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Effect of feed flavor and sweetener on growth performance, nutrient digestibility, blood profile, and diarrhea score in weaning pigs

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Abstract

A total of 120 weaning pigs [(Landrace × Yorkshire) × Duroc] with an average body weight of 8.95 ± 0.88 kg were used in this study to investigate the influence of supplementation of combined flavor and sweetener. The diets included: 1) TRT1, basal diet, 2) TRT2, TRT1 + 500 mg/kg flavor, 3) TRT3, TRT1 + 150 mg/kg sweetener, and 4) TRT4, TRT1 + 500 mg/kg flavor + 150 mg/kg sweetener. The TRT4 treatment produced a higher average daily gain (ADG) than that in the other treatments on d 0 - 14 and the whole experimental period ($p < 0.05$). The TRT1 and TRT2 treatments gave a lower average daily feed intake (ADFI) than that of TRT4 on d 0 - 14 and d 0 - 42 ($p < 0.05$). On d 15 - 42, TRT4 had a higher ADFI than that of TRT1 ($p < 0.05$). Compared to TRT1, dry matter (DM), gross energy (GE), and nitrogen (N) digestibility increased in pigs fed the TRT4 diet ($p < 0.05$) on d 14. Serum norepinephrine concentration in TRT1 treatment was higher ($p < 0.05$) than that in TRT4 treatment at 72 h after weaning. On d 0 - 7, diarrhea score decreased in TRT4 treatment compared with TRT1 and TRT2 treatments. In conclusion, flavor and sweetener complex addition improved ADG and ADFI as well as DM, N, and GE digestibility in weanling pigs. This supplementation in pigs' diets decreased the serum norepinephrine concentration at 72 h after weaning and the diarrhea score during the first week of weaning.

Keywords: basal diet, feed intake, flavor, serum norepinephrine

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Introduction

Weaning is a stressful event for piglets; the changes in intestinal morphology and function induced by feed intake are the most important reasons for weaning stress (Spreeuwenberg et al., 2001). It has been well documented that essential oil and spray-dried plasma can serve as alternatives to increase the performance of weaning pigs (Park et al., 2016; Jang et al., 2016). Feed intake is greatly affected by the chemical senses of olfaction and taste (Jacela et al., 2010). Taste plays a major role in feed consumption, and taste is especially important when appetite is suppressed for some reason such as weaning stress (Hellekant and Danilova, 1999).

Flavor is the sensory impression of food or other substances. The chemical senses of taste and

smell determine the type of flavor and influences the palatability of food. Feed flavoring agents are added to enhance the smell and taste of the feed in order to stimulate feed intake, especially in post-weaning diets (Roura et al., 2008). Sweeteners are substances that enhance the sweetness in food and are categorized as bulk sweeteners and intense sweeteners. Hydrogenated glucose syrup and sorbitol, classified as bulk sweeteners, have the same calorific value as natural sugars and are used to enrich flavor in many processed foods. However, intense sweeteners, such as aspartame and saccharin, are often used as part of weight reducing diets because they do not have calories.

The inclusion of feed flavors has been shown to improve weight gain and apparent feed intake in the first three weeks after weaning (Torrallardona et al., 2001), and the inclusion of sweeteners, such as sucrose, lactose, dextrose, or artificial high intensity sweeteners increased feed intake and body weight gain (BWG) of newly weaned pigs (Lewis et al., 1955; Grinstead et al., 1961; Schlegel and Hall, 2006). Feed flavors enhance the feed intake of growing pigs (Lv et al., 2012), average daily feed intake (ADFI), and average daily gain (ADG) of weanling pigs (Wang et al., 2014). Therefore, it is essential that pigs are fed a diet that is highly palatable for optimal growth performance and production efficiency. However, some other studies failed to demonstrate the positive effect of dietary flavor or sweeteners on the performance of pigs (Forbes et al., 1995; Munro et al., 2000; Thacker et al., 2009).

Therefore, the objective of this study was to further evaluate the effect of flavor, sweetener, and the flavor and sweetener complex on growth performance, nutrient digestibility, blood profiles, and diarrhea score in weanling pigs.

Materials and Methods

Ethical considerations

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University.

