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ODOR MITIGATION IN GROWING PIGS USING TAPIOCA AS FEED REPLACER

Research Article

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ABSTRACT

This study was carried out with the objectives of to evaluate the effectiveness odor reduction of pigs using tapioca in diet. Initially we selected tapioca among 15 feed ingredients on the basis of reduced ammonia, indolic and phenolic compounds after 48 h *in vitro* fermentation. Finally, the excretion of major odor-causing compounds in response to dietary tapioca levels was investigated in this study, with 36 cross-bred [(L×Y)×D] growing-finishing pigs of an average initial body weight (BW) of 26.5±2.1 kg. The animals were fed a control diet and two treatment diets with 10 and 20% tapioca (as-fed basis) for different periods. The experimental period lasted 98 d. The fecal samples were analyzed for odor-causing compounds: 1) ammonia-N; 2) volatile sulfides 3) VFA, indole, skatole, *p*-cresol, and phenol. Dietary tapioca levels at 10 and 20% reduced ($p < 0.05$) the excretion of ammonia-N, total volatile sulfides, acetic acid, valeric acid, skatole, *p*-cresol and phenol. Excretion of propionic, butyric and total volatile fatty acid (VFA) was increased by the tapioca diets relative to the controls ($p < 0.05$), whereas no effect of treatment was noted for indole ($p > 0.05$). Fecal NH_3-N, volatile sulfides, acetate, valeric acid, skatole, *p*-cresole and phenol concentrations were reduced by 44.18, 19.81, 17.95, 16.60, 85.50, 75.36 and 52.78% for the 20% tapioca diet at week 14, respectively. Therefore, we conclude that dietary tapioca can aid in ameliorating odor from feces in growing-finishing pigs. Inclusion rates of tapioca at 10% and 20% (preferable) in diets may prove to be a practical strategy for decreasing odor from swine farms

Keywords: Indole, Odor compounds, Odor mitigation, Phenol, Swine odor, Tapioca

INTRODUCTION

Intensive and large confinement pig production has increased, which is beneficial to raising a pigs' performance due to the control of a constant indoor thermal condition. During rearing, odorous compounds generate which are hazardous to farmers and pigs **(Aarnink *et al.*, 1999; Kim *et al.*, 2005).** To alleviate the odor problem, strict environment regulations are continually being strengthened throughout the world **(Huang *et al.*, 2004)**. Odorous compounds associated with pig production include ammonia, sulfuric compounds, VFA, and phenolic compounds **(Schaefer, 1977; O'Neill and Phillips, 1992; Zahn *et al.*, 1997; Schiffman *et al.*, 2001).** Minimizing the amount of indigestible feed from suitable diet may be important for odor reduction **(van Kempen and Heugten, 2003).** Replacing part of the soluble starch in the diet increases N retention, and glucose specifically inhibits protein degradation **(Fulks *et al.*, 1975; Fuller *et al.*, 1977),** and may ameliorate odor excretion **(Jensen and Hansen, 2006).** Dietary modification or manipulations have been shown to potentially reduce odor generation in swine operations **(Honeyman, 1996; Sutton *et al.*, 1999).** Changing the dietary composition may

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inhibit certain bacterial groups in the GIT of pigs or alter the fermentation of existing bacteria to control odorous end products **(Sutton *et al.*, 1999).** Therefore, a convenient method to reduce the amount of malodorous compounds in manure might include the use of feed ingredients that result in an effective reduction in the compounds. Livestock producers are continually looking for new ingredients to include in diets to fulfill specific consumers' demands. Although conventional grains are the most widely used high energy feed type, unconventional carbohydrates often provide an alternative. Moreover, a concentrated carbohydrate source provided in a diet with high starch composition may improve the growth rate and carcass traits of pigs **(Camp *et al.*, 2003).** One of these is tapioca, which is a source of starch (62.0%) that has a nutritional value that allows for the replacement of partial concentrate ingredients; this might maximize efficiency for the expected characteristics **(Moehn *et al.*, 2006).** Although tapioca has been used as a livestock feed in some of the countries, there is little definitive information available regarding its effect on swine odor reduction. Thus, the amount of tapioca necessary for a sufficient reduction of odorous compounds therefore should be determined. Generally, the study intends to elucidate the composition of feeding diets in reducing malodorous compounds with a fermented carbohydrate. Thus, the objective of this study was to evaluate the effectiveness odor reduction via colonic fermentation under *in vitro* conditions in pigs.

