) days of age with body weight of (7.81 ± 0.82) kg were selected and randomly divided into 4 groups with 4 replicates per group and 8 piglets per replicate. Piglets in the control group (group A) were fed a basal diet without flavoring or sweetening agents, while those in the experimental groups received the basal diet supplemented with 0.2 g/kg saccharin sodium (group B), 0.2 g/kg umami enhancer compounds (group C), or 0.2 g/kg plant sweetener extract (group D). The trial lasted 28 days.

The results showed: (1) Compared with the control group, the feed-to-gain ratio of weaned piglets in groups B, C, and D during days 1-14 and days 1-28 was significantly reduced ($P < 0.01$). (2) Serum glucagon-like peptide-1 content in group C was significantly lower than in the control group ($P < 0.05$). (3) No significant differences were observed among all groups in the digestibility of organic matter, crude protein, ether extract, calcium, or phosphorus ($P > 0.05$). (4) Fecal counts of *Escherichia coli*, *Lactobacillus*, and *Bifidobacteria* did not differ significantly among groups ($P > 0.05$). These findings indicate that dietary supplementation with saccharin sodium, umami enhancer compounds, and plant sweetener extract can improve growth performance and modulate serum gastrointestinal peptide secretion in weaned piglets.

Key words: flavoring agents; weaned piglets; growth performance; serum gastrointestinal peptide indexes; nutrient digestibility; fecal microbes

Feed intake plays a crucial role in animal production and exhibits a strong linear correlation with the growth potential of piglets in swine production. Therefore, increasing feed intake in weaned piglets is essential for ensuring optimal growth performance. Due to pigs' sensitive taste perception, various flavoring agents are commonly added to piglet diets to enhance feed intake. Previous research has demonstrated that dietary sweeteners not only prevent post-weaning feed intake depression but also improve growth performance in weaned piglets. While

sweeteners are widely used in piglet diets, most commercial products rely primarily on saccharin sodium, which possesses an inherent metallic bitter taste that can compromise diet palatability. Similarly, monosodium glutamate (MSG) is commonly used as an umami enhancer in piglet diets, but excessive MSG can hinder piglet growth. The novel sweeteners and umami enhancers evaluated in this study are plant-derived extracts with enhanced safety profiles. Most existing research on sweeteners and umami enhancers has focused on growth performance in weaned piglets, with limited reports on serum gastrointestinal peptide indexes, nutrient digestibility, and fecal microbe numbers. Therefore, this experiment was conducted to investigate the effects of novel sweeteners and umami enhancers on these parameters in weaned piglets.

1.1 Experimental Materials

Saccharin sodium, umami enhancer compounds (code 79021Z, containing plant extracts, yeast hydrolysate, nucleotides, amino acids, and MSG), and plant sweetener extract (code 79028Z, containing plant extracts, yeast hydrolysate, nucleotides, and amino acids) were provided by Lucta (Guangzhou) Flavors Co., Ltd.

1.2 Experimental Design

This experiment employed a single-factor completely randomized design. One hundred twenty-eight healthy Large White piglets at (28 ± 2) days of age with body weight of (7.81 ± 0.82) kg were selected and randomly allocated to 4 groups based on sex and uniform average body weight, with 4 replicates per group and 8 piglets per replicate. The supplementation scheme for novel sweeteners and umami enhancers is presented in Table 1. The experimental period lasted 28 days.

Table 1 Novel sweetener and umami enhancer application scheme for experiment

Group	Diet Composition
Group A (control)	Basal diet
Group B	Basal diet + saccharin sodium (0.2 g/kg)
Group C	Basal diet + umami enhancer compounds (0.2 g/kg)
Group D	Basal diet + plant sweetener extract (0.2 g/kg)

1.3 Animal Management

The experiment was conducted at the Edmont breeding farm of Liaoning Debao Agriculture and Animal Husbandry Group. Experimental piglets were provided by Edmont Breeding Pig Co., Ltd. All four groups were housed in the same building with temperature maintained at $(25 \pm 3)^{\circ}\text{C}$ and good ventilation. Piglets were managed according to conventional procedures and normal immunization protocols, with free access to water and feed throughout the trial period.

1.4 Experimental Diets

A corn-soybean meal basal diet was formulated according to NRC (1998) nutrient requirements for swine. The composition and nutrient levels of the basal diet are shown in Table 2 .