Preparation of flavor and sweetener

The flavor and sweetener used in this study were prepared and supplied by DadHank Biotechnology Corporation, Chengdu, Sichuan, China. The flavor was comprised of the following: 10.22% linalool, 33.47% eugenol, 11.09% coconut aldehyde, 5.54% propylene glycol, 4.79% peach aldehyde, 9.52% anethole, 2.00% isoamy lisovalerate, 1.09% isoamyl butyrate, 1.75% ethyl maltol, 1.01% geranial, 0.35% phenylethyl butyrate, 3.81% ethyl vanillin, 0.89% γ-Decalactone, 3.19% strawberry aldehyde, 1.22% ethyl oleate, 3.59% decanoic acid, 2.33% ethyl butyrate, 1.41% butyric acid, 0.59% isopentyl acetate, 0.81% ethyl valerate, 0.12% α-Pinene, 0.90% ethyl acetyl acetate, and 0.31% nerol. In addition, the chemical composition of anise flavor was detected by gas chromatography/mass spectrometry. The sweetener was produced by chemical composition 70% sodium saccharin, 5% eohesperidin dihydrochalcone, and 25% carrier (silica).

Experimental design, animals, and housing

A total of 120 weaning pigs [(Landrace × Yorkshire) × Duroc] with an average body weight (BW) of 8.95 ± 0.88 kg was used in a 42-d experiment. Pigs were randomly allotted to 4 treatments according to their BW and litters (6 pens per treatment and 5 pigs per pen). The diets included: 1) TRT1, basal diet; 2) TRT2, TRT1 + 500 mg/kg flavor; 3) TRT3, TRT1 + 150 mg/kg sweetener; and 4) TRT4, TRT1 + 500 mg/kg flavor + 150 mg/kg sweetener. The diets were

formulated to meet or exceed National Research Council (NRC) nutrient requirements (2012) (Table 1). All pigs were allowed *ad libitum* access to feed and water throughout the experiment, and were housed in an environmentally-controlled room, which provided 0.26 m^2/ pig. Temperature during week 1 was maintained at 32°C and was lowered by 2.5°C each week thereafter.

Table 1. Compositions of basal weanling pig diets (as-fed basis).

Items	Phase 1 (d 1 to 14)	Phase 2 (d 15 to 42)
Ingredient, %		
Extruded corn	35.50	60.20
Soybean meal (48% CP)	19.80	21.55
Corn gluten meal	3.00	-
Fish meal (66% CP)	5.30	4.00
Whey	18.6	4.40
Plasma Powder	-	1.53
Lactose	10.00	-
Beef tallow	5.00	5.00
L-Lys HCl (78%)	0.25	0.30
DL-Met (50%)	0.30	0.30
L-Thr (89%)	0.15	0.15
Calcium phosphate	1.20	1.52
Limestone	0.40	0.55
Vitamin premix[y]	0.10	0.10
Trace mineral premix[z]	0.20	0.20
NaCl	0.20	0.20
Analyzed composition		
ME, kcal/kg	3,560	3,480
Crude protein, %	20.93	20.86
Lys, %	1.39	1.39
Met, %	0.67	0.64
Ca, %	0.90	0.89
Total P, %	0.73	0.72
Crude fiber,%	1.14	1.45

[y]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.
[z]Provided per kg of complete diet: Cu (as $CuSO_4 \cdot 5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.
Pigs were randomly allotted to one of four treatments with six replicate pens.

Sampling and measurements

Individual pig BW and pen feed intake were recorded at the beginning and the end of each dietary phase and used to calculate ADG, ADFI, and gain to feed ratio (G : F). Chromium oxide (0.2%) was added to the diet as an indigestible marker (Fenton and Fenton 1979) at each phase for 7 d before fecal collection to determine apparent total tract digestibility (ATTD) of dry matter (DM), N (nitrogen), and gross energy (GE). On the last 2 d of the experiment, fecal samples were collected from 2 pigs daily in each pen. All feed and fecal samples were stored at -20°C until analysis.

Before chemical analysis, fecal samples were dried at 57°C for 72 h, after which they were ground to pass through a 1-mm screen. All feed and fecal samples were analyzed for DM (Method 930.15; AOAC, 2007), CP (Method 990.03; AOAC, 2007), and crude fat (Method 920.39; AOAC, 2007). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu UV-1201, Shimadzu, Kyoto, Japan). The GE was analyzed by an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). The ATTD was then calculated using the following formula according to Stein et al. (2006): Digestibility (%) = {1 - [(Nf × Cd)/(Nd × Cf)]} × 100, where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