MATERIALS AND METHODS

Animals, housing, diets and experimental design. The experiment was conducted with a total of 36 male pigs [(Landrace × Yorkshire) × Duroc]. Each treatment and control group included three pens with four pigs. The average live weight was 26.53±2.10 kg at the beginning of the experiment and 114.13±3.16 kg at the time of slaughter. Fully slatted floor pens in the pig house at the Animal Environment Division research farm, National Institute of Animal Science (NIAS), Suwon, Korea were individually sealed off from the rest of the building. Fundamentally, an automatic controller adjusted the wall ventilation rate based on the inside room temperature and the humidity. The average temperature and relative humidity of the house during the experimental period were 20.0±0.59°C and 60.0±2.8% (mean±SD), respectively. The pigs were provided balanced diet at 5.5% of BW/d and supplied fresh water throughout the experiment. The diets were divided into grower (20-50kg), early finisher (50-80kg) and late finisher (80-120kg), and tapioca levels were provided at 10 and 20% (Table 1). The composition of the diets and their calculated chemical composition were prepared and supplied during the experimental period in accordance with the NRC guideline (1998), which is shown in Table 1. The research protocol, including procedures for the care and treatment of the animals, was reviewed and approved by the Animal Care Committee at the NIAS. The animals used in this experiment were cared for in accordance with the guidelines established by NIAS (Korea).

Table 1: Diet formulation and nutrient content of the experimental diets for growing finishing pigs at different stages (as fed-basis)

Live weight (kg)	Grower (20 ~ 50)			Early finisher (50 ~ 80)			Late finisher (80 ~ 120)		
Item/ Diets	Control	Tapioca 10%	Tapioca 20%	Control	Tapioca 10%	Tapioca 20%	Control	Tapioca 10%	Tapioca 20%
Ingredients, %									
Soybean meal	19.07	22.73	25.66	11.99	16.54	19.18	3.94	9.84	12.49
Corn	68.09	50.73	45.91	76.23	54.65	49.98	68.31	56.28	50.19
Palm meal	-	-	-	-	-	-	-	2.50	5.00
Tapioca	-	10.00	20.00	-	10.00	20.00	-	10.00	20.00
Lupine seed	6.48	-	-	6.36	-	-	8.06	-	-
Wheat grain	-	8.03	1.09	2.48	11.00	4.22	10.81	12.85	4.52
Grobig SW	0.35	0.35	0.35	0.50	0.50	0.50	0.50	0.50	0.50

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
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Journal of Biotechnology and Biosafety

Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Enzyme[1)]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Probiotics[2)]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	-	-	0.02	-	-	-	-	0.03	0.06
Lysine	0.19	0.17	0.13	0.14	0.10	0.07	0.17	0.11	0.11
Limestone	0.84	0.84	0.60	0.82	0.77	0.54	0.86	0.86	0.44
Molasses	2.47	2.96	3.00	0.32	3.00	3.00	4.00	3.68	4.00
DCP	0.77	0.57	0.81	0.54	0.33	0.57	0.23	0.11	0.52
Soybean oil	1.12	3.00	1.81	-	2.49	1.32	2.50	2.62	1.55
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient content[3)]									
DM, %	89.64	89.65	89.64	89.67	89.66	89.68	89.68	89.68	89.69
CP, %	16.16	15.90	16.00	13.80	13.80	13.80	11.50	11.70	11.50
DE, kcal/kg	3,450	3,450	3,450	3,400	3,400	3,400	3,400	3,400	3,400
CF, %	4.00	4.00	4.00	4.00	4.00	4.00	4.30	4.30	4.30
Ca, %	0.60	0.60	0.60	0.50	0.50	0.50	0.45	0.45	0.45
P, %	0.50	0.50	0.50	0.45	0.45	0.45	0.40	0.40	0.40
Lysine, %	0.95	0.95	0.95	0.75	0.75	0.75	0.60	0.60	0.60
Methionine, %	0.25	0.25	0.25	0.23	0.22	0.22	0.19	0.21	0.21
Threonine, %	0.60	0.59	0.59	0.51	0.50	0.50	0.40	0.41	0.40
Tryptophan, %	0.17	0.19	0.20	0.14	0.16	0.16	0.11	0.13	0.13

Vit.-Min. premix provided 3.5g per kg of diet containing 1,600,000 IU of vit. A, 300,000 IU of vit D_3, 800 IU of vit E, 132mg of vit K_3, 1,000mg of vit B_2, 1,200 mg of vit. B_{12}, 2,000mg of niacin, 60mg of folic acid, 35,000mg of choline chloride, 800mg of pantothenic calcium, 9,000mg of Zn, 12,000mg of Mn, 4,000mg of Fe, 500mg of Cu, 6,000mg of I, and 100mg of Co.; [1)] Optipos; [2)] Natufomen mix. [3)] Calculated values.

