Original Article

Antioxidant and Acidifier Properties of Roselle (*Hibicus sabdariffa* Linn.) Calyx Powder on Lipid Peroxidation, Nutrient Digestibility and Growth Performance in Fattening Pigs

Suwanna Kijparkorn*   Uttra Jamikorn   Somporn Wangsoonean   Punyaphat Ittitanawong

Abstract

The study was conducted to investigate an antioxidant and acidifier properties of Roselle in fattening pigs. Twenty crossbred pigs were randomly allocated to 4 treatments with 5 replicates. Four diets were calculated to be isocaloric and isonitrogenous: control diet, control diet supplemented with antibiotic (Chlortetracycline) 50 mg/kg or acidifier (Fra® Acid Dry) 4 g/kg and diet containing Roselle calyx powder at the level of 40 g/kg. Pigs were raised in individual concrete pen and received diet *ad libitum* for 8 weeks. Body weight, feed intake, blood constituent and thiobarbituric acid-reactive substances (TBARS) were measured in two consecutive periods at 4th and 8th week. Fecal content was collected at the end of the experiment to determine the coefficient of apparent total tract digestibility (CATTD). The blood constituent data was not significant difference (p>0.05) but the neutrophils to lymphocytes ratio was more likely high in both periods. TBARS value was not differed (p>0.05) among treatments. Roselle had the highest CATTD value of ether extract and significant difference from the others (p<0.05). Roselle also gave the highest CATTD value of phosphorous when compared to control and acidifier (p<0.05) but was not significantly different with antibiotic (p>0.05). Feed conversion ratio (FCR) was significant differences among treatment groups in the second period (p<0.05) and the best FCR was Roselle group but not significant difference when compared to control and antibiotic. The result implied that Roselle at the level of 40 g/kg could not clearly show neither antioxidant nor acidifier properties in fattening pigs.

Keywords : acidifier, antioxidant, digestibility, fattening pigs, growth performance, Roselle

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บทคัดย่อ

คุณสมบัติในการเป็นสารต้านออกซิเดชั่นและสารเสริมกรดของกลีบเลี้ยงกระเจี๊ยบแดงผง (*Hibicus sabdariffa* Linn.) ต่อการเกิดเปอร์ออกซิเดชั่นของไขมัน การย่อยได้ของสารอาหาร และสมรรถนะการเจริญเติบโต ในสุกรขุน

