Asian-Aust. J. Anim. Sci.
Vol. 21, No. 4 : 574 - 581
April 2008

www.ajas.info

The Effect of Roselle (*Hibicus sabdariffa* Linn.) Calyx as Antioxidant and Acidifier on Growth Performance in Postweaning Pigs

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ABSTRACT : Two experiments, involving a total of 100 crossbred pigs (Hampshire×Landrace×Duroc) aged 5 weeks, were used to evaluate the effect of Roselle (*Hibicus sabdariffa* Linn.) calyx as an antioxidant and acidifier on growth performance. Experiment 1: growth performance response of pigs fed basal corn-soy diet was compared with that of pigs consuming diets that contained 4, 8 and 12% Roselle in powder form, 4 g/kg acidifier (Fra® Acid Dry) or 100 mg/kg antibiotic (Aurofac®). All diets were isocaloric and isonitrogenous. Twenty-four castrated male and twelve female pigs were randomly allocated into 6 treatments which comprised 3 replicates (2 male and 1 female) of 2 pigs each. The results demonstrated no beneficial weight gain and feed intake response among treatments at 7, 9 and 11 weeks of age ($p>0.05$). However, feed-to-gain ratios (FCR) of Roselle-fed groups were significantly different from the basal-diet group at 7 weeks of age ($p<0.05$). Pigs fed with 8% Roselle had the lowest FCR. Therefore, 8% was set as an appropriate level of Roselle in feed. Experiment 2 was conducted to determine antioxidant and acidifier properties. Pigs were fed similar diets to Experiment 1 except that Roselle was fed to only one treatment at the level of 8%. Sixty-four piglets, 32 castrated male and female each were randomly allocated into 4 treatments which comprised 4 replicates (2 male and 2 female) of 4 pigs each. Body weight was measured. At 7 and 9 weeks of age, four pigs from each treatment were randomly selected. The pH in their gastrointestinal tract, pepsin activity in stomach mucosa, trypsin activity in the pancreas and protein and fat digestibility in the ileum were measured to investigate acidifier properties. For antioxidant properties, malondialdehyde (MDA) and glutathione concentration in plasma and liver were used as parameters. The results showed no significant difference in weight gain, pH or pepsin activity among all treatments at both ages ($p>0.05$). Nevertheless, trypsin activity and fat digestibility of the Roselle-fed group were higher than the basal-diet group at 7 weeks of age ($p<0.05$). Glutathione in plasma was significantly different between antibiotic-fed and basal-diet groups ($p<0.05$). Significant differences were found only in some parameters and between the basal-diet group and the other groups. Therefore, it cannot be precisely concluded that Roselle in powder form has clear acidifier and antioxidant properties in postweaning pigs. Further studies should be undertaken to clearly confirm both properties. (**Key Words :** Roselle, Antioxidant, Acidifier, Growth Performances, Enzyme Activity, Malondialdehyde, Ileal Digestibility, Postweaning Pigs)

INTRODUCTION

The postweaning lag period is a result of limited digestive and absorptive capacity due to insufficient production of hydrochloric acid (HCl), pancreatic enzymes and sudden changes in feed consistency and intake (Cranwell, 1995). Therefore, digestion of proteins and populations of beneficial bacteria are minimized and harmful bacteria are exhibited (Doyle, 2001). Incorporated with environmental stress from changing place and society may lead to higher risk of diarrhea, retarded growth and, in some instances, morbidity and death (Partanen and Mroz, 1999); this increases costs due to extra medication. To cope with these problems, antibiotics (Mahady, 2002) and alternative feed additives, such as organic and inorganic acids (Gauthier, 2002), etc. have been used.

Roselle (*Hibicus sabdariffa* L.) is an interesting herb ingredient because its petals consist of anthocyanin pigment which has many properties corresponding to biological activities such as antioxidant activity (Tseng et al., 1997; Wang et al., 2000; Liu et al., 2002; Tsai et al., 2002; Ali et al., 2003) and inhibition of pathogenic bacteria (Chu et al., 1987; Kramonwan and Narumon, 2001). For antioxidant properties, results have demonstrated that dried calyx

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Received April 30, 2007; Accepted October 11, 2007

Table 1. Chemical composition of Roselle (DM, %)