The *in vitro* experiment was a completely randomized arrangement with 15 treatments containing 0.2 g of any of the different feed ingredients from grain, bran, and other some available carbohydrate (CHO) source were added to each of the serum bottles along with 10 ml of fecal slurry. Each of the treatments was prepared in triplicate and anaerobically incubated for 48 h. According to **Sutton *et al.*, (1999),** sulfur- and phenolic-containing VOC and ammonia are the most predominant odorous compounds in fresh feces, and therefore were quantified under *in vitro* conditions for purposes of identifying the most effective odor-reducing dietary ingredient. The total experimental period was 98 days for this study, with 7 d allowed for dietary adaptation and the last week (14th wk) used for the collection of fecal samples. The experimental animals were designed as mentioned above. Indolic and phenolic odor compounds were measured at the 50 kg, 80 kg, and 120 kg stages. Growth performance was measured for 0-4 wk, 5-8 wk, 9-12 wk, and 13-14 wk of age. Meat characteristics, pH, ammonia nitrogen, sulfide compounds, and VFA were measured at 14th wk of age. Three replicates for each of the parameters were conducted, and their averaged data were considered the representative value.

In vitro incubations. Salt media were prepared in accordance with the methods of **Jensen *et al.*, (1995) and Wang *et al.*, (2004).** This media was pH-adjusted (6.0±0.3) and autoclaved. Fresh feces were randomly collected directly from the anus of pigs and transferred to a thermo flask at 37°C in vacuo. The pigs behaved normally and remained in good health throughout the experimental fecal collection, which was generally completed within 15 minutes in order to prevent contamination. The collected feces were introduced at 10% (w/v) into salt media and the prepared suspensions were transferred in a stomacher for homogenization. The resultant homogenate was filtered through a 4-folded sterile cheese cloth to remove the crude particle material and 10 mL of

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the prepared anaerobic buffer-slurry was transferred into 20 mL serum bottles under a constant flow of O_2-free CO_2 **(Wang *et al.*, 2004).** Fifteen feed ingredients at a weight of 0.2 g, which were obtained from the research institute of DAEHO Co. Ltd., Korea were added according to the treatments prior to the introduction of the buffer-slurry. Serum bottles were sealed with butyl rubber stoppers. Immediately prior to the initiation of incubations, the gas phase of each serum bottle was changed to O_2-free CO_2 by three successive cycles of evacuation and refilled with CO_2, using a manifold fitted to a vacuum pump and a cylinder of CO_2 in the anaerobic gassing system. Bottles were then placed in a HB 201SF shaking incubator (Hanbaek Scientific Co., Korea) set to 50 rpm at 37°C for 48 h. Volatile organic compound (phenol, *p*-cresol, indole, skatole and NH_3–N) concentrations were analyzed after the completion of *in vitro* fermentation from each of the serum bottles.

Analyses of odor compounds for both samples under in vitro and in vivo conditions. Fecal samples were analyzed for DM content according to AOAC procedures (1993). For the determination of fecal pH, approximately 2.0 g of fresh feces was weighed into a glass beaker and mixed with 50 ml of distilled and deionized water. Fecal pH values were measured at room temperature (20°C) as the mixture was stirred on a magnetic stirring plate using a Pinnacle series M530p pH meter (Schott Instruments, Mainz, Germany). For the determination of fecal NH_3-N content, a 2.0 g freshly frozen sample, mixed with 30 ml of distilled and deionized water, was homogenized using a Power Gen homogenizer (700D, Fisher Scientific) at 10,000 rpm for 2 min and centrifuged for 15 min at 800 ×*g*. The supernatant was removed, and an aliquot of the sample was taken for the determination of total NH_3-N content via spectrophotometric analysis at 625 nm according to **Weatherburn (1967).**

For the determination of total fecal volatile sulfide content, a 2.0-g freshly frozen sample, mixed with 25 ml of cadmium hydroxide (18 m*M* cadmium sulfate and 7.5 m*M* sodium hydroxide) solution was homogenized with the above homogenizer in the same condition. The supernatant was removed and analyzed for total volatile sulfide content in terms of hydrogen sulfide (H_2S) units via spectrophotometric analysis at 670 nm using sodium sulfide (Na_2S) as the standard compound in accordance with the methods described by Jacobs et al. (1957).

For the analyses of volatile fatty acids, fresh or pulverized freshly frozen fecal samples (10 g) were mixed and homogenized with 100 mL of previously prepared anaerobic salt media. 5ml of this mixed slurry or *in vitro* sample, 1 mL 25% metaphosphoric acid, and 0.05 mL of saturated mercury chloride were placed in a 15 ml glass tube and mixed appropriately. The mixed solution was then centrifuged at 1197 ×g for 20 minutes and 1 mL supernatant was transferred to 1.5 mL tubes. The supernatant was subsequently centrifuged for 10 minutes at 14240 ×g and filtered through a 0.2µm filter. Filtrates were transferred to a 2.0 ml auto sampler vial for the analysis of short-chain VFA. The supernatant was analyzed via gas chromatography (6890N, Agilent, USA) with a Hewlett-Packard FID detector (Agilent Technologies Inc., Wilmington). A HP-INNOWax capillary GC column (30 m × 0.25 mm i.d. ×□0.25 µm film thickness, Agilent Technologies Inc.) was used for chromatographic separation. The injection port of the GC was maintained at a temperature of 250°C. The column temperature was programmed to increase from 80 to 205°C at a rate of 10°C/min. The sample injection volume was 0.2 µL with a 10:1 split ratio. The major volatile fatty acids identified and well-quantified were acetic acid, propionic acid, butyric acid, and valeric acid with the relevant standard.