สุวรรณา กิจภากรณ์* อุตรา จามิกร สมพร แวงสูงเนิน ปัญพัสตร์ อิทธิธนาวงษ์

การศึกษาคุณสมบัติของกระเจี๊ยบแดงในการเป็นสารต้านออกซิเดชั่น และสารเสริมกรดในสุกรขุน โดยใช้สุกรลูกผสมจำนวน 20 ตัว สุ่มออกเป็น 4 กลุ่มๆ ละ 5 ตัว ได้รับอาหารทดลอง 4 ชนิดที่คำนวณให้มีระดับพลังงานและโปรตีนเท่ากัน ได้แก่ อาหารควบคุม อาหารควบคุมเสริมที่ด้วยสารปฏิชีวนะคลอเตตร้าไซคลิน 50 มก/กก. หรือ สารเสริมกรดฟรา® แอซิด ดราย 4 ก./กก. และ อาหารที่มีส่วนผสมของกระเจี๊ยบแดง 40 ก./กก. สุกรถูกเลี้ยงขังเดี่ยวและได้รับอาหารอย่างเต็มที่เป็นเวลา 8 สัปดาห์ ทำการวัดน้ำหนักตัว ปริมาณอาหารที่กิน ค่าส่วนประกอบของเลือด และค่าไธโอบาร์บิทูริคแอซิด (TBAR) ในสัปดาห์ที่ 4 และ 8 ของการทดลอง มูลสุกรถูกเก็บเมื่อสิ้นสุดการทดลองเพื่อนำไปหาสัมประสิทธิ์การย่อยได้ปรากฏในทางเดินอาหาร (CATTD) ผลการทดลองพบว่าส่วนประกอบของเลือดไม่มีความแตกต่างทางสถิติ (p>0.05) แต่ค่าสัดส่วนของนิวโทรฟิลต่อลิมโฟไซต์ค่อนข้างสูงในสัปดาห์ที่ 4 และ 8 ไม่พบความแตกต่างของค่า TBAR ระหว่างกลุ่มทดลอง (p>0.05) กระเจี๊ยบแดงให้ค่า CATTD ของไขมันสูงที่สุดและแตกต่างจากกลุ่มอื่นที่เหลือ (p<0.05) ขณะเดียวกันให้ค่า CATTD ของแร่ธาตุฟอสฟอรัสสูงที่สุดด้วยและสูงกว่ากลุ่มควบคุมและสารเสริมกรด (p<0.05) แต่ไม่แตกต่างจากสารปฏิชีวนะ (p>0.05) ด้านอัตราการแลกเนื้อ (FCR) พบความแตกต่างทางสถิติระหว่างกลุ่มทดลอง ในช่วง 5-8 สัปดาห์ (p<0.05) และกระเจี๊ยบแดงให้ค่า FCR ดีที่สุด แต่ไม่แตกต่างจากกลุ่มควบคุมและสารปฏิชีวนะ(p>0.05) จากผลดังกล่าวจึงสรุปได้ว่าการใช้กลีบเลี้ยงกระเจี๊ยบแดงผงในระดับ 40 ก./กก. ไม่สามารถแสดงคุณสมบัติในการเป็นสารต้านออกซิเดชั่นและสารเสริมกรดอย่างเด่นชัดในสุกรขุน

คำสำคัญ : สารเสริมกรด สารต้านออกซิเดชั่น การย่อยได้ สุกรขุน สมรรถนะการเจริญเติบโต กระเจี๊ยบแดง

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Introduction

Finishing pig raised in tropical climate could expose to stress and infections due to the inconsistency of temperature and humidity. In stressed pigs, both number and percentage of lymphocytes and eosinophils typically decrease but leukocytes and neutrophils increase and often resulting in reversal of neutrophils to lymphocytes ratio (N/L ratio), a stress indicator (Elbers et al., 1991; Evans, 2000). Antibiotics or chemotherapeutics are routinely used in the production line to decrease stress, promote growth and curing diseases. Regarding to food safety policy and the concerning about drug resistant of consumer thus the pig producers must be aware to use of those in feed. Natural products are an alternative product that can provide the same action such as Roselle, Chili, Turmeric, Garlic, Tea and Mangosteen etc.

Roselle (*Hibiscus sabdariffa* L.) is an interesting herb because its petal consist of polyphenolic and anthocyanin (Wong, 1989) which has antioxidant properties (Tseng et al., 2000; Ali et al., 2003; Odigie et al., 2003) and also has organic acids (Tseng et al., 1996). In finishing pig, functions of gastrointestinal tract are mature and can produce enough acid and enzymes to cope with the feed. However, the enzyme activities of trypsin, chymotrypsin and amylase were significantly decreased by the hot environment at 32°C (Hai et al., 2000). Mahan (2001) stated that stress causes the increasing of free radicals which consequently increase lipid peroxidation occurred at cell membranes; however, oxidation could be prevented by some antioxidant substances by donating reducing equivalents to inactivate reactive compounds. Wang et al. (2000) demonstrated that oral pretreatment of the dried Roselle calyx extract (100 and 200 mg/kg) for 5 days before a single dose of t-BHP (0.2 mmol/kg) significantly lowered the serum levels of hepatic enzymes markers and reduced oxidative liver damage by significantly decrease malondialdehyde (MDA) and increased glutathione in rat. The same results were supported by Tseng et al. (1997), Liu et al. (2002) and Ali et al. (2003). Moreover, Roselle calyx also consists of 27.4% organic acid as dry matter basis such as acetic, formic, malic, stearic and tartaric acid (Tseng et al., 1996). Organic acids are used to control the growth of pathogenic microorganisms (Chu et al., 1987; Kamonwan and Narumon, 2002), provide optimal gut environment and increase nutrient digestibility (Mroz et al., 2000[b]). The objectives of this study were to determine an antioxidant and acidifier properties of Roselle calyx powder on lipid peroxidation, coefficient of apparent total tract digestibility and growth performance in finishing pigs.

