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Effects of Fermented Diets Including Grape and Apple Pomace on Amino Acid Digestibility, Nitrogen Balance and Volatile Fatty Acid (VFA) Emission in Finishing Pigs

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Abstract: This study was conducted to investigate the fermented diet including grape and apple pomace on amino digestibility, nitrogen balance and volatile fatty acid in finishing pigs. A total of 24 finishing pigs ((Yorkshire x Landrace) x Duroc) with an average BW of 63.42±9.78 kg were used in this experiment. Pigs were allotted to 6 experiment diets according to initial BW. Dietary treatments were: CON (dry compound diet), (fermented liquid compound diet), GF10 (fermented liquid compound diet with 10% grape pomace), GF20 (fermented liquid compound diet with 20% grape pomace), AF10 (fermented liquid compound diet with 10% apple pomace) and AF20 (fermented liquid compound diet with 20% apple pomace). The DM, CP and CF digestibility was highest ($p<0.05$) in CON and F treatments among treatments. The DM, EE, ash and Ca digestibility was highest ($p<0.05$) in AF10 treatment among treatments. All the detected Essential amino acid and non-essential amino acids digestibility were highest ($p<0.05$) in F treatment among treatments. All the detected essential amino acid and non-essential amino acids digestibility were lowest ($p<0.05$) in AF20 treatment except leucine and and proline digestibility were lowest ($p<0.05$) in GF20 treatment among treatments. The total essential amino acids, total non-essential amino acids and total amino acid digestibility was highest ($p<0.05$) in CON and F treatments and lowest in AF20 treatment among treatments. The dietary Lactobacillus concentration was increased ($p<0.05$) as the order of treatments listed in table and the Enterobacteriaceae concentration was decreased ($p<0.05$) in reverse order. The fecal acetic acid, isobutyric acid and isovaleric acid emission were higher ($p<0.05$) in CON treatment than other treatments. The slurry acetic acid, butyric acid, isobutyric acid and isovaleric acid emission was higher ($p<0.05$) in AF10 treatment than other treatments. The feed intake and feces excretion were highest ($p<0.05$) in AF20 treatments among treatments. The feces (DM) excretion was highest in GF20 treatment and lowest ($p<0.05$) in F treatment among treatments. The Fecal nitrogen, urinary nitrogen, nitrogen retention, fecal nitrogen excretion ratio, urinary nitrogen excretion ratio and nitrogen retention ratio were highest in AF20 treatment, F treatment, AF10 treatment, AF20 treatment, CON treatment and AF10 treatment, respectively. In conclusion, the supplementation of grape and apple pomace in finishing pig diets limited the approximate chemical composition and amino acid digestibility. The supplementation of grape and apple pomace in finishing pig diets could benefit the beneficial bacterial and decreased VFA emission in feces. High apple pomace (20%) supplementation level caused high feces excretion. The low level (10%) of apple pomace supplementation improved the nitrogen retention.

Key words: Apple pomace, amino acid diegestibility, fermented diet, grape pomace, nitrogen balance

INTRODUCTION

Current Korean swine industry are facing a big crisis. The soaring oil and food price increased of feed cost, the competition of importing pork under FTA make this status much worse. Especially, most of the feed stuff rely on imports in current domestic market reflect the effort to increase domestic food self-sufficiency. Corn is used as energy source in feed and the most of it depends on import. The increase of feed price due to the increase import and transportation price is an inevitable reality. The trial was conducted to use apple and grape meal to partially replace the corn in liquid fermented diet. In 2005, the domestic apple and grape cultivated land area are 26,907 and 22,057 ha, take up 17.4 and 14.3% of the 154,717 ha total fruit cultivated area. In addition, the apple

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and grape crop production are 367,517 tons and 381,436 tons, take up 14.2 and 14.7% of the 2,592,950 tons total fruit crop production. The raw fruit consumption level is high in the country however >80% of raw grape are used for wine production in European countries such as France, Italy and Spain (Lee and Kim, 2006).

The apple meal and grape meal are obtained from apple and grape juice extraction which including shells, seeds and pulp by-products. Thus, the use of apple and grape meal in pig feed, increase domestic food self-sufficiency, decrease feed cost and reduce environment pollution. However, juice by-product might reduce the swine growth performance due to the regional and seasonal restriction corruption risk, high moisture and fiber content. These factors may limit the use of juice by-production swine feed.

Therefore, this experiment was conducted to investigate the effected of high moisture and fiber content apple and grape meal fermented by *Lactobacillus reuteri* on proximate composition and amino acid digestibility, feces and urine excretion, fecal microbial, nitrogen balance and the occurrence of volatile fatty acids in finishing pigs.