For the analyses of other volatile odor compounds, including indoles, skatole, *p*-cresol and phenols, fresh or pulverized freshly frozen fecal samples (10 g) were mixed and homogenized with 100 mL of previously prepared anaerobic salt media, which is relatively consistent with Jensen et al. (1995). The homogenates were centrifuged at 1,197 ×g for 20 minutes, and 3 ml supernatant or *in vitro* sample, 0.05ml of 4M NaOH and 3ml of chloroform were transferred to 20 ml glass vials and mixed properly. The mixed solutions were subsequently centrifuged at 1,197 ×g for 10 minutes and the supernatant was transferred to a 2.0 ml auto sampler vial for analysis. The supernatant was analyzed using a 6890N liquid-gas chromatography (Agilent, USA) with a Hewlett-Packard FID detector (Agilent Technologies Inc., Wilmington). A DB-5ms capillary GC column (30 m × 0.25 mm i.d. × 0.25 µm film thicknesses, Agilent Technologies Inc.) was used for chromatographic separation. The injection port of the GC was maintained at a temperature of 250°C.

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
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The column temperature was programmed to increase from 40 to 230°C at a rate of 10°C/min. Sample injection volume was 2 µL with a 5:1 split ratio and a column flow rate of 1.0 ml/min. Compound identities were further confirmed by comparing the retention time and mass spectra to those of authentic standard compounds in the solvent.

Statistical analysis. In the current study, all data were subjected to one-way ANOVA procedures for a completely randomized design using the general linear model (GLM) procedures (SAS Inst. Inc., Cary, NC) (SAS, 2003). The concentrations of odorous compounds data were compared and significant differences among means of treatment and control groups were assessed using Duncan's multiple range (comparison) tests. Variability in the data was expressed as the pooled mean values and standard error (SE) or standard error of the mean (SEM) via the MEANS procedure. The threshold for significance was $p<0.05$ for all measured variables.

RESULTS

There is limited research validating potential benefits of tapioca for pigs. The experimental knowledge on efficacy, possible modes of action, and aspects of application of phytogenic products of tapioca for swine and poultry are not clear. Zinn and DePeters (1991) previously reported that tapioca pellets can be used to replace up to 30% of dry matter intake in growing-finishing diets without adversely affecting the average daily gains of feedlot cattle. On the basis of their established theme, we decided to use tapioca levels of 10 and 20% in the diet, and comparatively lower percentages of tapioca were included in the pig feeding trial than in the cattle experiments, due to the smaller body size of the pigs.

Evaluation of feed ingredients on rate of volatile organic compounds (VOC) under in vitro conditions. In an effort to evaluate the specific rates of indolic, phenolic and ammonia-N production, *in vitro* incubations were conducted for 48 h using pig fecal slurry. The results presented in Table 2 show that tapioca, beet pulp, unhulled barley, corn grain, wheat bran, wheat grain, wheat flour and lupine seeds did not produce phenol, and in case of *p*-cresol, tapioca produced the lowest concentration among all of the feed ingredients. The production rate of indole was completely not detected in tapioca, sweet potato, beet pulp, unhulled barley, rye, rice bran, corn gluten, corn germs, corn grain, wheat bran, wheat grain, wheat flour, and lupine seeds (Table 2). Skatole concentrations were also undetectable in the tapioca, sweet potato, beet pulp, unhulled barley, rye, corn gluten, corn germs, corn grain, wheat grain, and wheat flour (Table 2). The lowest concentrations of NH_3-N were observed in tapioca, beat pulp, unhulled barley, rye, and corn grain (Table 2). Different feed ingredients affected significantly different effects ($p<0.05$) on VOC and NH_3-N production. Tapioca was selected on the basis of the VOC odor compounds evaluations from the *in vitro* tests, and was used for practical animal feeding in order to confirm the odor reduction.

Table 2. Comparison of volatile organic compound (VOC) production by different feed ingredients during pig fecal *in vitro* fermentation at 48 h

Feed ingredients	VOC, mg/L				
	phenol	p-cresol	indole	skatole	NH_3-N
Tapioca	0^{f}	9.62 ± 2.17^{f}	0^{b}	0^{d}	0^{h}
Sweet potato	0.70 ± 0.076^{c}	29.04 ± 1.83^{c}	0^{b}	0^{d}	61.0 ± 21.07^{f}
Beet pulp	0^{f}	24.12 ± 2.18^{d}	0^{b}	0^{d}	0^{h}
Replaced-milk	0^{f}	12.62 ± 0.22^{e}	0^{b}	0^{d}	0^{h}
Unhulled-barley	0^{f}	23.65 ± 1.28^{d}	0^{b}	0^{d}	0^{h}
Rye	0.02 ± 0.03^{e}	26.13 ± 2.60^{cd}	0^{b}	0^{d}	0^{h}
Rice bran	3.12 ± 0.30^{a}	26.16 ± 1.47^{cd}	0^{b}	0.19 ± 0.09^{c}	114.67 ± 20.52^{d}