Materials and Methods

Preparation of Roselle calyx powder: Dried calyx of Roselle was bought from the supplier who supplies food and pharmaceutical industry. Roselle was milled and passed through 2 mm screen by cutting mill machine. The calyx powder was analyzed for the total phenolic compound according to Duh and Yen (1997) method and nutritional content by proximate analysis (AOAC, 1990). Metabolizable energy of Roselle was calculated using DE equation (Noblet and Perez, 1993) and ME equation (May and Bell, 1971) as shown in Table 1. The Roselle calyx powder was kept at -20°C until mixing in the diet. Due to the research of Aphirakchatsakun et al. (2008) reported that no significant difference on daily gain, feed intake and feed conversion ratio (FCR) during the period of 7-9 and 9-11 weeks of age when fed with diet containing Roselle calyx powder at the level of 4, 8 and 12 kg/100 kg, respectively. Therefore, Roselle at the level of 4 kg/100 kg diet was chosen for this study.

Animal and management: A total of 20 crossbred pigs (Hampshire x Landrace X Duroc) weight 52±5 kg (8 females and 12 barrows) were used in the experiments. All pigs were randomly allotted into 4 treatments groups with 5 replicates (2 females and 3 barrows). Pigs were raised in an individual concrete pen (size 1x2 m.) with self-feeder and one nipple drinker in open house for 8 weeks. After 5 days of acclimatized period, all pigs were individually fed at 8.00 am and 15.00 p.m. in *ad-libitum* basis. Pigs had free access to water from the nipple. The temperature and relative humidity were recorded everyday at 08:00, 12:00 and 16:00 h. The average temperature and relative humidity of the entire experimental period were 29.45, 34.18, 34.64 °C and 74.75, 51.52, 50.94 % and 29.51, 33.14, 32.50 °C and 62, 48, 66 % at the first and second periods, respectively.

The Institutional Laboratory Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University, approved the experimental protocol.

Feed and feeding: Two-period diets for pigs at weight 50-80 and 80-100 kg were calculated to be isocaloric and isonitrogenous to meet NRC requirement (NRC, 1998). There were four treatment diets: control diet, antibiotic (Chlortetracycline) 50 mg/kg feed, acidifier (Fra® Acid Dry) 4 g/kg feed and diet containing Roselle calyx powder at the level of 4%. The reason for using acidifier at 4 g/kg instead of the recommendation level at 2 g/kg was to make its organic acids level can be more comparable to the level of organic acid in Roselle 10 g/kg which was calculated from the research of Tseng et al. (1996). The diet formula and chemical composition of the dietary treatments are shown in Table 2. There are minor discrepancies of chemical analysis of diets among treatment groups in both periods. At 7 days before ending of the experiment, 0.3% chromic oxide was mixed into the feed as an indigestible dietary marker.

Table 1 Chemical composition of Roselle calyx powder (fed basis)

Nutrient composition	Amount (kg/100 kg)
Dry matter	92.72
Crude protein	9.73
Ether extract	1.84
Crude fiber	11.17
Ash	8.29
Calcium	1.20
Total phosphorus	0.36
Total phenolic compound (mg/g)	21.64
Calculated ME, kcal/kg	2700.81

Table 2 The ingredient composition and chemical analysis of the experimental diets, kg/100 kg diet

