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Research article

Effect of pineapple stem starch in concentrate diet on rumen fermentation in beef cattle and *in situ* dry matter degradability

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Abstract

Importance of the work: Pineapple is one of the major crops grown in Thailand. Pineapple stem starch is a potential byproduct from the bromelain enzymes extraction process with pineapple stems used as feedstuff for ruminants. However, the rumen fermentation and dry matter digestibility of pineapple stem starch has not been reported.
Objectives: To evaluate the rumen fermentation characteristics and dry matter digestibility of pineapple stem starch.
Materials & Methods: Four ruminal-cannulated beef cattle were used to determine the effects of the starch in four feed ingredients on the fractional degradation rates, ruminal degradability of dry matter (DM) and fermentation products. Animals were offered a basal diet containing 40% starch source with either ground corn (GC), broken rice (BR), ground cassava (CA) or pineapple stem starch (PS) as treatment concentrates, supplemented with Napier grass silage (NS) as roughage sources. Each animal was offered 1.2% DM concentrate based on animal bodyweight with 4 kg DM of NS. A 4×4 Latin squared design was used. Non-linear regression was used to fit an asymptotic exponential model on the degradation kinetics of the dry matter loss percentage of the four substrates against the time of incubation.
Results: Dry matter intake, ruminal pH and ammonia-nitrogen (NH_3-N) were not affected by the starch source. PS and CA had higher total short-chain fatty acid concentrations than GC and BR. Ruminal digestibility of the concentrate diet was greater for PS compared to GC at 4–24 hr post-incubation ($p < 0.05$). However, CA produced more lactate and influenced the rate of disappearance in NS. PS had higher ruminal digestibility of the concentrate diet than in either BR or GC ($p < 0.05$).
Main finding: The results suggested that PS had potential as a starch source in a ruminant diet without any negative effects on feed intake or rumen fermentation.

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Introduction

Pineapple is the major crop grown in Thailand and is exported globally (Food and Agriculture Organization of the United Nations, 2020). The harvest of pineapple fruits generates a large volume of residues in the field such as stems and leaves; However, the pineapple stems contain a bromelain enzyme that can be extracted (Napper et al., 1994). Hale et al. (2005) reported that bromelain is a major proteolytic enzyme in *Ananas comosus* L. Merryl and it is valued highly for pharmaceutical and food uses (Latt et al., 2019). The residues from the bromelain extraction process are crushed pineapple stem (CPS) and pineapple stem starch (PS) amounting to 696,679 t and 77,409 t, respectively, per annum (unpublished data from Hong Mao Biochemicals Co., Ltd, 2020). Therefore, these by-products have potential as feedstuff in a ruminant diet. Hattakum et al. (2019). Pintadis et al. (2020) reported CPS contained high amounts of fiber—(25.2% dry matter (DM) of neutral detergent fiber (NDF) and 12.9% DM of acid detergent fiber (ADF)—and 41% DM of starch, which are suitable as roughage for steers. PS contains more starch than ground rice and also has a high amylose content (Nakthong et al., 2017). Moreover, PS contains no toxic residues because the bromelain extraction process does not use toxic chemicals (Pintadis et al., 2020). PS has potential as an energy source in the diets of ruminants. For example, Khongpradit et al. (2020) reported that PS induced desirable production responses in beef cattle, including increased total short-chain fatty acid (SCFA) in the rumen, as well as increased average daily gain and feed conversion ratio. The enhancement of rumen fermentation by PS could be attributable to the high activity of amylolytic *Ruminococcus bromii* being able to enhance growth performance of the animal.

Starch represents an energy source in ruminants; in terms of overall metabolizable energy yield, grain starch is best fermented in the rumen (Huntington, 1997). Rumen fermentation can supply 70–85% of an animal's energy supply and can be absorbed as volatile fatty acids, which are the main end-products of microbial fermentation (Weimer, 1998; Anantasook et al., 2013). In general, starch-effective digestion has a great impact on bovine performance. In addition, net energy may be confounded with starch sources and different total amounts or ratios (or both) of nutrients released in the rumen (Noziere et al., 2010). Whole grains of corn and rice are enclosed by a pericarp, which is extremely resistant to rumen microbial degradation (Dehghan-Banadaky et al., 2007). However, the degradation of starch is affected by the processing of raw materials and the intake of animals (Offner and Sauvant, 2004). The present study hypothesized that PS has different properties compared to other starch sources in terms of fermentation characteristics and ruminal degradability. Another study (Khongpradit et al., 2020) focused on the effect of PS as a starch source in a concentrate diet on the growth performance and rumen microbial profile of dairy cows. However, study of rumen fermentation characteristics and dry matter digestibility of PS as a starch source in concentrate diet in beef cows has not been reported. Thus, the objectives of the present study were to investigate the effect of PS as a starch source in a concentrate diet on rumen fermentation and *in situ* dry matter degradability.