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
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Corn-gluten	0.77±0.08^{c}	40.71±2.18^{bc}	0^{b}	0^{d}	154.0±5.86^{c}
Corn-germs	1.11±0.14^{b}	28.21±1.24^{c}	0^{b}	0^{d}	84.67±11.56^{e}
Corn grain	0^{f}	24.74±0.87^{d}	0^{b}	0^{d}	0^{h}
Wheat- bran (imported)	0^{f}	27.62±1.67^{cd}	0^{b}	1.26±0.27^{b}	69.67±27.42^{f}
Wheat- bran (Korean)	0^{f}	32.27±0.86^{c}	0^{b}	1.29±0.11^{b}	65.67±31.32^{f}
Wheat-grain	0^{f}	31.59±1.51^{c}	0^{b}	0^{d}	24.33±10.31^{g}
Wheat-flour	0^{f}	25.59±1.19^{d}	0^{b}	0^{d}	32.67±11.33^{g}
Wheat-gluten	0.58±0.11^{d}	81.08±3.63^{a}	8.90±0.1^{a}	14.42±0.6^{a}	1328.33±56.0^{a}
Lupine-seeds	0^{f}	52.11±2.09^{b}	0^{b}	1.24±0.03^{b}	240.67±42.39^{b}

Values presented as Mean±SE; ^{a,b,c,d,e,f,g,h} Mean (n=3) in the same rows with different superscripts differ (p<0.05).

Effects of dietary tapioca on odor compounds in feces. Table 4 shows the effects of tapioca level over the basal diet on the DM content, pH value, ammonia-nitrogen, volatile sulfide and VFA contents in feces at 14 weeks of age. The DM content of fresh feces did not differ significantly (p>0.05), but fecal pH, NH_3-N, sulfide and VFA contents differed significantly (p<0.05) as the result of the experimental diets. Fecal pH value, NH_3-N and sulfide concentration were lowest to highest in the 20%, 10% and 0% tapioca diets (in order). All of the VFA concentrations were comparatively higher in the tapioca diet than in the controls, with the exception of acetic and valeric acid concentrations, which were lower. The concentrations of propionic, butyric, and total VFA measured in the feces of the experimental pigs were higher in the 20% group than in the 10% group, whereas the acetic and valeric acid concentrations were slightly lower. Ammonia-N, volatile sulfide compounds, acetic acid, and valeric acid concentrations in the pig feces at 14 weeks were reduced by 34.65, 18.05, 16.94 and 9.07% with the 10% tapioca diet, and 44.18, 19.81, 17.95 and 16.60% for the 20% tapioca diet, respectively (Table 3).

Table 3. Effects of dietary tapioca on DM, pH, ammonia, volatile sulfides and VFA from the feces of growing pigs at 14 weeks of age

Item	Control	Tapioca		SEM[1]
		10%	20%	
DM content of fresh feces, %	31.53	31.84	31.90	0.01
Fecal pH	6.72^{a}	6.37^{b}	6.25^{b}	0.04
Fecal ammonia N, g/kg	20.69^{a}	13.52^{b}	11.55^{b}	0.60
Total fecal volatile sulfides, mg H_2S/kg	885.09^{a}	725.34^{b}	709.73^{c}	3.10
VFA, on the basis of fecal DM				
Acetic acid, mg/kg	414.63^{a}	344.39^{b}	340.22^{b}	1.58

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
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Propionic acid, mg/kg	823.32[c]	1197.63[b]	1260.41[a]	7.17
Butyric acid, mg/kg	238.75[c]	318.49[b]	332.87[a]	2.57
Valeric acid, mg/kg	228.99[a]	208.23[b]	190.99[c]	1.34
Total VFA, mg/kg	1705.69[b]	2068.74[ab]	2124.5[a]	9.08

[a,b,c] Mean (n=3) in the same rows with different superscripts differ ($p<0.05$); [1]Mean of the standard error.