Nutrients	Experiment 1	Experiment 2
Crude protein	10.27	10.48
Crude fat	1.98	1.84
Crude fiber	12.05	11.75
Ash	8.96	8.64
Calcium	1.29	1.22
Total phosphorus	0.39	0.38
Total phenolic compound (mg/g)	20.23	21.84
Calculated ME (kcal/kg)	2,578.38	2,634.63

extract of Roselle significantly decreased the leakage of lactate dehydrogenase, formation of MDA and liver damage induced by t-BHP (Tseng et al., 1997; Wang et al., 2000) and paracetamol (Ali et al., 2003) and increased glutathione (Wang et al., 2000) in rats. In addition, Roselle calyx consists of several organic acids such as acetic, formic, malic, stearic and tartaric acid (Nanthawan and Auranut, 1997). These organic acids may have the same mode of action as acidifier to provide optimal gut environment and to increase nutrient digestibility and growth performace (Gauthier, 2002). The objectives of this study were to determine a suitable dose of the Roselle calyx powder and its effects on growth performance, antioxidant activity in plasma and liver tissue, pH and activity of enzymes in the gastrointestinal tract and on digestibility of nutrients in the ileum.

MATERIALS AND METHODS

This study was approved by the Institutional Laboratory Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University.

Preparation of Roselle calyx powder

Dried calyx of Roselle was bought from the supplier who supplies the food and pharmaceutical industry. Roselle was milled and passed through a 2 mm screen by a cutting mill machine. The calyx powder was analyzed for total phenolic compound (Duh and Yen, 1997) and nutritional content by proximate analysis (AOAC, 1990) as showed in Table 1. Metabolizable energy of Roselle was calculated using DE (Noblet and Perez, 1993) and ME equations (May and Bell, 1971). The Roselle calyx powder was kept at -20°C until use.

Animal and management

Two experiments involved a total of 100 crossbred piglets (Hampshire×Landrace×Duroc) weaned at four weeks of age plus an adaptation period of 7 days. Initial body weights of all pigs in each group were not significantly different. All piglets received diets and water *ad libitum* for the whole period.

Experiment 1 : Thirty-six piglets, 24 castrated male and 12 female, were used. All animals were randomly allocated into 6 treatments which comprised 3 replicates (2 castrated male and 1 female) of 2 pigs each. All pigs were raised for 6 weeks.

Experiment 2 : Sixty-four piglets, 32 castrated male and female each, were used. All animals were randomly allocated into 4 treatments which comprised 4 replicates (2 castrated and 2 female) of 4 pigs each. All weaned pigs were raised in groups in 1.75×2.5 m pens on a concrete floor with one nipple waterer and a four-hole self-feeder per pen for four weeks. The temperature and relative humidity were recorded on a daily basis at 7 a.m. and 4 p.m. The average temperature and relative humidity over the entire experimental period was 26.36-30.90°C and 60.15-85.17% and 25.20-31.94°C and 51.32-85.71% in experiment 1 and 2, respectively.

Feed and feeding

All diets in both experiments were calculated to be isocaloric and isonitrogenous according to NRC requirements (1998).

Experiment 1 : There were six treatment diets: basal diet, diet containing Roselle calyx powder at the level of 4, 8, and 12% feed, acidifier (Fra® Acid Dry) at 4 g/kg feed, or antibiotic (Aurofac®) at 100 mg/kg feed. The diet formula and chemical analysis are shown in Table 2. All pigs were fed for 6 weeks.

Experiment 2 : There were four treatment diets: basal diet, diet with Roselle calyx powder at the level of 8% feed, acidifier at 4 g/kg feed, or antibiotic at 100 mg/kg feed. The same diet formula was used in both experiments. The pigs were fed for a period of 4 weeks during which, from day 10 to 14 and day 24 to 28, Celite, a source of acid insoluble ash (AIA), was added to all diets at 20 g/kg feed as an indigestible dietary marker. The use of 20 g/kg AIA for a 4-day adaptation period was enough for equilibrating the flow of digesta as it was used at twice normal concentration compared to the general use of 10 g/kg AIA for 5-7 days (Sales and Janssens, 2003).

Data collection

Data recording : Body weight and feed intake were measured every 2 weeks in both experiments.