MATERIALS AND METHODS

Experiment design, animal and diets: A total of 24 finishing pigs [(Yorkshire x Landrace) x Duroc)] with an average BW of 63.42±9.78 kg were used in a 7 days experiment. Pigs were allotted to 6 experiment diets according to initial BW. The pigs were placed in individual elevated solid-sided stainless steel metabolism cages (1.6×0.8 m^2) equipped with plastic slatted floors, stainless steel feeders and automatic water nipple drinkers. There was approximately 1 m between the cages to prevent cross-contamination of feces and urea samples. The temperature was maintained at approximately 25°C using thermostatically controlled heaters and exhaust fans. Dietary treatments were: CON (dry Compound diet), F (Fermented liquid compound diet), GF10 (fermented liquid compound diet with 10% grape pomace), GF20 (fermented liquid compound diet with 20% grape pomace), AF10 (fermented liquid compound diet with 10% apple pomace) and AF20 (fermented liquid compound diet with 20% apple pomace).

All diets were formulated to meet or exceed the nutrient requirements (NRC, 1998) for finishing pigs fed in a powder form. Composition of diets is shown in Table 1.

Fermented feed processing: The F treatment was based on control diet which supplemented 19.6% water (calculated by feed weight) and 0.2% probiotic (*Lactobacillus reuteri*, 1.2×10^6 cfu g^{-1} + Yeast culture, 1.2×10^6 cfu g^{-1}) and then fermented through anaerobic method. The chemical composition and amino acids profile of grape and apple pomace was shown in Table 2.

Table 1: Ingredient and chemical composition of basal diet

Ingredient (%)	Basal diet
Corn	62.84
Soybean meal	19.08
Wheat	6.41
Molasses	4.00
Wheat bran	1.57
Soy oil	3.00
Fish meal	0.43
Limestone	1.09
DCP	0.73
Salt	0.25
Lysine·HCl	0.25
Vit.-Min. Mix.[1]	0.25
Antibiotics	0.10
Total	100.00
Chemical composition	
Dry matter (%)	85.13
Gross energy (kcal kg^{-1})	4.01
Crude protein (%)	16.80
Ether extract (%)	5.42
Crude fiber (%)	3.08
Crude ash (%)	4.10
Ca (%)	0.84
Total P (%)	0.56

[1]Provided per kilogram of diet; 1,600,000 IU of Vitamin A, 300,000 IU of Vitamin D_3, 800 IU of Vitamin E, 132 mg of Vitamin K_3, 1,000 mg of Vitamin B_2, 1,200 mg of Vitamin B_{12}, 2,000 mg of niacin, 60 mg of folic acid, 35,000 mg of choline chloride, 800 mg of pantothenic calcium, 9,000 mg of Zn, 12,000 mg of Mn, 4,000 mg of Fe, 500 mg of Cu, 6,000 mg of I and 100 mg of Co. Analyzed values are given

Table 2: Chemical and amino acid composition of grape and apple pomace (DM basis)

Items	Grape meal	Apple meal
Chemical composition		
Dry matter (%)	29.23	15.43
Gross energy (kcal kg^{-1})	4.83	4.71
Crude protein (%)	8.26	7.49
Ether extract (%)	7.67	5.61
Crude fiber (%)	38.24	31.91
Crude ash (%)	2.25	2.09
Ca (%)	0.21	0.14
Total P (%)	0.23	0.15
Essential amino acids		
Arginine	0.47	0.40
Histidine	0.20	0.15
Isoleucine	0.28	0.26
Leucine	0.55	0.51
Lysine	0.33	0.37
Methionine	0.11	0.11
Phenylalanine	0.32	0.29
Threonine	0.30	0.29
Valine	0.36	0.32
Non-essential amino acids		
Alanine	0.49	0.37
Asparatic acid	0.65	0.67
Cystine	0.15	0.15
Glutamic acid	1.47	1.19
Glycine	0.56	0.37
Proline	0.34	0.33
Serine	0.36	0.34
Tyrosine	0.16	0.19
Total amino acids	7.10	6.28

Analyzed values are given

Table 3: Chemical and amino acid composition of experimental diets (DM basis)