Ingredient	Body weight 50-80 kg.[1/]				Body weight 80-100 kg.[1/]			
	C	CTC	A	R	C	CTC	A	R
Broken rice	67.31	67.24	66.34	62.28	75.27	75.19	74.30	70.24
Rice bran	8.75	8.75	8.86	8.64	8.39	8.40	8.51	8.29
Soybean meal,44%	20.59	20.60	20.73	20.67	13.95	13.96	14.09	14.02
Rice bran oil	1.38	1.40	1.70	2.56	0.79	0.81	1.11	1.97
Dicalcium phosphate	0.61	0.61	0.61	0.59	0.52	0.52	0.52	0.50
Oyster shell	0.68	0.68	0.69	0.58	0.66	0.66	0.66	0.55
Salt	0.17	0.17	0.17	0.18	0.17	0.17	0.17	0.17
Premix[2/]	0.50	0.50	0.50	0.50	0.25	0.25	0.25	0.25
Antibiotic[3/]	-	0.03	-	-	-	0.03	-	-
Acidifier[4/]	-	-	0.40	-	-	-	0.40	-
Roselle[5/]	-	-	-	4.00	-	-	-	4.00
Chemical analysis (DM basis)								
Crude protein	18.49	18.32	18.49	18.29	14.41	14.54	14.63	14.30
Ether extract	4.74	4.74	5.08	5.41	3.42	3.50	3.77	4.70
Crude fiber	1.59	1.47	1.63	1.72	1.32	1.43	1.37	1.67
Ash	4.27	4.43	4.60	4.39	4.15	4.21	4.43	4.04
Calcium	0.48	0.45	0.46	0.48	0.51	0.50	0.47	0.46
Total phosphorus	0.72	0.77	0.71	0.78	0.53	0.57	0.54	0.55
Calculated ME, kcal/kg	3265	3265	3265	3265	3265	3265	3265	3265

[1]C: control, CTC: antibiotic, A: acidifier, R: Roselle

[2]Premix/kg diet of pig weight 50-80 and 80-100 kg contained: vitamin A 10,000, 5000 IU; D3 2,000, 1000 IU; E 20, 10 mg, K_3 0.5, 0.25 mg; B_1 2, 1 mg, B_2 5, 2.5 mg, B_6 3.5, 1.7 mg, B_{12} 30, 15 µg, C 50, 25 mg, d-biotin 50, 25 mg, nicotinic acid 20, 10 mg, calcium-d-pantothenate 10, 5 mg; folic acid 1.5, 0.7 mg; choline chloride 250, 125 mg; Cu 100, 50 mg; Zn 182.5, 91.2 mg; Mn 100, 50 mg, Fe 250, 125 mg, Se 0.5, 0.2 mg; Co 1, 0.5 mg; I 1, 0.5 mg, preservative 10, 0.5 mg and filler

[3]Antibiotic *(Chlortetracycline)*/kg diet contained: 50 mg

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

[5]Roselle calyx powder 40 g/kg

Data collection: Body weight and feed intake were measured in two consecutive periods at 4th and 8th week. Animals were restrained using a snout snare during the blood collection process. Two blood samples were collected from jugular vein at the beginning to establish the baseline data and at 4th and 8th week. One blood sample was stored at 5-10^{o}C until analyzed for blood constituent. Another blood sample was centrifuged at 1,500 rpm for 5 min and plasma was collected and stored at -80^{o}C until analysis for thiobarbituric acid-reactive substances. Fecal sample of each pig was collected from rectum at day 5th to 7th after the chromic oxide was fed. The collected fecal samples in each day were equally weighed, pooled, homogenized and kept at -20^{o}C until further analysis.

Samples analysis: Blood sample was analyzed for the amount of total red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), total white blood cell count (WBC) and differential lymphocyte count, followed the method of Villiers and Dunn (1998). Plasma was analyzed for the level of thiobarbituric acid-reactive substances (TBARS) suggested by Ohkawa et al. (1979). For diet and fecal content, nutrient compositions were analyzed using proximate analysis (AOAC, 1990) and chromic oxide was measured by spectrophotometer as described by Williams et al. (1962). The coefficient of apparent total tract digestibility (CATTD) of nutrients was calculated by using the following equation:

$$CATTD = (AF \times ID) / (AD \times IF)$$

where AF is the concentration of nutrient in feces (%); ID is the chromic oxide concentration in the assay diet (%); AD is the concentration of nutrient in the assay diet (%); and IF is the chromic oxide concentration in feces (%).