The effect of basal (control), 10% and 20% tapioca diets on indole, skatole, *p*-cresol and phenol production from feces of experimental pigs at the different stages (50, 80 and 120 kg BW) are provided in Table 4. Tapioca level in the diet exerted no effects on the fecal content and excretion for indole in all stages, skatole in 80 kg, *p*-cresol in 120 kg ($p>0.05$), but differed significantly ($p<0.05$) for skatole in 50 kg and 120 kg, *p*-cresol in 50 and 80 kg, and phenol in all stages of BW. The lowest skatole concentrations were observed in the 10% tapioca group at the 50 kg stage and the 20% group was lowest at the 120 kg stage. In the case of *p*-cresol, the 20% tapioca diet evidenced the lowest concentrations at the 50 kg stage and the 10% tapioca diet evidenced the lowest concentrations at the 80 kg stage. Phenol concentrations were lowest in the 20% tapioca-treated feces for all of the stages than 10% tapioca and control. Skatole concentrations were reduced in pig feces at 50, 80 and 120 kg by 48.10, 26.56 and 70.0% in the 10% tapioca diet, and 26.58, 35.94 and 85.55% for the 20% tapioca diet, respectively. With regard to *p*-cresol concentrations, the rates of reduction in pig feces at 50, 80 and 120 kg were 6.77, 87.04 and 11.54% for the 10% tapioca diet, and 54.13, 75.36 and 3.85% for the 20% tapioca diet, respectively. Moreover, phenol concentrations in feces were reduced at 50, 80 and 120 kg by 36.58, 73.80 and 47.22% for the 10% tapioca diet, and 46.34, 73.36 and 52.78% for the 20% tapioca diet, respectively (Table 4).

Table 4. Effects of dietary tapioca on indole, skatole, indole+skatole, p-cresol and phenol compounds from the feces of pigs at different live weight

Odor compounds, mg/kg	Live weight, kg	Control	Tapioca		SEM[1]
			10%	20%	
Indole					
	50	1.38	1.57	1.34	0.23
	80	0.20	0.19	0.14	0.05
	120	0.27	0.29	0.26	0.07
Skatole					
	50	1.58[a]	0.82[b]	1.16[ab]	0.62
	80	0.64	0.47	0.41	0.31
	120	0.9[a]	0.27[b]	0.13[c]	0.08
Indole+skatole					
	50	2.96	2.39	2.5	0.39
	80	0.84	0.66	0.55	0.20
	120	1.17[a]	0.56[b]	0.39[c]	0.16
p-cresol					
	50	1.33[a]	1.24[a]	0.61[b]	0.26
	80	5.48[a]	0.71[b]	1.35[b]	0.20
	120	0.52	0.46	0.50	0.11
Phenol					
	50	0.82[a]	0.52[b]	0.44[b]	0.03
	80	2.29[a]	0.60[b]	0.61[b]	0.04

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	120	1.08[a]	0.57[b]	0.51[c]	0.01

Values presented as [a,b,c] Mean (n=3) in the same rows with different superscripts differ (p<0.05); [1]Mean of the standard error.

DISCUSSION

Effect of tapioca diet on odor reduction. **Garry *et al.*, (2007)** reported previously that pigs offered a barley-based diet evidenced reduced odor and ammonia emissions as compared to pigs fed on wheat and other cereal diets; this is consistent with the present studies, in which we used tapioca rather than barley. The reduction of ammonia emissions in the pigs provided with tapioca-based diets compared to the control diets may be attributable to the beneficial effects of the starch and NSP in tapioca on the populations of bifidobacteria and lactobacilli. The inclusion of tapioca promotes carbohydrate-fermenting bacteria, which is similar to what was found in a study of a barley-based diet conducted by **O'Connell *et al.*, (2005).** In the large intestine, these fiber-degrading bacteria utilize ammonia as a substrate for microbial protein synthesis and are subsequently excreted in the feces. In addition, a high FC level stimulates bacterial activities in the hindgut of animals and in manure stores resulting in high short-chain VFA concentration and lower pH level and finally reduced ammonia emission **(Kendall *et al.*, 1999; Shriver *et al.*, 2003; Sutton *et al.*, 1997).** The reduction of the odorous compounds mentioned above might be reducing due to feeding with tapioca. The fermentation of tapioca in the cecum, colon, and rectum of pigs results in the production of short-chain fatty acids. Higher amounts of short-chain fatty acids reduce pH, which exerts a positive influence on the retention of ammonia in the feces and manure. This results in an improved environment in both the stable and in its surroundings **(Lenis and Jongbloed, 1999; Sutton *et al.*, 1999).** The emission of ammonia can be reduced further as the bacteria switch from protein fermentation to carbohydrate fermentation when fed with tapioca. Furthermore, as the bacteria grow, the nitrogen will be employed for the production of proteins in the bacterial biomass, and is therefore not available for the production of ammonia or odorous compounds.

