

Effects of Sweet-Fragrant-Umami Compound Palatant on Growth Performance, Serum Biochemical Indices, Nutrient Apparent Digestibility, and Fecal Microbiota in Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of sweet-fresh composite attractant on growth performance, serum biochemical parameters, nutrient apparent digestibility, and fecal microbial flora in weaned piglets. A total of 72 healthy Large White piglets at (43 ± 4) days of age with similar body weight were selected and randomly allocated to 3 groups, each comprising 3 replicates of 8 piglets. The control group was fed a basal diet, while the treatment groups were fed the basal diet supplemented with 1 and 2 kg/t of sweet-fresh composite attractant, respectively. The experimental period lasted 28 days. The results showed that: compared with the control group, dietary supplementation with 2 kg/t sweet-fresh composite attractant significantly increased the average daily feed intake and average daily gain of weaned piglets ($P < 0.05$); dietary supplementation with sweet-fresh composite attractant had no significant effect on nutrient apparent digestibility, serum biochemical parameters, and fecal microbial flora ($P > 0.05$). Therefore, dietary supplementation with 2 kg/t sweet-fresh composite attractant can increase the average daily feed intake and average daily gain of weaned piglets, thereby improving growth performance. Under the conditions of this experiment, dietary supplementation with 2 kg/t sweet-fresh composite attractant is recommended for weaned piglets.

Full Text

Effects of Aromatic, Sweetener and Flavor Enhancer Compound Feeding Attractant on Growth Performance, Serum Biochemical Indices, Nutrient Apparent Digestibility and Faecal Microflora of Weaning Piglets

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Abstract: This study investigated the effects of dietary supplementation with different levels of aromatic, sweetener and flavor enhancer compound feeding attractant (ASF-CFA) on growth performance, serum biochemical indices, nutrient apparent digestibility, and faecal microflora of weaning piglets. Seventy-two healthy Large White piglets at (43±\$4) days of age with similar body weight were selected and randomly allocated into three groups, with three replicates per group and eight piglets per replicate. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 1 kg/t and 2 kg/t ASF-CFA, respectively. The trial lasted for 28 days. The results showed that compared with the control group, dietary supplementation with 2 kg/t ASF-CFA significantly increased average daily feed intake (ADFI) and average daily gain (ADG) ($P < 0.05$), while ASF-CFA had no significant effects on serum biochemical indices, nutrient apparent digestibility, or faecal microflora ($P > 0.05$). It is concluded that dietary supplementation with 2 kg/t ASF-CFA can improve the growth performance of weaning piglets by increasing ADFI and ADG. Under the conditions of this experiment, the recommended supplemental level of ASF-CFA for weaning piglets is 2 kg/t.

Keywords: aromatic, sweetener and flavor compound feeding attractant; weaning piglets; growth performance; serum biochemical indices; nutrient apparent digestibility

Growth performance in piglets is directly related to feed intake, and feed palatability is a key factor influencing intake. The feeding process in mammals primarily relies on visual and gustatory stimulation from food. After food is delivered to the mouth via the lips, tongue, and teeth, animals evaluate it based on taste, olfactory stimulation, and oral tactile sensations, expelling unsuitable substances [1]. Therefore, increasing feed intake involves satisfying animals' preferences for visual, olfactory, and gustatory cues. Feeding attractants can attract animals' attention through their aroma, induce feed consumption, and ensure continued feeding through their taste properties. Additionally, they can stimulate sensory organs to trigger responses that, after brain processing, enhance digestive function and increase feed intake. Studies have demonstrated that dietary flavor additives can promote appetite and increase feed intake in

pigs [2-3]. Rezaei et al. [4] investigated the effects of different glutamate concentrations in weanling piglet diets and found that daily gain and feed efficiency were positively correlated with glutamate concentration, with 4% being the highest safe and effective supplementation level. While applications of flavoring agents, sweeteners, and umami agents are well-established, most research focuses on single or pairwise usage, with limited reports on combined use of all three. Therefore, this study aimed to investigate the effects of dietary ASF-CFA (aromatic, sweetener and flavor enhancer compound feeding attractant) on production performance, serum biochemical indices, nutrient apparent digestibility, and faecal microflora in nursery piglets, providing a scientific basis for ASF-CFA application in nursery piglet feed.

1.1 Experimental Materials

The ASF-CFA was provided by Lucta (Guangzhou) Flavors Co., Ltd. (product number 78456Z). The attractant had a cheese aroma, with sweetness provided by sodium saccharin and umami flavor provided by a compound of sodium glutamate, amino acids, nucleotides, plant extracts, and yeast hydrolysates.