Statistical analysis: Statistical analysis for all dependent variables was performed as a completely randomized design to determine the effect of treatments. Dependent variables, blood parameter, TBARS, coefficient of apparent total tract digestibility and growth performance were analyzed using one-way analysis of variance (ANOVA). Significance of variables among treatments was determined by Duncanís New Multiple Range Test at the level of $p< 0.05$.

Results

Blood constituent: Total red blood cell count (RBC), hemoglobin (Hb), pack cell volume (PCV) and total white blood cell count (WBC), were in the normal ranges which were 5.0-8.0 x 10^6/µL, 10.0-16.0 g/dL, 32-50% and 11-22x10^3/µL, respectively. While the neutrophils, lymphocytes and neutrophils to lymphocytes ratios (N/L ratio) were out of the normal range, which were 28-47% (average 37%), 39-62% (average 53%) and 0.69 respectively. At the starting, 4th and 8th week of the experiment, there was no significant difference (p>0.05) of all blood constituent and N/L ratio in all treatment groups (Table 3).

Lipid peroxidation: The thiobarbituric acid-reactive substances (TBARS) value in plasma, expressed as nmol of malondialdehyde (MDA) concentration/ml, was shown in Table 4. There was no significant difference of TBARS number in all treatment groups at the starting, 4th and 8th week of the experiment. However, at 4th week of experiment, TBARS number of control group showed the highest while acidifier group showed the lowest. At 8th week of experiment, TBARS number of Roselle group tended to be lower compared to control group but it was higher compared to antibiotic group.

Coefficient of Apparent Total Tract Digestibility (CATTD): As depicted in Table 5, there was no significant difference in CATTD value of protein in all treatment groups (p>0.05). For ether extract, the Roselle group had the highest CATTD value that significant difference from the others (p<0.05). Roselle group also had the highest CATTD value of phosphorous when compared to the control and acidifier groups (p<0.05) but was not significantly different with antibiotic group (p>0.05). Roselle group showed significant difference in CATTD value of almost all nutrients except CATTD value of

Table 3 Effect of Roselle on blood constituents and stress indicator

	RBC x10[6] cell/μL	Hb g/dL	PCV %	WBC x10[3]/μL	% of leukocyte neutrophil	% of leukocyte lymphocyte	N/L ratio
At starting							
Control	6.6±0.8	10.4±1.2	33.3±4.6	13.8±3.0	78.0±2.3	14.6±1.5	5.4±0.8
CTC	6.7±0.6	10.7±1.0	34.1±3.6	14.7±1.6	78.2±1.9	15.0±3.0	5.4±1.0
Acidifier	6.4±0.6	10.1±0.7	32.4±2.0	17.3±2.1	76.4±1.7	16.0±2.4	4.9±0.9
Roselle	6.6±0.4	10.6±0.4	33.6±1.6	16.0±3.6	78.6±1.3	16.2±1.3	4.9±0.4
P-value	0.914	0.723	0.871	0.237	0.292	0.607	0.584
At 4th week							
Control	7.3±0.6	12.0±0.6	36.9±2.5	13.2±3.8	75.2±2.9	18.4±1.5	4.1±0.4
CTC	7.0±0.5	12.0±0.7	36.3±2.3	15.5±2.9	75.6±5.2	16.0±3.3	4.9±1.2
Acidifier	6.7±0.5	11.4±0.6	34.3±1.8	18.6±2.3	75.4±4.9	15.0±4.5	5.4±1.7
Roselle	6.9±0.5	11.8±0.7	35.5±2.3	18.4±4.4	75.4±4.5	16.4±3.6	4.8±1.3
P-value	0.396	0.420	0.310	0.077	0.999	0.470	0.464
At 8th week							
Control	5.9±1.0	12.1±1.5	35.4±5.9	15.6±4.0	71.6±4.8	17.8±1.9	4.0±0.3
CTC	6.2±0.5	12.9±1.1	38.6±3.0	16.0±7.3	74.8±4.2	16.2±2.0	4.7±0.6
Acidifier	5.5±1.0	11.5±1.5	33.4±4.9	17.4±4.1	73.0±2.1	18.0±3.0	4.2±0.8
Roselle	5.4±1.2	11.5±2.4	33.8±7.4	14.1±2.2	74.8±2.7	18.4±1.5	4.1±0.4
P-value	0.494	0.479	0.457	0.763	0.456	0.430	0.302