Moreover, this investigation supports the hypothesis that FC in the diet can affect the emission of ammonia from pig fecal slurry. According to **Sommer and Husted (1995),** the pH of the slurry is of great importance for the emission of ammonia from pig slurry. Because the effect of pH on ammonia emission is profound, a minor change in pH can exert a large effect. In this study, the source and level of FC in the diet are factors that importantly affect the pH and ammonia emissions. Increasing amounts of FC in the diet enhanced the microbial activities in the hindgut of pigs, thus increasing VFA formation in the feces and slurry. This lowered the pH of the slurry and thereby reduced ammonia emissions. **Canh *et al.*, (1998)** demonstrated and confirmed this by showing that the inclusion of 30% dried sugar beet pulp in a pig diet lowered the slurry pH and the emission of ammonia from slurry, hence 20% tapioca diet resulted in an efficient reduction of odor in our experiment. The latter authors concluded that the slurry pH was related to slurry VFA concentrations. Present experimental findings are consistent with the above studies, although the ingredient supplied as a carbohydrate source differed. **Lynch *et al.*, (2007)** demonstrated that fecal pH was reduced significantly due to increases in total VFA, propionic and butyric acids concentrations; this is consistent with the present experimental results. The tapioca mixed diet is believed to work by either directly excluding harmful bacteria or by reducing intestinal pH to indirectly favor the development of other desirable health- promoting microorganisms that compete with harmful odor-producing bacteria to reduce their presence in the gut **(McKean, 2004).**

Although the short-chain acids are present in much higher concentrations and are more volatile, the VFAs with higher carbon numbers have a lower odor detection threshold, and are thus more offensive in nature **(Mackie, 1994).** Therefore, the high concentration of VFAs in swine manure may not necessarily causes high malodor intensity, as a large portion of the VFAs could be composed of short-chain acids with lower odor potential. A study conducted by **Zhu *et al.*, (1997)** appeared to provide some evidence to support this argument. The odorous nature of VFAs progresses from the pungent odors of formic and acetic acids to the distinctly unpleasant and offensive odors of the valeric and caproic acids **(Morrison, 1987).** In our studies, most of the bad odor-producing VFAs such as acetic and valeric acids were comparatively lower in the tapioca-added diet group than the control, which reflects the excretion of highly odorous compounds according to the above report.

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
ISSN 2322-0406
Journal of Biotechnology and Biosafety

Mason and Just (1976) found that feeding potato starch to pigs, which is known to be digested by large-intestinal bacteria **(Baker *et al.*, 1950)**, resulted in a higher proportion of butyric acid in the total VFA than for ingested maize, which is consistent with tapioca added diets. Furthermore, inulin consumption generally yields high levels of VFA in the rat model **(Levrat *et al.*, 1993; Younes *et al.*, 2001)** and pig model **(Rideout *et al.*, 2004).** However, the reduced fecal pH of the tapioca-mixed group observed in the current study increased fecal VFA content, which yielded results similar to those observed with inulin in the above-mentioned studies. However, the numerical increase in fecal VFA excretion in the tapioca-mixed group may nonetheless provide a biological explanation for the reduced fecal pH. **Van Beers-Schreurs *et al.*, (1998)** explained that the quantity of VFA depends on the amount and composition of the substrate, and on the types of microbes present within the cecum. Lower VFA concentrations reflect lower amounts of fermented substrate and lower quantities of microbial activity in the cecum of pigs, as well as lower energy intake. The present results showed comparatively higher favorable VFA (propionic and butyric acids), which is a good indication for the energy and health of animal.

Fakhoury *et al.*, (2000) identified sulfide compounds as being the most highly correlated with malodor, which may be reduced via the introduction of selective additives in the diet. Conversely, **Gibson *et al.*, (1988)** reported that SRB may perform an important role in the terminal stages of fermentation in the colon; in particular, their ability to utilize hydrogen may help to prevent excessive amounts of this gas from accumulating in the colonic lumen. In a related study, chicory supplement was shown to be effective in reducing (p<0.05) the sulfide content in the fresh slurry of growing-finishing pigs **(Rideout *et al.*, 2001).** Although we did not use the bacteria, feed additives, or chicory, our selected ingredient may help to stimulate those beneficiary bacteria to reduce sulfides or inhibit S-producing bacteria. Tapioca supplementation with both the 10% and 20% diet affect the fecal content and excretion of total volatile sulfides in the current study. Therefore, it appears that any reduction in the production and excretion of volatile sulfides in response to tapioca supplementation may be related to differences in the post-absorptive urinary loss of inorganic sulfur-containing compounds and the time period permitted for colonic fermentation during storage in the colon or large intestine.