Sample collection : Samples were collected only in Experiment 2. Four pigs from each treatment group were randomly selected in the 2nd and 4th week of the experimental period and a blood sample was collected from the cranial vena cava. The pigs were euthanized by an intravenous overdose of pentobarbital sodium. Immediately after slaughter, the abdominal cavity was opened and the entire gastrointestinal tract was removed. The pancreas and liver were carefully dissected and kept frozen at -70°C. The gastric pH was measured at the center of the fundic region

Table 2. The ingredient composition and chemical analysis of experimental diets

Ingredient	Amount (kg/100 kg diet)					
	Basal diet	Roselle 4%	Roselle 8%	Roselle 12%	Acidi-fier	Anti-biotic
Broken rice	38.48	33.59	28.75	23.85	37.65	38.43
Soybean meal	27.98	27.91	27.83	27.74	28.12	27.98
Full fat soybean meal	8.00	8.00	8.00	8.00	8.00	8.00
Rice bran	12.00	12.00	12.00	12.00	12.00	12.00
Fishmeal	3.00	3.00	3.00	3.00	3.00	3.00
Whey	5.00	5.00	5.00	5.00	5.00	5.00
Coconut oil	2.90	3.97	5.03	6.13	3.18	2.90
Mono-dicalcium phosphate	1.00	0.97	0.93	0.90	1.00	1.00
Salt	0.27	0.27	0.27	0.27	0.27	0.27
DL-methionine	0.02	0.03	0.05	0.06	0.02	0.02
Oystershell	0.86	0.76	0.65	0.55	0.85	0.86
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Roselle	-	4.00	8.00	12.00	-	-
Acidifier[2]	-	-	-	-	0.40	-
Antibiotic[3]	-	-	-	-	-	0.05
Chemical analysis (% DM)						
Crude protein	22.82	22.64	22.68	22.84	22.71	22.88
Crude fat	8.31	8.39	9.16	9.37	8.34	8.45
Crude fiber	2.34	2.72	3.00	3.32	2.42	2.38
Ash	6.77	6.80	7.40	7.26	7.16	6.85
Calcium	0.88	0.91	0.95	0.95	0.93	0.90
Total phosphorus	0.77	0.83	0.87	0.76	0.81	0.79
Calculated ME (kcal/kg)	3,265	3,265	3,265	3,265	3,265	3,265

[1] Premix/kg of diet: vitamin A 20,000 IU; vitamin D_3 4,000 IU; vitamin E 40 mg; vitamin K_3 4 mg; vitamin B_1 2 mg; vitamin B_2 10 mg; vitamin B_6 3 mg; vitamin B_{12} 0.03 mg; nicotinic acid 40 mg; calcium pantothenate 20 mg; choline choride 200 mg; folic acid 2 mg; biotin 0.1 mg; Fe 300 mg; Co 2 mg, Mn 80 mg; Cu 400 mg; Zn 224 mg; I 2 mg; Se 0.2 mg; ethoxyquin 100 mg; silicon dioxide 20 mg.

[2] Acidifier (Fra® Acid Dry)/kg diet: formic acid 0.86 g, lactic acid 0.74 g, citric acid 0.04 g, fumaric acid 0.4 g and carrier.

[3] Antibiotic (Aurofac®)/kg diet: chlortetracycline 100 mg.

and the intestinal pH was measured at 3 points (duodenum at 30.5 cm from upper duodenum, jejunum at 61 cm from pancreas and ileum at 30.5 cm before colon) using *in situ* measurement by a semi-solid glass electrode probe (Eutech instruments). The measurable points were opened and the probe was put into the digesta and was read when the sign showed up. The stomach was opened along the greater curvature and rinsed with cold saline solution. The opened stomach was laid flat on ice and the mucosa of the fundic region was scraped from the muscular layer using a glass slide, wrapped with tin foil and kept frozen at -70°C. The ileal content was collected on day 14 and 28 by gentle squeezing into plastic bottles with thumb and finger. The samples of ileal content from pigs in each replicate were dried at 60°C for twenty-four hours, ground and kept frozen at -20°C until analysis.