[1]Mean±SD (N=5)

Table 4 Effect of Roselle on thiobarbituric acid reactive substance (TBARS) in plasma[1/]

Observation	nmol MDA/ml Control	Antibiotic	Acidifier	Roselle	*P*-value
At starting	3.5±1.2	3.5±1.2	5.0±2.0	4.3±1.8	0.368
At 4th week	5.5±2.0	4.2±1.1	3.5±1.5	3.7±1.4	0.218
At 8th week	3.9±0.9	2.9±1.2	3.9±1.0	3.3±1.2	0.404

[1]Mean±SD (N=5)

Table 5 Effect of Roselle on coefficient of apparent total tract digestibility of nutrients (DM basis)[1,2]

Nutrient	Control	CTC	Acidifier	Roselle	*P*-value
Crude Protein	0.9±0.02	0.9±0.01	0.8±0.02	0.8±0.02	0.572
Ether extract	0.7±0.04[b]	0.7±0.03[b]	0.7±0.02[c]	0.8±0.02[a]	<0.000
Crude Fiber	0.4±0.09[a]	0.4±0.05[a]	0.3±0.04[b]	0.5±0.06[a]	<0.000
Ash	0.4±0.05[a]	0.5±0.05[a]	0.3±0.05[b]	0.5±0.02[a]	<0.000
Calcium	0.6±0.05[a,b]	0.6±0.03[a]	0.5±0.04[c]	0.5±0.03[b,c]	0.001
Total phosphorus	0.5±0.03[b]	0.6±0.02[a]	0.5±0.03[b]	0.6±0.04[a]	0.003

[1]Mean±SD (N=5)

[2 a,b,c]Means within the same row with different superscripts differ significantly ($p < .01$).

Table 6 Effect of Roselle on growth performance

Observation	Control	Antibiotic	Acidifier	Roselle	*P*-value
Started wt., kg.	51.5±3.1	52.0±4.4	51.4±3.4	51.8±2.5	0.994
Final wt., kg.	94.6±5.2	95.4±6.9	92.0±7.2	94.6±6.3	0.850
At 4th week of experimental period					
ADG, kg/head/day	1.0±0.1	0.9±0.1	1.0±0.2	0.9±0.1	0.897
ADFI, kg/head/day	2.4±0.3	2.4±0.4	2.5±0.4	2.3±0.2	0.748
FCR	2.5±0.1	2.6±0.2	2.6±0.1	2.4±0.3	0.321
At 8th week of experimental period					
ADG, kg/head/day	0.5±0.1	0.6±0.1	0.5±0.1	0.6±0.1	0.298
ADFI, kg/head/day	1.8±0.2	2.2±0.5	1.9±0.3	1.9±0.2	0.210
FCR	3.4±0.2[b]	3.6±0.4[a,b]	4.0±0.7[a]	3.2±0.3[b]	0.045
Overall period					
ADG, kg/head/day	0.8+0.1	0.8+0.1	0.7+0.1	0.8+0.1	0.807
ADFI, kg/head/day	2.1+0.1	2.3+0.4	2.2+0.3	2.1+0.1	0.555
FCR	2.8+0.1[B,C]	3.0+0.1[A,B]	3.1+0.1[A]	2.7+0.1[C]	0.006

[1]Mean±SD (N=5)

[A,B,C]Means within the same row with different superscripts differ highly significantly ($p<0.01$).