1.2 Experimental Design

A completely randomized block design was employed. Seventy-two Large White piglets at (43 ± 4) days of age with initial body weight of (8.07 ± 2.05) kg and good health status were selected and randomly divided into three groups, with three replicates per group and eight piglets per replicate. The control group received a basal diet, while the experimental groups received the basal diet supplemented with 1 kg/t and 2 kg/t ASF-CFA, respectively. The trial lasted for 28 days. Pelleted diets were formulated according to NRC (1998) nutrient requirements for early-weaned piglets. The composition and nutrient levels of the basal diet are shown in Table 1.

1.3 Feeding Management

The experiment was conducted from April 28 to May 25, 2015, at Liaoning Aidemeng Swine Breeding Co., Ltd. The pig house temperature was maintained at $(26 \pm 3)^\circ\text{C}$. All experimental pigs were housed in the same building with ad libitum access to feed and water, following the farm's routine management procedures.

1.4 Sample Collection

1.4.1 Faecal Sample Collection Four piglets with good growth and similar body condition were selected from each group. Fresh faecal samples were collected daily from 07:00 to 09:00 for three days before the end of the trial. Each piglet provided two fresh faecal samples of 200 g each, collected aseptically using sterile instruments and sealed in sterile plastic bags. One sample was mixed with 20 mL of 10% tartaric acid solution (to prevent ammonia volatilization)

for nutrient content analysis, while the other was stored at -20°C for microbial enumeration. Before laboratory analysis, the three-day samples from each piglet were thoroughly mixed, and subsamples were taken for analysis. On the final day of the trial, approximately 30 g of fresh faeces was collected from one piglet per replicate and stored in a refrigerator.

1.4.2 Blood Sample Collection On the final day of the trial, four piglets were randomly selected from each replicate and 10 mL of blood was collected from the anterior vena cava after overnight fasting. The blood was allowed to clot for 15 minutes, then centrifuged at 3,000 rpm for 10 minutes to obtain serum, which was stored at -20°C until analysis.

1.5 Measurement Indicators

1.5.1 Growth Performance Body weight was measured on the morning of day 1 and day 29 after overnight fasting to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). Faecal consistency was observed each morning to diagnose diarrhea and record incidence. Faeces were scored on a four-point scale: normal (0), soft (1), viscous (2), and watery (3), and the diarrhea index was calculated. Diarrhea rate was statistically analyzed with each pen as one replicate.

Diarrhea rate (%) = [(Number of diarrheic piglets) / (Total number of piglets × trial days)] × 100

Diarrhea index = Total diarrhea score / Number of piglets

1.5.2 Serum Biochemical Indices Serum growth hormone (GH), leptin (Lep), and cholecystokinin (CCK) concentrations were determined by radioimmunoassay using kits from Tianjin Jiuding Medical Bioengineering Co., Ltd., Beijing Hualibite Biotechnology Research Institute, and Shanghai Second Military Medical University Neurobiology Department, respectively, and measured using an Sn-69513 immunocounter according to the manufacturers' instructions. Glucagon-like peptide-1 (GLP-1) and ghrelin concentrations were determined by enzyme-linked immunosorbent assay using kits from Nanjing Baisenjia Biotechnology Co., Ltd., and measured using a Multiskan MK3 microplate reader according to the kit instructions.

1.5.3 Faecal Microbial Counts Plate counting was used to enumerate *Escherichia coli*, *Bifidobacteria*, and *Lactobacillus* in faecal samples. Fresh faecal samples were prepared as 10⁻¹ dilutions in a laminar flow cabinet (1.0 g sample in 9 g sterile saline). After thorough mixing, 1 mL of the dilution was combined with 9 mL sterile saline to prepare 10⁻² dilutions. This process was repeated to prepare serial dilutions from 10⁻³ to 10⁻⁷. Dilutions were inoculated onto LBS medium (HB0385), MacConkey medium (HB6238-1), and BL medium (HB0395) for selective enumeration of lactobacilli, *E. coli*, and bifidobacteria, respectively (media purchased from Qingdao Hope Bio-Technology Co., Ltd.). *E.*

coli was cultured aerobically for 24 h, while lactobacilli and bifidobacteria were cultured anaerobically for 36 h and 48 h, respectively. Each dilution was plated in triplicate, and the mean value was used as the final result, expressed as log colony-forming units per gram of faeces [$\log(\text{CFU/g})$].