Sample analysis

Plasma and liver tissue were analyzed for malondialdehyde formation using the thiobarbituric acid reaction method suggested by Ohkawa et al. (1979), while glutathione content was measured employing the method recommended by Beutler et al. (1963) and modified by Mayerly et al. (2000). The homogenate of pancreas was analyzed for trypsin activity using the method described by Rick (1965). The homogenate of fundic mucosa was analyzed for gastric pepsin activity using the method described by Rick (1965) with slight modifications. In brief, the mucosal proteolytic zymogens were activated by adjusting the mucosal supernatant to pH 2.0-2.5. The pepsin activity was measured at pH 1.5-2.5 using haemoglobin as a substrate. The 2.5 ml of substrate was added to 80 μl of homogenate and 400 μl of 0.01 N HCl and left at 25°C for 10 min. Then 5 ml of 5% trichloroacetic acid solution was added to stop the reaction. The solution was vigorously stirred and centrifuged at 4,000×g for 20 min and, subsequently, 2.5 ml supernatant was added into 5 ml 0.5 N NaOH and 1.5 ml 1:2 folin and ciocalteu phenol reagent. The optical density was read at 750 nm against a blank (distilled water) using a UV-VIS spectrophotometer (Shimadzu® UV 1201). Total tissue protein concentrations of gastric mucosa, pancreas and liver were determined using the Lowry (1951) method. For ileal content, acid-insoluble ash (celite) was measured as described by Choct and Annison (1992) and nutrient composition was analyzed using proximate analysis (AOAC, 1990).

Statistical analysis

Statistical analysis for all dependent variables was performed as a completely randomized design using one-

Table 3. Effect of Roselle on growth performance in Experiment 1[1]

Item	Basal diet	Roselle 4%	Roselle 8%	Roselle 12%	Acidifier	Antibiotic
No. of pigs	6	6	6	6	6	6
Initial wt. (kg)	7.03±0.58	7.23±1.52	7.35±0.53	7.14±0.50	7.82±1.02	7.48±1.03
Final wt. (kg)	28.67±1.08	30.70±4.76	31.03±3.48	30.63±0.32	33.07±3.00	31.90±2.25
Daily weight gain (kg)						
5-7 wks	0.36±0.03	0.39±0.10	0.41±0.01	0.42±0.03	0.43±0.36	0.44±0.07
7-9 wks	0.54±0.06	0.57±0.11	0.57±0.10	0.54±0.02	0.57±0.07	0.52±0.04
9-11 wks	0.64±0.09	0.71±0.03	0.71±0.13	0.72±0.03	0.79±0.05	0.78±0.06
5-11 wks	0.52±0.02	0.56±0.08	0.56±0.07	0.56±0.01	0.60±0.04	0.58±0.04
Daily feed intake (kg)						
5-7 wks	0.47±0.03	0.52±0.13	0.48±0.02	0.52±0.03	0.54±0.06	0.52±0.09
7-9 wks	0.78±0.11	0.86±0.16	0.82±0.05	0.83±0.03	0.86±0.08	0.77±0.05
9-11 wks	1.01±0.06	1.17±0.15	1.01±0.17	1.15±0.02	1.23±0.09	1.15±0.05
5-11 wks	0.75±0.05	0.85±0.14	0.79±0.08	0.83±0.02	0.87±0.06	0.81±0.06
Feed conversion ratio[2]						
5-7 wks	$1.31±0.03^{A}$	$1.32±0.02^{A}$	$1.17±0.03^{B}$	$1.24±0.06^{AB}$	$1.24±0.04^{AB}$	$1.18±0.09^{B}$
7-9 wks	1.46±0.06	1.50±0.01	1.46±0.14	1.52±0.11	1.50±0.08	1.49±0.10
9-11 wks	1.57±0.15	1.64±0.13	1.51±0.10	1.61±0.08	1.54±0.06	1.47±0.07
5-11 wks	1.46±0.07	1.51±0.04	1.40±0.04	1.49±0.06	1.46±0.04	1.40±0.04

[1] Mean±SD. [2] Mean in the same row with different superscripts differed significantly at p<0.05.

way analysis of variance (ANOVA) to determine the effects of treatments (SAS, 1989). Dependent variables were growth performance, gastrointestinal pH, pepsin and trypsin activity, ileal digestibility, MDA and glutathione concentration. Significant differences of variables among treatments were determined by Duncan's New Multiple Range Test at the level of p<0.05 (Steel and Torrie, 1980).