Items	CON[1]	F[1]	GF10[1]	GF20[1]	AF10[1]	AF20[1]
Chemical composition						
Dry matter (%)	85.13	63.88	70.27	56.59	59.86	40.07
Gross energy (kcal kg^{-1})	4,010	4,517	4,562	4,627	4,510	4.51
Crude protein (%)	16.80	18.54	16.94	16.96	17.31	15.49
Ether extract (%)	5.420	5.980	5.870	6.990	6.170	5.68
Crude fiber (%)	3.080	3.210	7.220	6.820	6.020	9.56
Crude ash (%)	4.100	4.600	4.210	4.280	4.370	3.80
Ca (%)	0.840	0.990	0.900	0.840	0.920	0.77
Total P (%)	0.560	0.650	0.570	0.560	0.570	0.49
Essential amino acids						
Arginine	1.030	1.110	1.000	1.030	1.000	0.85
Histidine	0.390	0.440	0.400	0.410	0.400	0.34
Isoleucine	0.610	0.720	0.640	0.640	0.640	0.56
Leucine	1.560	1.880	1.610	1.600	1.660	1.40
Lysine	1.000	0.930	0.780	0.860	0.710	0.61
Methionine	0.250	0.260	0.240	0.250	0.230	0.22
Phenylalanine	0.800	0.930	0.820	0.820	0.850	0.69
Threonine	0.630	0.730	0.650	0.660	0.670	0.57
Valine	0.740	0.880	0.750	0.780	0.790	0.66
Non-essential amino acids						
Alanine	0.870	1.040	0.890	0.940	0.920	0.77
Asparatic acid	1.580	1.780	1.590	1.600	1.650	1.41
Cystine	0.260	0.270	0.260	0.260	0.250	0.23
Glutamic acid	3.040	3.440	3.160	3.120	3.190	2.61
Glycine	0.690	0.810	0.730	0.790	0.750	0.63
Proline	1.150	1.360	1.180	1.160	1.230	0.92
Serine	0.810	0.930	0.830	0.840	0.870	0.72
Tyrosine	0.440	0.520	0.370	0.440	0.430	0.38
Total amino acids	15.83	18.01	15.88	16.18	16.23	13.54

[1]CON: Dry Compound diet; F:Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermentedliquid compound diet with 20% apple pomace. Analyzed values are given

The juice by-product feed was formulated by mixing control diet and juice by-productive (DM) at ratios of 9:1 and 8:2, respectively. Thereafter 0.2% probiotic (*Lactobacillus reuteri*, 1.2×10^{-6} cfu g^{-1} + Yeast culture, 1.2×10^{6} cfu g^{-1}) was supplemented in these diets and then anaerobic fermented at 25°C for 10 days. Dietary chemical composition and amino acid profile was shown in Table 3.

Sampling and measurements: Chromium Oxide (Cr_2O_3) was added at 0.20% of diet as an indigestible marker to calculate digestibility coefficients. At the end of the experiment, faecal grab samples were taken randomly from at least two pigs in each pen. After collection, faecal samples were dried at 70°C for 72 h and finely ground to pass through a 1 mm screen. Then all feed and feces samples were frozen and stored in a refrigerator at -20°C until analysis. The procedures used for determination of DM and N digestibilities were according to the methods of AOAC (1995). Chromium was analyzed by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) according to the method of Williams.

Urine and feces were collected separately from each of the pigs during the final 7 days (two times per day) with urine being collected in a bucket via a funnel positioned below the cage and feces was collected in a plastic sample bag. All urine samples were stored immediately at 4°C until used and feces samples were stored immediately at 20°C until use. The urine and slurry were collected to determine the fatty acid and ammonia concentration. Urine was used directly for the analysis but the feces and slurry samples were diluted with 1000 mL mineral medium at 1:200 before analyze. The ratio of feces to the urine was determined by the excreted feces and urine during metabolism experiment.

Table 4: Gas chromatography condition of volitile fatty acids

Instruments	Parameters	Values
Injection	Injection volume	0.2 µL
Inlet	Temperature	250°C
	Split ratio	10:1
Column	HP-INNOWax	
	Flow	1.0 mL min^{-1}
		80°C for 2 min
		increased 20°C min^{-1}
		to°C120°C
Oven	Temperature program	120°C for 0 min increased
		10°C min^{-1} to 205°C
		205°C for 2 min
Detector	Temperature	FID 250°C

Volatile fatty acid was analyzed by the Gas Chromatography (6890 N. agilent, USA). Samples were pre-treated before analysis. Briefly, 20 mL samples were incubated in the headspace glass vial at 37 mL for 72 h after which 5 mL pre-treated samples were collected and mixed with 50 µL, saturated $HgCl_2$ mL, 1 mL 25% HPO_3. The mixer were centrifugated at 3500 set for 20 min and then the supernatant was stored at -20°C until analysis. Before the analysis, the supernatant were centrifugated at 12,000 rpm again for 10 min and then the supernatant were filtered with syringe filter (Puradisc 25AS 0.2 µm, Whatman, UK) to a 2 mL autosampler vial and then the sample were transferred to the GC machines. The GC conditions are shown in Table 4.