Materials and Methods

Ethics statements

The study was conducted at the Ruminant Research Unit, Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand. The animal study protocol was reviewed by the Institutional Animal Care and Use Committee of Kasetsart University, Thailand, following the Guidelines of Animal Care and Use under the Ethical Review Board of the Office of the National Research Council of Thailand (ACKU62-AGK-007).

Animal and experimental diet

Four ruminal-canulated Brahman crossbred cattle (501±30 kg bodyweight) were used in a 4×4 balanced Latin square. Each experimental period lasted 21 d, including 14 d for adaption. In each period, animals were randomly assigned to one of four treatments: 1) ground corn (GC); 2) ground cassava (CA); 3) broken rice (BR); and 4) pineapple stem starch (PS), as shown in Table 1. The experimental diets contained 40% of starch source with either GC, CA, BR or PS as concentrate and Napier grass silage (NS) as a roughage source. The pineapple stem starch was obtained from Hong Mao Biochemicals Co., Ltd., Rayong, Thailand. Napier grass silage was prepared at the farm located within Kasetsart University, Nakhon Pathom, Thailand and harvested at 70 days. Napier grass was chopped to about 2–3 cm in length and ensiled in a plastic bag container for 21 d. The animals were individually offered 1.2% DM

Table 1 Ingredients of experimental diet (dry matter)

Item	Concentration (% dry matter)				Napier grass silage
	GC	BR	CA	PS	
Ingredient					
Formulated concentrate	57.54	56.91	55.85	54.65	
Ground corn	37.76	-	-	-	
Broken rice	-	37.30	-	-	
Ground cassava	-	-	37.48	-	
Pineapple stem starch	-	-	-	38.50	
Palm oil	-	0.25	1.25	1.31	
Molasses	4.70	4.66	4.61	4.60	
Urea	-	0.88	0.81	0.94	
Total	100.00	100.00	100.00	100.00	
Chemical composition					
Dry matter (DM), (%)	91.14±1.03	91.25±0.95	91.35±0.88	90.96±0.64	20.42±0.83
Crude protein (%DM)	17.22±0.95	17.39±0.60	17.77±0.41	17.27±0.42	5.44±0.71
Ether extract (%DM)	1.55±0.04	1.16±1.29	1.82±0.11	1.55±0.01	1.23±0.36
Neutral detergent fiber, (%DM)	38.97±0.70	40.35±4.08	40.56±3.80	41.89±4.69	72.29±1.81
Acid detergent fiber, (%DM)	20.45±1.14	24.44±3.01	23.66±3.56	21.30±0.29	57.06±0.52
Acid detergent lignin, (%DM)	4.41±0.11	5.08±0.41	5.22±0.16	4.85±0.39	11.23±0.18
Ash, (%DM)	7.11±0.46	7.13±0.26	8.37±1.97	7.49±0.28	10.65±0.68
Calcium, (%DM)	1.01±0.06	1.00±0.01	1.09±0.01	1.13±0.02	0.32±0.03
Phosphorus, (%DM)	0.79±0.03	0.80±0.00	0.80±0.00	0.77±0.01	0.29±0.03
Total carbohydrates†	74.12±1.45	74.32±0.94	72.04±2.27	73.69±0.69	-

GC = ground corn; BR = ground broken rice; CA = ground cassava; PS = pineapple stem starch; †Total carbohydrates were calculated based on 100 – (%Crude protein + %Ether extract + %Ash) (Sniffen et al., 1992)
Chemical composition values shown as mean ± SD

concentrate based on animal bodyweight with 4 kg DM of NS. The animals were raised in individual pens (2.5 m × 4 m) during the adaptive phase. They were fed twice daily at 0800 hours and 1600 hours and had free access to fresh water. Samples of each concentrate and the Napier grass silage were analyzed for crude protein, ether extract, NDF, ADF, acid detergent lignin, ash, calcium and phosphorus (Association of Official Analytical Chemists, 2016) in each period. Feed samples were stored at -20°C prior to further laboratory analyses.