Blood samples were collected from the cervical vein into K_3EDTA containing non-heparinized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) from 2 pigs (1 gilt and 1 barrow) in each pen at 24, 48, and 72 h after weaning, and the same pigs were sampled each time. After collection, the samples for serum were centrifuged (3,000 × g) for 30 min at 4°C and the serum was stored at -20°C until being analyzed for cortisol, norepinephrine, and epinephrine concentrations. Serum cortisol concentrations were determined using a standardized solid phase radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The norepinephrine and epinephrine were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection, as described previously by Hay and Mormède (1997). Briefly, samples were loaded onto cationic columns, and the catecholamines were eluted with boric acid. The eluates were assayed via HPLC with electrochemical detection with an oxidizing potential of + 0.65 V. The intra- and inter-assay CV were 7.0% and 7.1% for norepinephrine and 6.5% and 11.6% for epinephrine, respectively.

Subjective diarrhea scores were recorded daily from d 0 to 7 and d 8 to 14 by the same person and were based on the following scoring system from 1 to 5 (Hu et al., 2012): 1 = hard feces; 2 = firm well formed feces; 3 = soft and partially formed feces; 4 = loose, semi-liquid feces; and 5 = watery feces. Scores were recorded on a pen basis after observations of individual pigs and the appearance of feces in the pen.

Statistical analysis

Data were analyzed using the GLM Procedure of SAS as a randomized complete block design (SAS Inst. Inc., Cary, NC). The pen served as the experimental unit. Differences among treatment means were determined using the Tukey's range test. A probability level of $p < 0.05$ was considered to be significant.

Results

Compared with the basal diet, the diet supplemented with flavor and sweetener enhanced the ADG from d 0 to 14 and for the overall experimental period and ADFI in the whole experiment ($p < 0.05$) (Table 2). Pigs supplied a diet with flavor had higher ADFI during d 0 to 14 and d 0 to 42. There were no dietary treatment effects on G : F throughout the study. Compared with basal diet, the supplementation of flavor or sweetener did not enhance the ADFI, ADG, and G : F during the whole study. No significant differences were found between TRT2 and the three other treatments. The ADFI in TRT4 were higher than those in TRT2 during d 0 to 14 and the overall experiment.

Compared with TRT1, the ATTD of DM, GE, and N increased in pigs fed the TRT4 diet ($p < 0.05$) on d 14 (Table 3). However, no differences were observed among the TRT1, TRT2, and TRT3 treatments. On d 42, there were no

Table 2. Effect of flavor and sweetener on growth performance of weanling pigs[y].

Items	TRT1	TRT2	TRT3	TRT4	SE[z]	p-value
d 0 to 14						
ADG, g	302b	333ab	344ab	377a	14.32	0.024
ADFI, g	391b	424b	433ab	471a	11.48	0.022
G : F	0.77	0.79	0.79	0.80	0.04	0.970
d 15 to 42						
ADG, g	529	544	561	592	18.50	0.111
ADFI, g	826b	835ab	863ab	881a	11.16	0.023
G : F	0.64	0.65	0.65	0.67	0.02	0.770
d 0 to 42						
ADG, g	454b	468ab	495ab	521a	13.45	0.026
ADFI, g	681b	698b	720ab	744a	10.21	0.002
G : F	0.67	0.67	0.69	0.70	0.02	0.683

[y]Dietary treatments were: TRT1, basal diet; TRT2, TRT1 + 500 mg/kg flavor; TRT3, TRT1 + 150 mg/kg sweetener; TRT4, TRT1+ 500 mg/kg flavor + 150 mg/kg sweetener.
[z]SE, standard error.
a,b: Means in the same row with different superscripts differ ($p < 0.05$).
Pigs were randomly allotted to one of four treatments with six replicate pens.

Table 3. Effect of flavor and sweetener on nutrient digestibility of weanling pigs[y].