Viable counts of microflora were measured by plating serial 10 fold dilutions onto MRS agar plates for Lactobacillus bacteria, DHL agar plates for Enterobacteriaceae, PD agar plates for Yeast. MRS agarplates were incubated for 48 h at 37°C under anacrobic conditions, DHL and PD agar plates were incubated for 24 h at 37°C under aerobic conditions.

Statistical analyses: All data were subjected to the GLM procedures of SAS (1996) as a randomized complete block design with each pen serving as the experimental unit. Variability in the data was expressed as the pooled Standard Error (SE) and a $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Nutrient digestibility: Nutrient digestibility was shown in Table 5. DM digestibility was higher ($p<0.05$) in CON, F and AF10 treatments than other treatments, pig fed GF20 and AF20 treatments have the lowest ($p<0.05$) DM digestibility. A highest digestibility of CP were observed ($p<0.05$) in CON and F treatments and was lowest ($p<0.05$) AF20 treatment.

The CF digestibility were highest ($p<0.05$) in CON and F treatments and was lowest ($p<0.05$) in AF20 treatment. However, EE digestibility was different among treatments which has an order as AF10 (68.68%), AF20 (63.32%), F (59.99%), CON (59.16%), GF10 (43.68%), GF20 (2.48%) ($p<0.05$). Ash digestibility in AF20 was lower ($p<0.05$) than other treatments. Ca digestibility was higher ($p<0.05$) in F and AF10 treatments than AF20 treatment. But no different ($p>0.05$) was observed in P digestibility. Energy digestibility was different among treatments which has an order as F (86.75%), CON (85.90%), AF10 (83.30%), GF10 (79.85%), AF20 (73.91%) and GF20 (72.92%) ($p<0.05$).

Varel *et al.* (1984) reported that alfalfa supplementation at 35% in growing-finishing pig increased cellulolytic bacteria and cellulase activity. However, fiber level 3, 6, 9% in feed supplementation linearly decrease DM and energy digestibility (Kim *et al.*, 1992; Dilger *et al.*, 2004), earlier studies have suggested that growing pigs fed high fiber diets decrease ileal and fecal DM, CP and energy digestibility (Sauer *et al.*, 1991; Wilfart *et al.*, 2007). Also, Jung reported that finishing pigs diet including fiber at 3, 7, 11 and 15% linearly decrease stomach, small intestinal, cecum and colon digesta dry matter value, digestive tract transit time and DM, CP, EE and CF digestibility, this maybe due to the reduction of reaction time that cause by enzyme and microbial in digestive tract, similar results were found in this experiment.

Hong and Lindberg (2007) reported that growing pig fed fermented feed increased CP and CF ileal digestibility and total tract CP digestibility, Scholten *et al.* (1999) reported that growing pig fed fermented compound feed improved ADG and G/F but no different was found in between CON and treatments.

Table 5: Effects of fermented diets including grape and apple pomace on nutrient digestibility in finishing pigs[1]

Items	CON[2]	F[2]	GF10[2]	GF20[2]	AF10[2]	AF20[2]	SE[3]
DM	86.71[a]	87.83[a]	81.91[b]	76.30[c]	85.53[a]	77.94[c]	0.75
CP	85.32[a]	86.28[a]	78.22[b]	74.41[c]	81.10[b]	66.14[d]	1.22
CF	81.96[a]	80.37[a]	64.86[bc]	61.28[c]	70.13[b]	44.86[d]	2.43
EE	59.16[b]	59.99[ab]	43.68[c]	2.48[d]	68.68[a]	63.32[ab]	3.19
Ash	57.35[a]	61.76[a]	56.74[a]	57.84[a]	61.40[a]	46.85[b]	2.56
Ca	48.97[ab]	55.62[a]	52.86[ab]	51.48[ab]	55.74[a]	39.79[b]	4.24
P	51.00	56.21	52.83	57.23	60.55	49.62	3.41
Energy	85.90[ab]	86.75[a]	79.85[c]	72.92[d]	83.30[b]	73.91[d]	0.92

[1]Twenty four pigs with an average initial body weight of 63.42±9.78 kg. [2]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [3]Pooled standard error. [a-d]Means in the same row with different superscripts differ ($p<0.05$)

Amino acid digestibility: Amino acid digestibility was shown in Table 6. Lysine, methionine and threonine was higher ($p<0.05$) in CON (88.19, 83.32, 83.39%) and F (86.98, 84.73, 85.41%) treatments than other treatments. Buraczewska *et al.* (2007) reported that growing pig fed diets including 4 and 8% pectin linearly decreased lysine and methionine digestibility, threonine and tryptophan digestibility was decreased in pigs fed pectin supplemented diet, in this experiment digestibility was different among juice by-product supplemented treatments however amino acid digestibility was decreased in juice by-product supplemented treatments.