RESULT AND DISCUSSION

Experiment 1

The results demonstrated that Roselle had no benefit on daily gain and feed intake in all periods except for feed-to-gain ratio (FCR). At 5-7 weeks of age, the FCR of the pigs receiving 8% Roselle was similar to that for antibiotics but these results were better than those for the basal diet group (p<0.05). During 7-9 and 9-11 weeks of age, Roselle did not show any significant effect in all parameters (Table 3). The effects of Roselle as acidifier and antibiotic on growth performance were obvious only during 5-7 weeks of age. This could be because of immaturity of the digestive system as suggested by Wilson and Leibholz (1981) who found that the development of gastric pH of the weaning pig is low in acidity for the first 40 days. Therefore, the improvement with Roselle can be explained by the presence of organic acids (Nanthawan and Auranut, 1997) which help maintain optimal pH, activate function of proteolytic enzymes (Thaela et al., 1988; Peris and Calafat, 1994) and, consequently, improve protein digestibility (Kemme, 1988). Roselle also has anti-microbial properties (Chu et al., 1987) but might not be as strong as antibiotic. The acids can penetrate the microbial cell wall and disrupt the normal action of certain types of bacteria including *Salmonella* spp., *E coli*, *Clostridium* spp and *Listeria* spp. (Dibner and Buttin, 2002). Moreover the results might have been caused by phenolic compounds in Roselle, such as protocatechuic acid and anthocyanin, which are reported to have antioxidant properties (Wang et al., 2000). It could be argued that the decrease in FCR was due to the effect of higher coconut oil in the Roselle diet (5%) compared to the other groups (3%) since Pettigrew and Moser (1991), who reported studies in which a constant protein-to-energy ratio was maintained, found no response in growth rate, a reduction in feed intake and improvement in gain-to-feed ratio when fat was added. In the present study, all feeds were formulated at constant protein-to-energy ratio; thus, the effect of coconut oil on FCR could be dismissed. Therefore, the 2nd experiment was conducted to prove these explanations except for anti-microbial properties. Roselle at the level of 8% was chosen based on the finding that FCR at 5-7 week improved by 10.68 and 5.34% in the 8 and 12% Roselle-fed group, respectively, over the basal-diet group, whereas daily weight gain improved by almost the same percentage (13.13 and 14.90 for 8 and 12% Roselle) over the basal diet.

Experiment 2

Acidifier properties : Gastrointestinal pH, enzyme activity and ileal digestibility were used as parameters to show acidifier properties. No significant difference was found in gastric pH and pepsin activity in both periods (Table 4); this indicated that the concentration of organic acids in Roselle and acidifier used in this experiment might be too weak to decrease pH and, consequently, to promote pepsin activity. The optimal pH for pepsin activity is 2-3.5

Table 4. Effect of Roselle on pH and enzyme activity of the gastrointestinal tract in Experiment 2[1]

Item	Basal diet	Roselle 8%	Acidifier	Antibiotic
Number of samples	4	4	4	4
At 7 weeks of age				
pH: Stomach	5.21±0.25	4.02±0.88	4.54±0.55	4.66±0.40
Duodenum	5.83±0.32	5.97±0.39	5.98±0.43	5.87±0.28
Jejunum	5.56±0.42	5.62±0.54	5.88±0.44	5.33±0.51
Ileum	6.68±0.25	6.91±0.17	6.72±0.39	6.84±0.31
Pepsin activity (PU^{Hb}/mg protein)[2]	0.48±0.10	0.79±0.15	0.63±0.25	0.57±0.11
Trypsin activity (U/mg protein)[3, 4]	1.43±0.28^{B}	2.21±0.35^{A}	2.36±0.35^{A}	2.22±0.37^{A}
At 9 weeks of age				
pH: Stomach	3.65±1.03	3.04±0.31	4.11±0.48	3.97±0.25
Duodenum	5.81±0.47	5.16±0.19	5.96±0.28	5.42±0.53
Jejunum	5.58±0.59	5.30±0.29	5.65±0.45	5.85±0.45
Ileum	6.89±0.27	6.79±0.14	6.82±0.11	6.56±0.10
Pepsin activity (PU^{Hb}/mg protein)	0.69±0.05	0.74±0.10	0.90±0.15	0.80±0.21
Trypsin activity (U/mg protein)	2.14±0.36	2.04±0.35	2.26±0.34	2.38±0.23

[1] Mean±SD. [2] $PU^{Hb}\times10^{3}$ = 1 mmol tyrosine-equivalents liberated/minute at 35.5°C.
[3] U = the amount of 1 μmol hydrolyzed substrate per minute.
[4] Mean in the same row with different superscripts differed significantly at p<0.05.