Items	TRT1	TRT2	TRT3	TRT4	SE[z]	p-value
d 14						
N, %	82.6b	84.3ab	84.6ab	87.6a	1.22	0.049
DM, %	82.6b	85.0ab	85.8ab	87.8a	1.46	0.052
Energy,%	81.4b	83.6ab	84.1ab	86.6a	1.25	0.050
d 42						
N, %	79.0	79.1	79.6	80.8	1.39	0.783
DM, %	77.6	79.0	80.8	81.4	2.26	0.647
Energy,%	78.4	79.2	79.7	81.8	1.61	0.535

[y]Dietary treatments were: TRT1, basal diet; TRT2, TRT1+ 500 mg/kg flavor; TRT3, TRT1+ 150 mg/kg sweetener; TRT4, TRT1+ 500 mg/kg flavor + 150 mg/kg sweetener.
[z]SE, standard error.
a,b: Means in the same row with different superscripts differ ($p < 0.05$).
Pigs were randomly allotted to one of four treatments with six replicate pens.

differences in the ATTD of DM, GE, and N among dietary treatments. Serum norepinephrine concentration in TRT1 treatment was higher ($p < 0.05$) than that in TRT4 treatment at 72 h after weaning (Table 4). No effects ($p > 0.05$) were detected in cortisol and epinephrine concentrations at 72 h nor were they detected for cortisol, epinephrine, and norepinephrine concentrations at 24 h and 48 h after weaning.

On d 0 to 7, diarrhea scores were decreased in TRT4 treatment compared to TRT1 and TRT2 treatments, but there were no differences among the dietary treatments on d 8 to 14 (Table 5).

Table 4. Effect of flavor and sweetener on blood profiles of weanling pigs[y].

Items	TRT1	TRT2	TRT3	TRT4	SE[z]	p-value
24h						
Cortisol, μg/dL	5.11	4.60	4.63	4.25	0.43	0.593
Epinephrine, pg/mL	172	165	169	149	14.25	0.415
Norepinephrine, pg/mL	218	205	206	194	10.33	0.504
48h						
Cortisol, μg/dL	4.34	4.04	4.29	4.08	0.36	0.912
Epinephrine, pg/mL	158	150	155	142	8.57	0.212
Norepinephrine, pg/mL	209	189	202	184	17.96	0.754
72h						
Cortisol, μg/dL	4.10	4.00	4.07	3.69	0.14	0.397
Epinephrine, pg/mL	145	136	138	120	13.56	0.311
Norepinephrine, pg/mL	211a	164ab	173ab	123b	18.87	0.052

[y]Dietary treatments were: TRT1, basal diet; TRT2, TRT1+ 500 mg/kg flavor; TRT3, TRT1+ 150 mg/kg sweetener; TRT4, TRT1+ 500 mg/kg flavor + 150 mg/kg sweetener.
[z]SE, standard error.
a,b: Means in the same row with different superscripts differ ($p < 0.05$).
Pigs were randomly allotted to one of four treatments with six replicate pens.

Table 5. Effect of flavor and sweetener on diarrhea score of weanling pigs[y].

Items	TRT1	TRT2	TRT3	TRT4	SE[z]	p-value
d 0 to 7	4.08a	4.02a	4.00ab	3.84b	0.04	0.013
d 8 to 14	3.30	3.30	3.26	3.26	0.03	0.737

[y]Dietary treatments were: TRT1, basal diet; TRT2, TRT1+ 500 mg/kg flavor; TRT3, TRT1+ 150 mg/kg sweetener; TRT4, TRT1+ 500 mg/kg flavor + 150 mg/kg sweetener.
[z]SE, standard error.
a,b: Means in the same row with different superscripts differ ($p < 0.05$).
Pigs were randomly allotted to one of four treatments with six replicate pens.

Discussion

Weaned pigs have shown preferences for diets containing flavor (Diaz et al., 1956; Aldinger et al., 1961). In the current study, inclusion of sweetener or flavor did not have any effect on growth performance which is consistent with the finding of Munro et al. (2000) who reported that sweetener Stevia (*Stevia rebaudiana*) at 83.3, 167, or 334 mg/kg had no effect on ADG. In contrast, Schlegel and Hall (2006) indicated a positive effect of dietary sweeteners on pig performance during d 12 to 25 and d 26 to 46 after weaning. No adverse effect on feed consumption and feed to gain ratio was observed with the addition of sweetener in the diet. Sterket et al. (2008) noted that weaning pigs need a certain period of time to exhibit the clear effects of dietary sweeteners on performance. Pigs preferred sucrose to glucose or saccharin in free-choice tests, but the performance of pigs offered feed with sucrose did not differ from that of pigs offered feed with glucose (Aldinger et al., 1959). This could explain the reason for the lack of effect on ADG despite the increased ADFI during d 15 to 42 in our study. Several studies concluded that the time of flavor exposure influences feed intake after weaning. Thus, it is possible that postnatal flavor exposure of pigs could have no effect on feed preference which would be consistent with our studies (Oostindjer et al., 2010; Wang et al., 2014). However, in the current study, pigs fed diets containing both 500 mg/kg flavor and 150 mg/kg sweetener led to increased ADG