[a,b]Means within the same row with different superscripts differ significantly ($p<0.05$).

calcium when compared to the acidifier group. Acidifier group had the lowest CATTD of all nutrients.

Growth performance: The starting weight of all pigs among the treatment groups were not differed ($p>0.05$). Weight gain and average daily gain were also not significant difference in both periods ($p>0.05$). Feed conversion ratio showed significant difference in second period ($p<0.05$) but Roselle fed group was not significant difference with control and antibiotic group. In over all period, the highly significant difference was found in FCR ($p<0.01$) and the best FCR was the Roselle group but was not significant difference when compared to control group (Table 6).

Discussion

Health status: The hematological data in this experiment showed the same health status of all pigs in all treatment groups at starting. Almost blood parameters were in the normal range except for neutrophils and lymphocytes when compared to the data as published by Jain (1993) and Thorn (2000). Evans (2000) stated that the high temperature induces corticosteroid release, consequently, decrease lymphocytes, increase neutrophils in both number and percentage, and resulting in reversal of neutrophils-to-lymphocytes ratio (N/L ratio). In agreement with Elbers et al. (1991) the increasing of N/L ratio in the animal could relate to the physiological respond to stress. In this experiment, the N/L ratio was high in both periods, so it indicated that all pigs were in a physiological stress condition. This might due to the high and fluctuation of temperature between the experimental periods. The temperature in the experiment varied from 29.48°C at 8.00 h to 33.66 and 33.57°C at 12:00 and 16:00 h, respectively. Hyun et al. (2005) reported that cycling-high from 28°C to 34°C can decrease lymphocytes, increase neutrophils concentration and consequently caused significantly increase N/L ratio than constant thermoneutral (24°C). There was no significant difference of N/L ratio in all treatment groups ($p>0.05$). Although, the antibiotic was supplemented, the improvement of N/L ratio was not observed. It might be implied that the physiological stress in pigs was too high therefore, the level of antibiotic as growth promoter used in this experiment could not show any improvement.

Antioxidant properties: In the present study, an antioxidant property of Roselle was not clearly demonstrated. It was possible that in this experiment the active ingredients in Roselle powder might not dissociate well in the intestine of pigs. Wang et al. (2000) stated that the Roselle extract at the concentrations of 0.1 and 0.2 mg/ml in hepatotoxicity rat induced by tert-butyl hydroperoxide (t-BHP) significantly decreased the formation of MDA and it showed similar result as the report of Tseng et al. (1997). Tee et al. (2002) also confirmed that the Hibiscus extract had stronger antioxidant activity than BHA and β-carotene. On the other hand, the reason that the Roselle group did not clearly shown superiority over the other might be because of a too high level of physiological stress which increased lipid peroxidation, so the level of phenolic compound in Roselle group was not enough to combat.

Coefficient of Apparent Total Tract Digestibility (CATTD): Partanen and Mroz (1999) and Partanen et al. (2002) reported that organic acids appear to be potentially alternate the prophylactic and promote growth in growing and finishing pigs. In present experiment, diet inclusion with Roselle that contains organic acids showed no beneficial effects on CATTD of protein. It might because the level of Roselle was too low or the organic acids in Roselle did not dissolve well in stomach, thus it could not decrease pH and consequently could not promote enzyme pepsin activity. For CATTD of ether extract, the group that showed highest value and significant difference from the others was the Roselle group. The reason should be due to the oil content in feed formula of Roselle group. The inclusion rate of rice bran oil that was used as an energy source in Roselle diet that was 2.5 times higher than control diet and rice bran oil contains high level of unsaturated fatty acid, which has high digestibility (Azain, 2001). On the other hand, it might because of the assimilation and alteration of fat by hindgut microflora (Yen, 2001). No significant difference was found in the CATTD of crude fiber, ash, and calcium between the Roselle and control group. However, the CATTD of phosphorus in Roselle group was better than the control. This might because organic acids contained in Roselle can bind various cations along the intestinal tract and may act as chelating agents, then resulting in the increasing of intestinal absorption of minerals (Ravindran and Kornegay, 1993).