(Cranwell, 1995) and its activity declines rapidly when pH rises above 3-6 and remains inactive at pH 6 (Partanen and Mroz, 1999). However, the lowest pH in the experiment was only 4.02 in the Roselle group which was too high to show any advantage. The *in situ* method used to measure pH directly from the stomach and intestine in this experiment may have caused very large variation and resulted in no significant difference, although no support could be found in the literature for this explanation. Thus, it needs to be confirmed whether this method is effective by comparing it concurrently with the accepted method. However, the pH of end products of pepsin digestion of Roselle, acidifier and antibiotic groups were low enough to stimulate trypsin activity (p<0.05) compared to the basal-diet group. Partanen and Mroz (1999) mentioned that the low pH of digesta and the end-products of pepsin digestion entering the duodenum are involved in the stimulation of pancreatic secretion of enzymes and bicarbonate. Also, Thaela et al. (1998) reported that supplementation of 2.5% lactic acid in weaning pigs increased trypsin and chymotrypsin activity in the pancreas. At 9 weeks of age, addition of 8% Roselle failed to produce a response in proteolytic enzyme because pigs can produce adequate HCl to maintain stomach pH at this age, and gastric pH was lower than at 7 weeks of age in all groups.

To determine ileal digestibility, protein and fat were examined. The results showed no positive effect of 8% Roselle, acidifier and antibiotic on protein digestibility (Table 5) even though trypsin activity increased. This result was similar to Kil et al. (2006) in the acidifier supplementation research in weaned piglets by using 3 different organic acids (fumaric, formic, lactic acid) but contradicted the finding of Kemme (1998). He reported that an addition of 0.6% of mixed acids (formic: propionic acid = 75:25) caused an improvement in digestibility of crude protein in weaning pigs which was supported by data of Kirchgessner and Roth (1982) when the diets contained 1.5 to 2% fumaric acid. However, a similar effect was shown on apparent digestibility of protein and dry matter at 4 weeks of age when 1, 2% fumaric or citric acids were supplemented in the diet (Falkowski and Aherne, 1984). The contrast might be due to low concentration, source and efficacy of organic acid, dietary buffering capacity as well as the age of pigs used (Ravindran and Kornegay, 1993). In addition, there are many luminal and brush border enzymes responsible for protein digestion.

For fat digestion, the effect of both 8% Roselle and antibiotic clearly improved fat digestibility at 7 weeks of

Table 5. Effect of Roselle on ileal digestibility of nutrients in Experiment 2 [1,2]

Nutrients	Basal diet	Roselle 8%	Acidifier	Antibiotic
At 7 weeks of age				
Protein (%)	81.39±3.04 (3)	81.47±1.53 (2)	82.20±00.91 (4)	82.44±0.48 (2)
Fat (%)	81.02±1.89^{B} (4)	85.83±0.73^{A} (2)	83.18±1.66^{AB} (4)	85.30±0.46^{A} (2)
At 9 weeks of age				
Protein (%)	74.90±12.37(2)	78.68±5.93 (2)	85.91±7.36 (4)	88.13 (1)
Fat (%)	85.67±5.60 (2)	88.62±5.89 (2)	76.95±14.13 (4)	83.86 (1)

[1] Mean±SD. [2] Numbers in parenthesis are the quantity of analyzed sample.

age compared to the basal-diet group ($p<0.05$). There are 2 hypotheses: it might be because of 1) higher level of coconut oil as an energy source in the Roselle diet which is easy to digest; or 2) an increase in lipase enzyme activity. Coconut oil is more digestible in earlier postweaning pigs as it is high in medium-chain fatty acids (Stahly, 1984; Cera et al., 1989). Unfortunately, lipase activity was not determined. Since ileal content samples could be collected only in very small amounts and were mostly high in water content, the samples were inadequate for analysis of all parameters. Thus, the data were insufficient to draw statistical conclusions. Further investigation is needed to confirm the nutrient digestibility results and to enable firm conclusions.

Antioxidant properties : Malondialdehyde (MDA) formation and glutathione concentration, a marker of lipid peroxidation, were used as parameters to show antioxidant properties. The plasma and liver tissue MDA and glutathione are depicted in Table 6. No significant difference was found in MDA at both ages. The result was in contrast to Wang et al. (2000) who reported that extract of calyx Roselle at concentrations of 0.1 and 0.2 mg/ml in studies of hepatotoxicity, induced by *tert*-butyl hydroperoxide (t-BHP) in rats, significantly decreased the formation of MDA, similarly to that reported by Tseng et al. (1997). The level of glutathione in plasma of pigs fed with antibiotic showed significant differences ($p<0.05$) when compared with the basal diet, whereas no significant differences were detected when compared to acidifier and Roselle at 7 weeks of age. The level of glutathione in liver tissue of pigs in all groups was not significantly different ($p>0.05$). The decreased level of glutathione in plasma of the basal diet group indicates that there was an increasing generation of free radicals and the glutathione was depleted during the process of combating oxidative stress (Gupta et al., 2003). At 9 weeks of age, there were no significant differences in plasma and liver tissue glutathione of all treatment diets ($p>0.05$). It is possible that the observed antioxidant effect was limited because the calyx Roselle used in this experiment was in crude powder form. However, the biological effects of phenolic compounds in the calyx of Roselle have not so far been well elucidated. The present investigation is the first report showing the antioxidant properties of Roselle in intact cell systems in weaning pigs and there further research is required to investigate and confirm this result.