Table 2 Composition and nutrient levels of the basal diet (air-dry basis), %

Item	Content
Ingredients	
Corn	58.00
Common soybean meal	25.00
Extruded soybean	5.00
Imported fish meal	4.00
Limestone	1.00
Calcium hydrogen phosphate	1.80
Salt	0.30
Premix ¹	4.90
Total	100.00
Nutrient levels²	
Metabolizable energy, MJ/kg	13.50
Crude protein	20.00
Calcium	0.85
Available phosphorus	0.45
Digestible lysine	1.25
Digestible methionine	0.36
Digestible threonine	0.78
Digestible tryptophan	0.22
Salt	0.30

¹The premix provided per kg of diet: VA 8,000 IU, VD₃ 1,228 IU, VE 15 IU, VK₃ 3.0 mg, VB₁ 1.3 mg, VB₂ 3.1 mg, VB₆ 1.2 mg, calcium pantothenate 13.4 mg, choline chloride 500 mg, biotin 0.11 mg, niacin 25 mg, folic acid 0.68 mg, VB₁₂ 0.03 mg, Fe (as ferrous sulfate) 120 mg, Cu (as copper sulfate) 10 mg, Zn (as zinc sulfate) 130 mg, Mn (as manganese sulfate) 100 mg, I (as potassium iodide) 0.3 mg, Se (as sodium selenite) 0.3 mg, Lys · HCl (78%) 3 g, Met 2.5 g, L-Thr 6 g, L-Try 1.5 g.

²Metabolizable energy and digestible amino acids were calculated values, while others were measured values.

1.5 Measurements

1.5.1 Growth Performance Piglets were individually weighed after overnight fasting on day 0, day 15, and day 28 to calculate average daily gain (ADG). Feed intake was recorded daily by replicate to calculate average

daily feed intake (ADFI) based on daily feed provision, remaining feed, and wastage. Feed-to-gain ratio (F/G) was calculated from ADFI and ADG. Fecal consistency was monitored daily to record diarrheic individuals and calculate diarrhea rate. Mortality and culling events were recorded, with immediate feed settlement and weighing of removed piglets.

1.5.2 Serum Gastrointestinal Peptide Indexes On the final day of the experiment, one piglet per replicate was randomly selected for blood collection. Five milliliters of blood were drawn from the anterior vena cava using vacuum tubes, allowed to clot for over 30 minutes, then centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -20°C for analysis. Measured parameters included cholecystokinin (CCK), leptin (LP), glucagon-like peptide-1 (GLP-1), and ghrelin. CCK was measured by radioimmunoassay using an Sn-69513 immunization counter according to kit instructions (kit purchased from the Department of Neurobiology, Second Military Medical University, Shanghai). LP, GLP-1, and ghrelin were measured by enzyme-linked immunosorbent assay using a Multiskan MK3 microplate reader according to kit instructions (kits purchased from Nanjing Bisenjia Biotechnology Co., Ltd.).

1.5.3 Nutrient Digestibility On the final day, approximately 200 g of feces were collected from each replicate, mixed thoroughly, and treated with 10% hydrochloric acid solution to prevent ammonia volatilization before storage at -20°C. Contents of organic matter (OM), crude protein (CP), ether extract (EE), calcium (Ca), and phosphorus (P) in diets and feces were determined using conventional feed analysis methods. Apparent nutrient digestibility was calculated using the internal indicator method with 2 mol/L hydrochloric acid-insoluble ash (2N-AIA) as the indicator:

$$X = 100 - [(b \times c) / (a \times d)] \times 100$$

Where: X = nutrient digestibility (%); a = nutrient content in diet (%); b = nutrient content in feces (%); c = 2N-AIA content in diet (%); d = 2N-AIA content in feces (%).

1.5.4 Fecal Microbe Enumeration On the final day, fresh feces were collected from one randomly selected piglet per replicate using sterile bags, sealed, labeled, and stored at 4°C for analysis. *Escherichia coli*, *Lactobacillus*, and *Bifidobacteria* counts were determined by plate spreading method. All culture media were purchased from Qingdao High-tech Industrial Park Hope Biotechnology Co., Ltd. Specific media were used for each organism: MacConkey agar (HB6238-1) for *E. coli*, *Lactobacillus* selective agar (LBS medium, HB0385) for *Lactobacillus*, and *Bifidobacteria* agar (BBL medium, HB0395) for *Bifidobacteria*. *E. coli* was cultured aerobically at 37°C for 24 hours, while *Lactobacillus* and *Bifidobacteria* were cultured anaerobically at 37°C for 48 hours. Results were expressed as log₁₀ colony-forming units per gram of feces [lg(CFU/g)].

1.6 Data Processing and Statistical Analysis Experimental data were processed using Excel and analyzed by one-way ANOVA using SPSS 19.0 software. Duncan's multiple comparison test was applied when significant differences were detected. Significance was set at $P < 0.05$ and extreme significance at $P < 0.01$. Data are presented as "mean \pm standard deviation."