Also, total essential amino acid, total non-essentail amino aicd and total amino acid were higher ($p<0.05$) in CON (88.07, 88.54, 88.33%) and F (89.39, 89.84, 89.64%) treatments and lowest ($p<0.05$) in AF20 (70.03, 70.51, 70.30%) treatment.

Sauer *et al.* (1991) reported that growing pigs fed fiber supplemented diets decrease all amino acid digestibility, Mitaru *et al.* (1984) reported that high fiber content in diet

Table 6: Effects of fermented diets including grape and apple pomace on amino acids digestibility in finishing pigs

Items	CON[1]	F[1]	GF10[1]	GF20[1]	AF10[1]	AF20[1]	SE[2]
Essential amino acids							
Arginine	93.55[a]	93.92[a]	89.26[b]	86.82[c]	90.00[b]	81.45[d]	0.70
Histidine	93.45[a]	93.82[a]	86.24[c]	82.17[d]	89.41[b]	80.25[d]	0.86
Isoleucine	86.24[a]	88.52[a]	80.85[b]	77.68[b]	81.60[b]	66.77[c]	1.33
Leucine	86.87[ab]	89.26[a]	80.73[bc]	77.86[d]	83.98[bc]	71.97[e]	1.25
Lysine	88.19[a]	86.98[a]	78.09[b]	76.52[b]	75.89[b]	57.09[c]	1.70
Methionine	83.32[ab]	84.73[a]	76.38[bc]	70.48[cd]	75.31[c]	65.10[d]	2.40
Phenylalanine	88.51[ab]	90.25[a]	83.11[cd]	80.27[d]	85.82[bc]	74.15[e]	1.01
Threonine	83.39[ab]	85.41[a]	76.57[cd]	73.35[d]	78.97[bc]	61.02[e]	1.59
Valine	86.59[a]	88.73[a]	79.39[bc]	76.59[c]	82.46[b]	65.67[d]	1.31
Non-essential amino acids							
Alanine	82.29[ab]	85.23[a]	75.13[c]	73.99[c]	78.10[bc]	58.25[d]	1.81
Asparatic acid	87.53[a]	88.73[a]	81.88[bc]	79.39[c]	83.68[b]	70.05[d]	1.11
Cystine	86.66[a]	85.89[a]	76.51[b]	67.53[c]	77.88[b]	64.07[c]	1.34
Glutamic acid	91.27[a]	92.27[a]	86.02[b]	82.67[c]	88.34[b]	76.83[d]	0.86
Glycine	84.37[a]	86.42[a]	75.50[bc]	71.70[c]	79.31[b]	61.18[d]	1.42
Proline	91.83[a]	91.89[a]	80.14[c]	74.64[d]	86.42[b]	69.53[e]	1.14
Serine	87.99[ab]	89.44[a]	82.35[c]	79.55[c]	85.32[b]	72.71[d]	0.97
Tyrosine	85.77[ab]	89.51[a]	79.21[c]	81.29[bc]	81.23[bc]	71.16[d]	1.86
Total essential amino acids	88.07[a]	89.39[a]	81.59[bc]	78.70[c]	83.40[b]	70.03[d]	1.16
Total non-essential amine acids	88.54[a]	89.84[a]	81.70[bc]	78.47[c]	84.62[b]	70.51[d]	1.08
Total amino acids	88.33[a]	89.64[a]	81.65[bc]	78.57[c]	84.10[b]	70.30[d]	1.12

[1]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20,: Fermented liquid compound diet with 20% grape pomace; AF 10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [2]Pooled standard error. [a-e]Means in the same row with different superscripts differ ($p<0.05$)

Table 7: Lactobacillus, enterobacteriaceae and yeast count of diets and fecal microflora in finishing pigs

Items	*Lactobacillus reuteri*[1]	CON[2]	F[2]	GF10[2]	GF20[2]	AF10[2]	AF20[2]	SE[3]
Diet (log cf μg^{-1})								
Lactobacillus	5.79^{f}	0.00^{f}	7.58^{c}	7.18^{e}	7.64^{b}	7.43^{d}	8.45^{a}	6.04
Enterobacteriaceae	3.76^{a}	3.65^{b}	3.11^{c}	2.11^{d}	0.00^{d}	0.00^{d}	0.00^{d}	1.94
Yeast	5.67^{f}	4.30^{f}	7.48^{a}	6.89^{d}	7.45^{b}	6.18^{e}	7.08^{c}	5.45
Fecal (log cf μg^{-1})								
Lactobacillus	-	6.06	6.61	6.96	6.53	6.92	6.59	7.05
Enterobacteriaceae	-	5.87	5.85	4.95	5.19	5.58	5.55	6.27
Yeast	-	3.53^{b}	5.02^{ab}	4.36^{b}	5.39^{a}	4.43^{b}	5.11^{b}	4.97