Growth performances : Roselle diet did not show any advantage of average daily gain in both periods (Table 7). The result was similar to Experiment 1. Kommera et al. (2006) demonstrated that growth of nursery pigs fed with phytobiotics (PEP1000-1) and organic acids did not differ from those both with and without antibiotics. Also, Kil et al.

Table 6. Effect of Roselle on malondialdehyde and glutathione in Experiment 2[1]

Lipid peroxidation marker	Control	Roselle 8%	Acidifier	Antibiotic
Malondialdehyde				
At 7th wk				
Plasma (nmol/ml)	4.57±0.33	3.64±0.97	4.07±0.57	3.71±1.98
Liver tissue nmol/mg protein	3.20±1.33	2.15±1.14	3.07±1.26	3.60±2.26
At 9th wk				
Plasma (nmol/ml)	4.28±1.21	3.91±0.77	4.66±1.23	4.12±0.83
Liver tissue nmol/mg protein	3.36±1.40	2.69±0.79	3.34±2.44	3.35±1.83
Glutathione				
At 7th wk				
Plasma (nmol/ml)	6.59±2.31[B]	9.42±2.31[AB]	10.60±0.38[AB]	13.42±1.61[A]
Liver tissue nmol/mg protein	5.58±0.97	7.15±1.81	6.32±1.22	5.90±2.03
At 9th wk				
Plasma (nmol/ml)	6.12±c2.93	10.60±2.81	11.30±2.43	12.25±3.69
Liver tissue nmol/mg protein	5.35±1.49	7.45±2.92	8.95±0.85	6.58±0.74

[1] Mean±SD. [2] Mean in the same row with different superscripts differed significantly at $p<0.05$.

Table 7. Effect of Roselle on growth performances in Experiment 2[1]

Parameter	Basal diet	Roselle 8%	Acidifier	Antibiotic
Number of pigs	16	16	16	16
Initial wt. (kg)	7.61±0.04	7.62±0.07	7.58±0.06	7.58±0.07
Final wt. (kg)	20.84±0.60	21.38±1.67	20.58±1.51	21.14±1.11
Average daily gain (kg)				
5-7 weeks	0.30±0.04	0.33±0.02	0.291±0.04	0.30±0.03
7-9 weeks	0.63±0.03	0.65±0.06	0.64±0.11	0.66±0.05
5-9 weeks	0.47±0.02	0.49±0.06	0.46±0.05	0.48±0.04

[1] Mean±SD.

(2006) who found no advantage in daily gain, feed intake and gain to feed ratio when 3 different organic acids and 1 inorganic acid were supplemented. Unfortunately, feed conversion ratio was not determined because some errors were detected in feed intake data. However, numerically. Roselle gave the best average daily gain.

IMPLICATION

Roselle might have an acidifier property as indicated by significant differences in some parameters between the basal-diet and Roselle-fed groups. Roselle has the ability to increase trypsin activity and fat digestibility and improve FCR. Moreover, Roselle can compete with an acidifier and antibiotics at 5-7 weeks of age. For antioxidant property, MDA and glutathione showed only a positive trend. The ambiguity was caused by the fact that some parameters did not clearly show significant differences because the experimental units used in this study were limited. Therefore, it cannot be precisely concluded that Roselle in powder form has clear acidifier and antioxidant properties in the postweaning pig. Further studies are needed to clearly confirm both properties. An extracted form of Roselle might be a better alternative towards obtaining unequivocal results.

ACKNOWLEDGMENT

This research was supported by a grant from Graduate school, Chulalongkorn University, Bangkok, Thailand. Acidifier (Fra® Acid Dry) was sponsored by Vet Agritech Co., Ltd.

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