1.5.4 Nutrient Apparent Digestibility The internal indicator method was used to determine crude protein (CP), ether extract (EE), calcium (Ca), phosphorus (P), and crude ash content in feed and faecal samples according to *Feed Analysis and Feed Quality Detection Technology* [5], and nutrient apparent digestibility was calculated.

1.6 Data Processing and Analysis

Data were analyzed using the general linear model univariate procedure in SPSS 22.0 software for completely randomized block design analysis of variance. Duncan's multiple range test was used for post-hoc comparisons. Results are expressed as "mean \pm standard deviation." Differences were considered significant at $P < 0.05$.

2.1 Effects of Dietary ASF-CFA on Growth Performance of Weaning Piglets

As shown in Table 2, dietary supplementation with 2 kg/t ASF-CFA significantly increased ADFI and ADG in weaning piglets compared with the control group ($P < 0.05$), with improvements of 14.8% and 18.8%, respectively. ASF-CFA supplementation had no significant effects on feed-to-gain ratio, diarrhea rate, or diarrhea index ($P > 0.05$). Supplementation with 1 kg/t ASF-CFA did not significantly affect ADFI, ADG, feed-to-gain ratio, diarrhea rate, or diarrhea index compared with the control group ($P > 0.05$).

2.2 Effects of Dietary ASF-CFA on Serum Biochemical Indices of Weaning Piglets

As shown in Table 3, dietary ASF-CFA supplementation had no significant effects on serum concentrations of growth hormone, leptin, cholecystokinin, glucagon-like peptide-1, or ghrelin in weaning piglets ($P > 0.05$).

2.3 Effects of Dietary ASF-CFA on Faecal Microbe Number of Weaning Piglets

As shown in Table 4, dietary ASF-CFA supplementation had no significant effects on the numbers of *E. coli*, bifidobacteria, or lactobacilli in faeces of weaning piglets ($P > 0.05$).

2.4 Effects of Dietary ASF-CFA on Nutrient Apparent Digestibility of Weaning Piglets

As shown in Table 5 , dietary ASF-CFA supplementation had no significant effects on apparent digestibility of crude protein, ether extract, organic matter, calcium, or phosphorus in weaning piglets ($P>0.05$).

Numerous studies have reported on the effects of feeding attractants on pig feed intake, though most have focused on single flavor components. Gao et al. [6] reported that flavor additives could improve piglet growth performance, and many studies have demonstrated that dietary sweeteners can increase ADFI and ADG in piglets [7-9]. Combined use of sweeteners and flavor additives in piglet diets has also been shown to increase ADFI and ADG [10]. Umami agents can enhance the effects of other attractants, with sodium glutamate primarily acting after conversion to glutamic acid under gastric acid conditions. Dietary glutamate supplementation has been shown to improve ADG in weaned piglets [11-12]. The results of the current study with ASF-CFA supplementation in weaning piglets are consistent with these findings. The improvements in ADFI and ADG may be attributed to the flavor additive improving diet aroma, while sweeteners and umami agents enhanced palatability, collectively increasing the attractiveness of the diet to weaning piglets and thereby improving growth performance.

Sodium glutamate in the umami component is converted to glutamic acid under gastric acid conditions, which can maintain normal physiological function and intestinal structure in weaning piglets [13]. Furthermore, glutamic acid can be further converted to glutathione, which provides protective effects when intestinal mucosa suffers oxidative damage [14]. Lü et al. [15] found that dietary flavor and sweetener additives could improve diarrhea rates in piglets, and Peng [16] reported that umami additives could ameliorate diarrhea in weaned piglets. Intestinal microflora balance also influences diarrhea rates. In this study, ASF-CFA supplementation did not affect diarrhea rates or faecal microbial numbers, possibly because the piglets had already passed the weaning stress period and adapted to the nursery diet, with intestinal physiology largely restored to normal levels, thereby limiting the potential of ASF-CFA to promote gastrointestinal development and microbial balance during the post-weaning period.

Serum biochemical indices reflect physiological status and fluctuate dynamically within normal ranges according to the animal' s condition, with values within normal intervals indicating healthy status. The current results demonstrate that ASF-CFA supplementation did not significantly affect serum biochemical indices in piglets.