[1]Probiotics, *Lactobacillus reuteri* 1.2×10^{6} cfu g^{-1} + Yeast culture 1.2×10^{6} cfu g^{-1}, [2]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [3]Pooled standard error. [a-f]Means in the same row with different superscripts differ (p<0.05)

decrease amino acid digestibility, similar result was found in this experiment. This maybe due to the adsorption of amino acid and peptide by fibrin (Mitaru *et al.*, 1984) which cause improved secretion of intestinal mucosa cells (Schneeman *et al.*, 1982). The result of reduced digestibility of amino acids was various and depending on the type and concentration of fiber researchers used.

Fecal microbial: Fecal microbial was shown in Table 7. The lactobacillus concentration in juice by-product supplemented diets were higher (p<0.05) than lactobacillus reuteri supplementation diet. Also, lactobacillus concentration was different (p<0.05) among treatments which has an order as AF20 (8.45), GF20 (7.64), F (7.58), AF10 (7.43), GF10 (7.18) and *Lactobacillus reuteri* (5.79) but there was no Lactobacillus concentration existed in CON diet.

Enterobacteriaceae concentration was different (p<0.05) among treatments which has an order as *Lactobacillus reuteri* (3.76), CON (3.65), F (3.11) and GF10 (2.11) but no Enterobacteriaceae observed in GF20, AF10, AF20 treatments.

Yeast concentration was different (p<0.05) among treatments which has an order as F (7.48), GF20 (7.45), AF20 (7.08), GF10 (6.89), AF10 (6.18), *Lactobacillus reuteri* (5.67) and CON (4.30).

After a anaerobic fementation process the lactobacillus and yeast was increased in juice by-product supplemented diet compared with the inoculated bacterial level with the decreased Enterobacteriaceae.

Canibe *et al.* (2007) reported that the lactobacillus level was increased from 4.2-9.6 log cfu g^{-1} and yeast was increased from 30-7.2 lof cfu g^{-1} in normal feed via fermentation however the Enterobacteriaceae was decreased from 5.4-3.5 log cfu g^{-1}, similar result was found in this experiment. In addition, fermentation treatments significantly reduced the Enterobacteriaceae level which is due to than low pH caused by the proliferation of lactobacillus.

Table 8: Effects of fermented diets including grape and apple pomace on volitile fatty acids from feces, urine and slurry

Items (ppm)	CON[1]	F[1]	GF10[1]	GF20[1]	AF10[1]	AF20[1]	SE[2]
Feces							
Acetic acid	306^{a}	187^{bc}	193^{bc}	134^{c}	234^{ab}	186^{bc}	28.5
Propionic acid	150^{a}	97^{ab}	91^{ab}	45^{b}	113^{ab}	70^{b}	20.6
Butyric acid	110	80	81	57	87	82	19.0
Isobutyric acid	26^{a}	18^{b}	17^{b}	13^{b}	18^{b}	17^{b}	2.4
Valeric acid	41^{a}	23^{ab}	22^{ab}	13^{b}	18^{ab}	17^{b}	7.1
Isovaleric acid	43^{a}	27^{b}	28^{b}	18^{b}	25^{b}	24^{b}	4.1
Ammonia	178	159	170	159	171	164	10.3
Urine							
Acetic acid	269	299	269	348	224	224	74.3
Propionic acid	15	15	23	53	12	20	17.0
Butyric acid	7	8	13	19	3	14	9.3
Isobutyric acid	0	0	0	0	0	0	0.0
Valeric acid	43	10	32	31	41	19	15.4
Isovaleric acid	7	12	14	22	9	15	5.4
Ammonia	2,344	2,246	1,082	1,808	1,440	1,079	484.2
Slurry							
Acetic acid	3,084	3,258	3,863	2,452	5,048	3,492	798.5
Propionic acid	384^{b}	700^{b}	654^{b}	486^{b}	1,929^{a}	692^{b}	230.2
Butyric acid	249^{b}	582^{b}	804^{b}	504^{b}	2,200^{a}	604^{b}	314.4
Isobutyric acid	53^{b}	134^{b}	128^{b}	67^{b}	229^{a}	106^{b}	27.2
Valeric acid	37^{b}	84^{ab}	102^{ab}	84^{ab}	201^{a}	85^{ab}	46.1
Isovaleric acid	109^{b}	243^{ab}	216^{b}	110^{b}	358^{a}	189^{b}	44.2
Ammonia	4,217	5,234	2,819	3,603	3,921	2,573	1,022.1