Surprisingly, the Roselle and control group showed better CATTD of all nutrients than acidifier group. These results contradicted to the finding of Mroz et al. (2000[b]) that adding 13.5 g of formic acid/kg diet in growing-finishing pigs can improve CATTD of DM, OM, CP and GE ($p<0.05$). While Mroz et al. (2000[a]) found only tendency in ileal digestibility of CP when adding 13.8 g of formic acid/kg diet for growing-finishing pig. These discrepancy responses of acidifier on CATTD could be due to the differences in inclusion dose and type of organic acid and in dietary composition (Ravindran and Kornegay, 1993). Omogbenigun et al. (2003) also stated that the effectiveness of organic acid in diets of pigs was poor and variable. In addition, 4 g/kg of acidifier for very long period (8 weeks) was used in present study instead of the recommendation dose of 2 g/kg in fattening pig could also be accounted for the failure of acidifier to promote growth performance. Maxwell et al. (1970) reported the high incidences of gastrointestinal ulceration when the pH in the stomach was decreased to the very low level and this could impair normal digestive functions of pigs too. Therefore, the overall digestibility observed in this group was inferior compared to the control and Roselle group.

Growth performance: No significant difference of weight gain and average daily gain was found in all groups for both periods. Similar result was reported by Aphirakchatsakun (2008). Incorporated with the finding of Pettigrew and Moser (1991), who reported that at a constant protein-to-energy ratio, no response in growth rate, feed intake, and gain-to-feed ratio was shown even though fat was added. Antibiotic group also did not show any significant improvement in ADG of fattening pig. In contrast to the report of Hong et al. (2004), diet supplemented with 100 mg/kg CTC and sulfathiazole complex can significantly increase ADG compared to control diet in finishing pigs ($p<0.03$). High stress of pigs due to hot climate or too low concentration of antibiotic supplemented in present experiment could be the reasons. Feed intake of pigs in Roselle group was slightly decreased in first period. This might because of the changing in color and odor of diet due to Roselle powder inclusion. However, the feed intake of all pigs in both periods were also lower than the estimated level from NRC, (1998) which were 2.57 and 3.07 kg/day in each period respectively. The reason was that the physiological stress in the experimental periods could suppress feed intake in pigs (McGlone et al., 1987)

The experiment revealed that the Roselle showed the highest potential to improve FCR in fattening pigs, although it was not significantly different when compared to the control group. This might due to its property that can improve nutrient digestibility. The result of poorer FCR of pigs in the antibiotic group compared to the Roselle group while it was not different when compared to the control group could be explained by the low inclusion rate, so it could not show the expected potency. In addition, it could be due to the rather clean environment in this experiment than the commercial unit so that the major mode of action of antibiotic to reduce pathogen load could not be performed and this could show different result if it was use in the commercial farm level (Cromwell, 2001). In acidifier group, the poorer CATTD value could be the cause of poorer FCR of pigs in this experiment. However, there was more than one factor affected the potency of acidifier. Ravindran and Kornegay (1993) demonstrated that the inconsistency results were due to various factors, including age of animals, composition of basal diet, differences in the type and dose of acid used, and existing levels of performance could be attributed.

Roselle calyx powder at the level of 40 g/kg could not clearly show antioxidant and acidifier properties in fattening pigs. Further studies should be investigate in higher doses or another form such as crude extract mixed in feed or dissolved in water. Moreover, the dissolvability of active ingredients (phenolic compound or anthocyanin and organic acid) in gastrointestinal tract might be determined and the experiment should be implementing in the field condition.

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