2.1 Effects of Dietary Novel Sweetener and Umami Enhancer on Growth Performance of Weaned Piglets

As shown in Table 3, ADFI and ADG of weaned piglets did not differ significantly among groups at any experimental stage ($P > 0.05$). However, F/G was reduced in all treatment groups compared with the control group. Specifically, F/G during days 1-14 and days 1-28 was extremely significantly decreased in all treatment groups ($P < 0.01$), with group B showing the lowest F/G at 16.87% and 15.05% reductions compared with the control group for the respective periods. During days 15-28, no significant differences in F/G were observed among groups ($P > 0.05$). Diarrhea rates also did not differ significantly among groups ($P > 0.05$).

Table 3 Effects of dietary novel sweetener and umami enhancer on growth performance of weaned piglets

Item	Group A	Group B	Group C	Group D	P-value
Initial weight, kg	7.82 \pm 0.88	7.81 \pm 0.89	7.81 \pm 0.88	7.81 \pm 0.88	—
Finalweight, kg	15.86 \pm 1.81	17.61 \pm 2.06	16.81 \pm 0.35	17.30 \pm 2.25	—
ADFI, g/d	418.38 \pm 55.05	424.65 \pm 79.28	435.36 \pm 51.07	424.96 \pm 94.79	> 0.05
ADG, g/d	253.46 \pm 45.10	308.26 \pm 59.11	308.71 \pm 28.63	305.36 \pm 60.62	> 0.05
F/G	1.66 \pm 0.08 ^a	1.38 \pm 0.07 ^c	1.41 \pm 0.05 ^c	1.39 \pm 0.07 ^c	< 0.001
*Diarrhearate, %	10.05 \pm 1.39	11.39 \pm 1.65	10.49 \pm 2.43	10.49 \pm 2.43	> 0.05

, In the same row, values with different letter superscripts differ extremely significantly ($P < 0.01$). The same applies below.

2.2 Effects of Dietary Novel Sweetener and Umami Enhancer on Serum Gastrointestinal Peptide Indexes of Weaned Piglets

As shown in Table 4, serum CCK, LP, and ghrelin concentrations did not differ significantly among groups ($P > 0.05$). However, serum GLP-1 concentration in group C was significantly lower than in the control group ($P < 0.05$), representing a 38.03% reduction.

Table 4 Effects of dietary novel sweetener and umami enhancer on serum gastrointestinal peptide indexes of weaned piglets

Item	Group A	Group B	Group C	Group D	P-value
CCK, pg/mL	45.32±13.22	37.53±10.10	42.52±4.31	39.78±7.40	>
LP, ng/mL	4.70±1.01	4.39±0.25	4.68±0.76	4.51±0.28	>
GLP-1, pmol/L	1.70±0.38 ^a	1.35±0.12 ^{ab}	1.06±0.22 ^b	1.53±0.08 ^a	<
Ghrelin, ng/L	450.45±52.54	487.55±17.09	468.32±28.76	465.13±28.86	

, Values with different letter superscripts differ significantly ($P < 0.05$).

2.3 Effects of Dietary Novel Sweetener and Umami Enhancer on Nutrient Digestibility of Weaned Piglets

As shown in Table 5, digestibility of OM, CP, EE, Ca, and P did not differ significantly among groups ($P > 0.05$).

Table 5 Effects of dietary novel sweetener and umami enhancer on nutrient digestibility of weaned piglets, %

Item	Group A	Group B	Group C	Group D	P-value
OM	79.27±2.43	84.81±5.57	81.57±3.30	82.42±4.93	>
CP	71.42±2.71	78.42±4.37	73.35±4.89	74.88±6.98	>
EE	63.45±7.46	70.37±3.46	65.45±8.98	64.69±10.59	>
Ca	40.64±7.06	49.69±8.18	46.39±13.97	48.45±7.52	>
P	44.80±6.04	58.64±6.90	51.47±8.98	52.76±12.68	

2.4 Effects of Dietary Novel Sweetener and Umami Enhancer on Fecal Microbe Number of Weaned Piglets

As shown in Table 6, fecal counts of Escherichia coli, Lactobacillus, and Bifidobacteria did not differ significantly among groups ($P > 0.05$).