[1]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [2]Pooled standard error. [a-c]Means in the same row with different superscripts differ (p<0.05)

The fecal Lactobacillus and Enterobacteriaceae level were not influenced (p>0.05) by the dietary treatments, the fecal yeast level was higher (p<0.05) in GF20 treatment than CON, GF10, AF10 and AF20 treatments. Canibe *et al.* (2007) reported that the lactic acid bacteria and Enterobacteriaceae levels in normal diet and fermented diet were not affected but yeast was higher in fermented diet than normal diet. Similar result was found in this experiment.

VFA and ammonia from feces, urine and slurry: VFA and ammonia from feces, urine and slurry was shown in Table 8. Fecal acetic acid was different (p<0.05) among treatments which has an order as CON (306), AF10 (234), GF10 (193), F (187), AF20 (186), GF20 (134), CON treatment was higher than other treatments. Propionic acid concentration was higher (p<0.05) in CON treatment than those in GF20 and AF20 tratments. Butyric acid concentration was not influenced (p>0.05) by juice by product supplementation, iso-butric acid and iso-valeric acid concentration was higher (p<0.05) in CON treatment than other treatments. Valeric acid concentration was higher (p<0.05) in CON treatment than in GF20 and AF20 treatments. However, ammonia concentration was not affected (p>0.05) by juice by-product supplementation.

Table 9: Effects of fermented diets including grape and apple pomace on feces and urine excretion in finishing pigs

Items	CON[1]	F[1]	GF10[1]	GF20[1]	AF10[1]	AF20[1]	SE[2]
Feed intake (kg day^{-1})	1.87^{d}	2.38^{bc}	2.16^{cd}	2.68^{b}	2.56^{bc}	3.84^{a}	0.16
Feed intake (DM/kg/day)	1.59	1.52	1.52	1.52	1.54	1.54	0.10
Feces (kg day^{-1})	0.58^{c}	0.53^{c}	0.76^{c}	1.03^{b}	0.75^{c}	1.28^{a}	0.08
Feces (DM/kg/day)	0.21^{cd}	0.19^{d}	0.28^{bc}	0.37^{a}	0.22^{cd}	0.34^{ab}	0.02
Urine (L day^{-1})	3.39	5.39	5.66	4.84	4.36	1.87	1.98

[1]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [2]Pooled standard error. [a-d]Means in the same row with different superscripts differ (p<0.05)

Generally speaking dietary fiber supplementation increased the volatile fatty acids (Kass *et al*., 1980). Farnworth *et al*. (1995) reported that the Helianthus tuberosus supplementation in weanling pig diets at 0, 1, 3 and 6%, respectively. The result indicated that acetic acid, propionic acid, butyric acid, iso-butyric acid and Iso-valeric acid concentration increased from 0-3% supplementation level and decreased at 6% supplementation level, this experiment result indicated that VFA was decreased in GF10 and AF10 treatments compared with GF20 and AF20 treatments.

Urine VFA and ammonia concentration was not affected (p>0.05) by dietary treatments. However, the ammonia emission concentration was higher from urine than feces.

Slurry acetic acid has no difference (p>0.05) among dietary treatments. However, Propionic acid, butyric acid and iso-butyric acid concentration was higher (p<0.05) in AF10 treatment than other treatments. Valeric acid concentration was higher (p<0.05) in AF10 treatment than in CON treatment. Iso-valeric acid concentration was higher (p<0.05) in AF10 treatment than CON, GF10, GF20 and AF20 treatments. However, ammonia concentration was not affected (p>0.05) by dietary treatments.

Varel *et al*. (1984) reported that 35% alpalpa was supplemented in growing-finishing pig diet decreased ammonia-N emission, this experiment the urine and slurry ammonia emission was decreased in juice by-product supplmented treatments compare with in CON and F treatments. The main source of ammonia is urea in pig urine, the urease exist in feces could decomposed urea into ammonia and carbon dioxide (Stevens *et al*., 1989). The higher ammonia emission in slurry than feces and urine maybe due to exist of urease in slurry.