during d 0 to 14 and the overall trial period. ADFI was also improved during d 0 to 14, d 15 to 42, and d 0 to 42, but no significant effect in feed efficiency was observed, which was consistent with Liu et al. (2008) and Hof (1999). Nevertheless, Lv et al. (2012) failed to observe any synergistic effect between flavor and sweetener. The possible reason may be due to the different dosages they used. The researchers mixed two kinds of flavors and one sweetener. Excessive rexposure can have a negative effect on taste and could change the physiological state of the perceiver and even cause aversive behaviour (Lv et al., 2012).

In our study, inclusion of both 500 mg/kg flavor and 150 mg/kg sweetener increased the ATTD of DM, GE and N compared to TRT1 treatment on d 14. However, no difference was observed on d 42. The low nutrient intake during the first day after weaning is a major contribution to the impaired intestinal function and integrity generally observed after weaning (McCrackenet et al., 1995; Spreeuwenberg et al., 2001). The flavor and sweetener complex treatment increased the ADFI during d 0 to 14 in the current study. This indicates the complex had the synergistic effects of increasing feed intake, and thereby contributing to the development of the gastrointestinal mucosa. The development of gastrointestinal mucosa enhanced nutrient digestibility during the early period of weaning in our study. With the maturation of the gastrointestinal tract, a better digestion of dietary cereals and a higher absorption of nutrients are observed (Graham et al., 1986). Stewart et al. (2010) also reported that crude protein (CP) and amino acid (AA) digestibility increased during the post-weaning period, possibly due to increased enzyme secretion or maturation of the gastrointestinal tract. In the current study, the effect of flavor and sweetener was higher in early period after weaning than later periods because of the maturation of the gastrointestinal tract at later stages. That's why no difference was observed in nutrient digestibility among the dietary treatments on d 42. Researches on the effect of flavoring agents or sweeteners on nutrient digestibility are still limited in pigs and further studies are warranted.

Piglets have to adjust to receiving dry feed after weaning. The sudden change from liquid feed to solid feed often leads to diarrhea, a reduction in feed intake, and a decrease in weight gain during the first week post-weaning. Diarrhea after weaning is a multi-factorial problem. Part of it is due to the compensatory eating after a period of underfeeding while the intestinal villi cannot absorb nutrients optimally (Ball and Aherne, 1982). The compromised intestinal functioning, caused by low food intake after weaning, further increases susceptibility to pathogens, which in turn can also cause diarrhea (Pluske et al., 1997). The ingestion of small amounts of feed immediately after weaning may be sufficient to prevent problems leading to diarrhea (McCracken et al., 1999). Taken together, the cause of diarrhea may be associated to feed intake. Furthermore, the stress levels of the animals may also affect intestinal integrity, prevalence of diarrhea, and subsequently, growth performance of the animals in the post-weaning period. In our study, inclusion of flavor and sweetener complex diet in the first week after weaning reduced diarrhea scores and improved feed intake. Additionally, stress increases intestinal permeability, leading to an increased risk of intestinal inflammation and a further loss of intestinal integrity (Soderholm and Perdue, 2001). If the presence of a flavor or sweetener reduces stress levels of animals, it is likely that gut functioning will be less impaired and the digestibility of nutrient will be improved. In the current study, cortisol and epinephrine concentrations were similar among the treatments at 24 h, 48 h, and 72 h after weaning, but norepinephrine concentrations was decreased at 72 h after weaning in flavor and sweetener complex treatment compared with basal diet treatment. The lower level of norepinephrine hormone indicates that flavor or sweetener had the beneficial effect of reducing stress in weanling pigs. The benefits of flavor and sweetener in decreasing first week post-weaning stress might have reduced diarrhea scores in the current study. Oostindjer et al. (2010) also reported that piglets that were prenatally exposed to anise flavor had diarrhea problem for a fewer number of days compared with non-exposed animals.

Conclusion

In conclusion, flavor and sweetener complex addition improved the performance during the first 14 d after weaning. It decreased the stress and diarrhea during the first week after weaning as well as the ATTD of DM, N, and GE in the early weaning period, and decreased serum norepinephrine concentrations at 72 h after weaning. It can be concluded that the combination of flavor and sweetener can improve the performance of weaning pigs and decrease the stress and diarrhea.

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