Table 6 Effects of dietary novel sweetener and umami enhancer on fecal microbe number of weaned piglets, lg(CFU/g)

Item	Group A	Group B	Group C	Group D	P-value
Escherichia coli	7.31±0.30	7.03±0.26	7.23±0.27	7.10±0.12	> 0.05
<i>Lactobacillus</i>	7.02±0.11	7.13±0.31	7.01±0.25	7.11±0.17	> 0.05
<i>Bifidobacteria</i>	5.82±0.47	6.11±0.18	5.84±0.36	5.95±0.29	> 0.05

3.1 Effects on Growth Performance

Weaning and diet changes can cause severe stress in piglets, leading to diarrhea. Dietary flavoring agents can stimulate feed intake, thereby alleviating stress and improving diarrhea conditions. Sweeteners and umami enhancers primarily enhance feed intake by stimulating taste perception and promoting appetite. Rezaei et al. reported that dietary umami enhancer improved growth performance, increased daily gain and feed conversion efficiency, and reduced diarrhea rates in weaned piglets. Supplementing finishing pig diets with 0.1% MSG increased feed intake, shortened the finishing period, and improved daily gain by 8%. Peng and Xiao et al. found that dietary umami enhancer improved growth performance and reduced diarrhea rates in weaned piglets. Yang et al. demonstrated that dietary sweetener supplementation significantly increased feed intake and weight gain, thereby improving growth performance and diarrhea conditions. Mou reported that sweeteners stimulated feed intake and promoted growth in weaned piglets, while Zhang showed they improved diarrhea incidence. The current results, showing that saccharin sodium, umami enhancer compounds, and plant sweetener extract extremely significantly reduced F/G during days 1-14 and days 1-28, align with these previous findings, confirming that novel sweeteners and umami enhancers improve growth performance in weaned piglets.

3.2 Effects on Serum Gastrointestinal Peptide Indexes

GLP-1 is a peptide derived from proglucagon cleavage, synthesized and secreted by L-cells in the distal small intestine and colon. It enhances insulin synthesis and secretion, suppresses glucagon production, delays gastric emptying, and regulates energy and nutrient metabolism. GLP-1 inhibits gastrointestinal motility through vagal nerves, GLP-1 receptors, and afferent nerve fibers, thereby slowing nutrient entry into the small intestine, reducing absorption rate, and decreasing feed intake. Studies in humans have shown that exogenous GLP-1 administration reduces food intake in both healthy and obese individuals. Näs-lund et al. reported that subcutaneous GLP-1 injections in obese subjects for 5 days reduced body weight by 0.55%. Meeran et al. demonstrated that peripheral GLP-1 infusion in rats decreased food intake and body weight. In this study, saccharin sodium and plant sweetener extract groups showed reduced serum GLP-1 levels compared with the control group, consistent with ADFI and ADG

results. The umami enhancer group exhibited significantly reduced GLP-1 levels, though their ADFI and ADG were lower than the other treatment groups. This discrepancy may be attributed to differences in flavoring agent composition or individual variation in GLP-1 response among piglets, warranting further investigation.

3.3 Effects on Nutrient Digestibility

Nutrient digestibility and utilization in piglets vary with breed, sex, and age. Diet composition, absorption capacity, and metabolic differences can significantly affect CP and EE digestibility. Since growth performance is closely linked to digestibility, improved nutrient digestibility should theoretically enhance growth. Mou reported that dietary sweeteners significantly improved DM, CP, EE, and OM digestibility in piglets, while Liu showed they enhanced GE, CP, and DM digestibility. In contrast, this study found no significant differences in OM, CP, EE, Ca, or P digestibility among groups supplemented with saccharin sodium, umami enhancer compounds, or plant sweetener extract. These divergent results may be due to differences in sweetener and umami enhancer types.

3.4 Effects on Fecal Microbe Numbers

Post-weaning diarrhea, primarily caused by enterotoxigenic *Escherichia coli* (accounting for 80% of cases), represents the most common and severe health challenge in weaned piglets, causing major economic losses. Certain *E. coli* strains possess adhesion factors that enable colonization of intestinal epithelium, while yeast extracts can inhibit this colonization and reduce diarrhea incidence while modulating intestinal microecological balance. *Lactobacillus* and *Bifidobacteria*, as beneficial gut microbes, can competitively inhibit *E. coli* proliferation when present in high numbers, thereby maintaining intestinal microflora balance. However, this study found no significant effects of saccharin sodium, umami enhancer compounds, or plant sweetener extract on fecal microbe numbers.

Conclusions

1. Dietary supplementation with saccharin sodium, umami enhancer compounds, and plant sweetener extract improved growth performance in weaned piglets, as evidenced by extremely significant reductions in F/G during days 1-14 and days 1-28.
2. Dietary umami enhancer compounds significantly reduced serum GLP-1 concentration in weaned piglets.

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¹Footnote marker from original text.

Note: Figure translations are in progress. See original paper for figures.

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