Nutrient digestibility depends not only on feed ingredient composition and processing technology but also on the animal' s own condition. The apparent digestibility values in this study were lower than those reported by Chen et al. [17] and Zhu et al. [18], possibly due to differences in pig breed and sampling methods. However, as this study used a uniform pig breed and consistent sampling

methodology, the results remain meaningful for reference. Dietary ASF-CFA supplementation did not significantly affect nutrient apparent digestibility in weaning piglets, consistent with Liu et al. [11] who reported no effect of glutamate supplementation on feed utilization.

Dietary supplementation with 2 kg/t cheese-flavored ASF-CFA significantly increased ADFI and ADG in weaning piglets. Under the conditions of this experiment, the recommended supplemental level of ASF-CFA in piglet diets is 2 kg/t.

References

- [1] OUYANG W Q. *Animal Physiology*[M]. 2nd ed. Beijing: Science Press, 2012: 135.
- [2] WANG Q, ZHANG G Z. Effects of feed flavor on production performance of piglets[J]. *Shandong Journal of Animal Science and Veterinary Medicine*, 2014, 35(6): 13-14.
- [3] ZHANG J Q. Comparison of feeding effects of pig feed flavors on piglets[J]. *Guizhou Journal of Animal Husbandry and Veterinary Medicine*, 2003, 27(2): 8-8.
- [4] REZAEI R, KNABE D A, TEKWE C D, et al. Dietary supplementation with monosodium glutamate is safe and improves growth performance in post-weaning pigs[J]. *Amino Acids*, 2013, 44(3): 911-923.
- [5] YANG S. *Feed Analysis and Feed Quality Detection Technology*[M]. Beijing: Beijing Agricultural University Press, 1993: 20-34.
- [6] GAO J R, SHAO S L, QIAO X T. Effect report of adding flavor MF-12 to piglet diets[J]. *Swine Production*, 1995(2): 2-3.
- [7] ZHU L, WANG G, DONG B, et al. Effects of sweetener neotame on diet preference, performance and hematological and biochemical parameters of weaned piglets[J]. *Animal Feed Science and Technology*, 2016, 214: 86-94.
- [8] NAIM M, RONEN T, STRIEM B J, et al. Adenylate cyclase responses to sucrose stimulation in membranes of pig circumvallate taste papillae[J]. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 1991, 100(3): 455-458.
- [9] STERK A, SCHLEGEL P, MUL A J, et al. Effects of sweeteners on individual feed intake characteristics and performance in group-housed weanling pigs[J]. *Journal of Animal Science*, 2008, 86(11): 2990-2997.
- [10] LIU S, CHEN Y L, SUN S D. Effects of several sweeteners and flavors on feed intake and weight gain of piglets[J]. *Acta Agriculturae Boreali-occidentalis Sinica*, 2008, 17(3): 73-77.
- [11] LIU T, PENG J. Effects of dietary glutamine and glutamate supplementation on growth performance of weaned piglets[J]. *Animal Husbandry and Feed*, 2003(9): 7-9.
- [12] KONG X F, ZHOU X L, FENG Z M, et al. Dietary supplementation with monosodium L-glutamate modifies lipid composition and gene expression related to lipid metabolism in growing pigs fed a normal- or high-fat diet[J]. *Livestock*

Science, 2015, 180: 247-252.

[13] SOUBA W W, SMITH R J, WILMORE D W. Glutamine metabolism by the intestinal tract[J]. Journal of Parenteral and Enteral Nutrition, 1985, 9(5): 608-617.

[14] ZHANG J M, WANG L D, GAO Z C, et al. Effects of dietary glutamine supplementation on antioxidant capacity in early-weaned piglets[J]. Acta Veterinaria et Zootechnica Sinica, 2002, 33(2): 105-109.

[15] LÜ J R, LEI Y, ZHANG K Y. Effects of different doses of sweetener on piglet performance[J]. Feed Industry, 2011, 32(20): 29-31.

[16] PENG Z Z. Effects of glutamate on nutrition and intestinal nervous system of weaned piglets[D]. Master's thesis. Nanchang: Nanchang University, 2012: 11.

[17] CHEN E Y, YANG Y F, QIAO J G. Study on effects of different low-protein diets supplemented with lysine, methionine, threonine and tryptophan in piglets[J]. Acta Agriculturae Universitatis Jiangxiensis, 2005, 27(5): 764-768.

[18] ZHU W T, LUO Q J, WANG F L, et al. Effects of four acidifiers including citric acid on weight gain and diet digestibility in weaned piglets[J]. China Animal Husbandry and Veterinary Medicine, 2004, 31(4): 13-16.

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