Feces and urine excretion: Feces and urine excretion was shown in Table 9. ADFI was different (p<0.05) among treatments which has an order as AF20 (3.84), GF20 (2.68), AF10 (2.56), F (2.38), GF10 (2.16), CON (1.87). ADFI (DM) was not affected (p>0.05) by dietary treatments. Average daily feces excretion was higher (p<0.05) in AF20 treatment than other treatments. However, average daily feces (DM) excretion was different (p<0.05) among treatments which has an order as GF20 (0.37), AF20 (0.34), GF10 (0.28), AF10 (0.22), CON (0.21), F (0.19). Average daily urine excretion was not affected (p>0.05) by dietary treatments. The grape seed takes up >50% of dry grape meal, the grape seed can not be chewed by pig due to its small particle size. Thus, grape seed directly go to digestive tract and hardly be digested. The supplementation of juice by-product increased the feces excretion was due to the high fiber content in these diets.

Table 10: Effects of fermented diets including grape and apple pomace on nitrogen balance in finishing pigs

Items	CON[1]	F[1]	GF10[1]	GF20[1]	AF10[1]	AF20[1]	SE[2]
Intake N (g day^{-1})	42.81	45.11	41.04	41.23	42.51	38.14	2.86
Fecal N (g day^{-1})	6.87^{cd}	6.02^{d}	8.68^{bc}	10.27^{ab}	7.80^{cd}	12.37^{a}	0.75
Urinary N (g day^{-1})	18.24^{ab}	19.26^{a}	12.45^{b}	11.99^{b}	11.90^{b}	12.22^{b}	2.05
N Retention (g day^{-1})	17.70^{bc}	19.83^{ab}	19.91^{ab}	18.97^{ab}	22.81^{a}	13.55^{c}	1.45
Fecal N excretion ratio (%)	15.89^{de}	13.17^{e}	20.99^{c}	24.76^{b}	18.22^{cd}	32.50^{a}	1.22
Urinary N excretion ratio (%)	42.93^{a}	41.72^{ab}	30.30^{c}	29.59^{c}	27.70^{c}	31.26^{bc}	3.53
N retention ratio (%)	41.19^{bc}	45.11^{abc}	48.71^{ab}	45.66^{abc}	54.08^{a}	36.25^{c}	2.99

[1]CON: Dry compound diet; F: Fermented liquid compound diet; GF10: Fermented liquid compound diet with 10% grape pomace; GF20: Fermented liquid compound diet with 20% grape pomace; AF10: Fermented liquid compound diet with 10% apple pomace; AF20: Fermented liquid compound diet with 20% apple pomace. [2]Pooled standard error.[a-e]Means in the same row with different superscripts differ (p<0.05)

Nitrogen balance: Nitrogen balance was shown in Table 10. Intake N was not affected (p>0.05) by dietary treatments. Fecal N was different (p<0.05) among treatments which has an order as AF20 (12.37), GF20 (10.27), GF10 (8.68), AF10 (7.80), CON (6.87), F (6.02). Fecal nitrogen excrerition is associated with the amino acid digestibility (Sauer *et al*., 1991) in this experiment, comparison between amino acid digestibility and nitrogen excretion confirmed this respective.

Urinary N was higher (p<0.05) in F treatment than GF10, GF20, AF10 and AF20 treatments. N retention was different (p<0.05) among treatments which has an order as AF10 (22.81), GF10 (19.91), F (19.83), GF20 (18.97), CON (17.70), AF20 (13.55).

Fecal N excretion ratio was different (p<0.05) among treatments which has an order as AF20 (32.50), GF20 (24.76), GF10 (20.99), AF10 (18.22), CON (15.89), F (13.17). Urinary N excretion ratio was highest (p<0.05) in CON and F treatment however lowest (p<0.05) in GF10, GF20, AF10 treatments. N retention ratio was higher (p<0.05) in AF10 treatment than in CON and AF20 treatments.

Generally speaking the fiber supplementation increase fecal and urine nitrogen excretion; Varel *et al.* (1984), Morgan and Whittemore (1988) and Zervas and Zijlstra (2002) reported that dietary soybean hull and beet pulp supplementation increase fecal nitrogen excretion ratio and decrease urine nitrogen excretion ratio no matter via restricted feeding or free feeding. In addition, Canh *et al.* (1997) reported that finishing pigs fed with high fiber diet decreased urinary urea, increase fecal nitrogen excretion ratio and decrease urinary nitrogen excretion ratio. The result was in agreement with this report.

The fermented diet was more effective than normal diet, the supplementation of juice by-product in fermented feed have negative effect on digestibility and increase feces excretion. However, the juice by product supplementation at 10% was more effective than at 20% in digestibility, feces and urine excretion and nitrogen retention ratio. Further experiment need to be carried out to investigate the optical supplementation level of juice by-product.

CONCLUSION

In this study, fermented feed improved nutrient digestibility, the juice by product supplementation at 10% was more effective than at 20% in nutrient digestibility, feces and urine excretion and nitrogen retention ratio.

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