Hansen *et al.*, (2006) and Jensen and **Hansen (2006)** previously demonstrated that the reduction of skatole compounds is significant in terms of odor reduction and that dietary chicory had a positive influence on it; the present study, dietary tapioca appeared to elicit a similar response. In another study, **Hawe et al., (1992)** reported that increased dietary sugar beet pulp increased the daily elimination of skatole and indole concentrations in the feces; hence, our experimental ingredient (tapioca) reduced skatole rather than indole. Moreover, the dietary addition of potato starch was previously shown to affect skatole-producing bacteria, but the indole-producing bacteria remained unaffected **(Chen *et al.*, 2007);** similar results were observed in this study. The reasons for the unchanged indole concentration, however, remain unclear. The concomitant increase in fecal-N loss and the reduction in fecal skatole excretion may appear contradictory, but it can be reconciled in light of the principal factors that potentially regulate skatole production in the hindgut. Two important factors that regulate intestinal skatole production are the activity of the resident proteolytic bacteria and colonic pH **(Jensen *et al.*, 1995).** Previous studies by **Knarreborg et al., (2002)** have demonstrated that the production of protein metabolites from microbial fermentation may be reduced via the addition of an alternative energy source from CHO. Thus, the preferential metabolism of tapioca by carbohydrate-fermenting bacteria may have reduced the activity of the proteolytic bacteria and effectively attenuated the breakdown of tryptophan into skatole **(Yokoyama and Carlson, 1979; Jensen *et al.*, 1995).** Furthermore, microbial proteases have been shown to function optimally at neutral or alkaline pH **(MacFarlane and Cummings, 1991).** The lower fecal pH associated with the tapioca group, although not entirely reflective of the conditions in the large bowel, may nonetheless explain the reduced excretion of skatole. As indole is also generated by the microbial degradation of tryptophan, it is surprising that tapioca supplementation did not affect the excretion of indole to the same extent as did skatole. However, because the factors influencing the relative production of indole and skatole yet to be fully elucidated **(Jensen *et al.*, 1995),** more research will be necessary to clarify the role of tapioca in the production of tryptophan metabolites.

More than 90% of urinary and fecal phenols consist of *p*-cresol, the remainder being made up by phenol and, to a lesser extent, 4-ethyl phenol.

JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
ISSN 2322-0406

Phenolic compounds such as phenols and *p*-cresols are generated from the microbial degradation of tyrosine and phenylalanine in the intestinal tracts of animals **(Ishaque *et al.*, 1985).** Although little is currently known regarding the factors that control the metabolism of aromatic amino acids in the large intestine, increasing dietary protein intake results in higher levels of amino acid fermentation in the colon, as indicated by urinary phenol excretion and fecal ammonia concentrations. This effect was largely reduced by increasing the amount of fermentable carbohydrates in the diet **(Mackie *et al.*, 1998).** The addition of cellulose to the diet tended to reduce sulfide compounds, phenolic compounds, and ammonia-nitrogen concentrations relative to the control diet **(Hankins *et al.*, 2000).** Our present study showed a similar declining trend with regard to the odorous compounds, although we employed tapioca rather than cellulose. The addition of 10% and 20% tapioca to a standard diet reduced phenolic compounds, especially methyl phenol and *p*-cresol, in freshly excreted feces relative to feces from pigs fed on the control diet.

Byrne and Hansen (2010, unpublished data) reported that a sensory analysis of the meat from the experimental pigs demonstrated that the addition of chicory to the diets resulted in overall improvement in eating quality. Similar opinion results were reported by **McKean (2004),** who determined that the desired effect of the tapioca was to improve weight gain and feed efficiency by improving gut digestion and reducing pathogenic organism loads. Although the tapioca-supplemented diet employed in the present study had little effect on growth performance and meat quality according to the previous findings (above), our principal objective was to reduce odor reduction and maintain the growth performance and meat quality at least similar to control levels, without any adverse affect. Fortunately, we measured better carcass weight along with odor reduction, which also was superior in the tapioca group than in the control group. It is not necessary to completely eliminate the presence of odorous compounds in the colon and rectum of pigs in order to reduce the impact of malodor on ambient air quality. An effective reduction should be sufficient to improve the ambient air quality to an acceptable level. The amounts of tapioca (20%) necessary for a sufficient reduction of odorous compounds in the fecal contents of pigs were determined in this study, a 10-25% inclusion level of tapioca feed ingredient in the swine diet was recommended by **Moehn et al., (2006).** According to our results, the amounts of odorous compounds were satisfactorily reduced by the tapioca supplementation method which may prove efficient.

CONCLUSION AND RECOMMENDATION

Dietary level of tapioca with 10 and 20% diets significantly affected the fecal content and excretion of NH_3, volatile sulfides, VFA, skatole, *p*-cresol and phenol in growing-finishing pigs. Thus, it appears likely that dietary supplementation with tapioca may alter the patterns of microbial fermentation in the large intestine to reduce the biogenesis and excretion of major odor-causing compounds in pigs during feeding. Furthermore, although tapioca supplementation at 10% appeared to be effective in reducing fecal odor compounds, 20% appears to be a generally more effective level, all factors considered. The results of this study allow us to confidently prescribe tapioca as an alternative feed ingredient to reduce odorous compounds. Further research will be required to clarify the mechanism(s) by which dietary tapioca reduces the fecal excretion of odorous compounds in growing-finishing pigs.

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JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
ISSN 2322-0406
Journal of Biotechnology and Biosafety

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JOURNAL OF BIOTECHNOLOGY AND BIOSAFETY
Volume 6, Issue 5, September-October, 2018, 557-569
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