Data collection and analysis

The ruminal pH was determined from the four fistulated cattle at 0 hr, 4 hr and 8 hr after feeding on days 15, 16 and 17 of each period. On each day, rumen digesta samples were collected from the cranial, caudal, left lateral and right lateral areas of the rumen. After sampling, the rumen digesta samples were mixed thoroughly and then strained through two layers of cheesecloth into a plastic container. Rumen fluid samples were immediately measured for pH using a handheld portable pH meter (Oakton WD 35634–30; USA) and 50 mL of sample was collected and frozen (-20°C) prior to further laboratory analyses.

Ruminal ammonia nitrogen (NH_3-N) was analyzed using a phenol-hypochlorite assay based on spectrophotometric detection at 660 nm (Khongpradit et al., 2020).

SCFA concentration determination was performed using gas chromatography; samples were centrifuged at 10,000×*g* for 10 min at 4°C and then 100 µL of supernatant was mixed with 20 µL of 25% metaphosphoric acid and kept at 4°C (overnight). Samples were centrifuged at 10,000×*g* for 10 min at 4°C. Subsequently, supernatant (50 µL) was mixed with crotonic acid (50 µL). Then, the samples were transferred into vials for gas chromatography injection (Shimadzu; Japan) equipped with an ULBON HR–20M fused silica capillary column (0.53 mm internal diameter × 30 m length, 3.0 µm film; Shinwa; Japan). The procedures were as described in Watabe et al. (2018).

The lactic acid concentration in the ruminal fluid of the animals fed the GC, CA, BR or PS diets were determined spectrophotometrically using a commercially available kit (Megazyme; Ireland).

In sacco dry matter degradability

The degradability of concentrate supplementation with GC, CA, BR or PS was determined using the nylon bag technique (Ørskov and McDonald, 1979). NS and each concentrate diet were dried and ground to pass through a 2 mm screen. The treatment diets and NS samples (8±0.5 g) were weighed into 7 cm × 15 cm nylon bags with a pore size of 50 µm and sewn closed using nylon thread. The nylon bags were inserted into the rumen at different time points. The incubation times were 0 hr, 4 hr, 8 hr, 12 hr, 18 hr, 24 hr, 48 hr and 72 hr post-feeding on days 18, 19 and 20 of each period. Immediately after their retrieval, all bags were washed with tap water and the samples were dried at 100°C. The kinetic parameters of DM degradation were estimated using the model proposed by Ørskov and McDonald (1979): $Y = a + b\ (1–e^{–ct})$, where Y is the fraction degraded over time t, a is the soluble fraction and b is the potentially degradable fraction all in expressed as a percentage, c is the rate of disappearance of component b per hour, t is the incubation time in hours and the sum of the fractions a and b equates to the plateau value of the curve or the maximal degradable fraction, referred to the a + b fraction. Ruminal effective degradability was estimated using the following formula: $ED = a + [b × c / (c + k)]$ (Nadirah et al., 2011), where k is the independent variable (in hours) and k at 0.05 $hr^{–1}$ represents the ruminal turnover constant (Pirmohammadi et al., 2006).

Statistical analysis

The degradation kinetics of incubation were investigated based on non-linear fixed-effects regression models (percentage of DM loss) of the four starch sources against the time of incubation in the reticulo-rumen, using the R software (version 4.0.5; R Core Team, 2021). The R package with “easynls” (Kaps and Lamberson, 2009) was applied according to the equation of Ørskov and McDonald (1979).

All data were analyzed using analysis of variance based on a 4×4 Latin square design using the general linear model procedure of the R software (version 4.0.5; R Core Team, 2021). Data were analyzed using the statistical model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + \varepsilon_{ijk}$$

where Y_{ijk} is the observation from animal *i*, receiving diet *k*, in period *j*; μ is the overall mean; C_i is the effect of cattle (i = 1 to 4); P_j is the effect of the period (j = 1 to 4); T_k is the effect of treatment (k = 1 to 4; ground corn, ground broken rice, ground cassava, pineapple stem starch, respectively); and ε_{ijk} is the residual (assumed to be normally distributed). Treatment means were compared using Tukey’s test and the differences were declared significantly at $p < 0.05$.

Results

Feed intake

Animal feed intake is shown in Table 2. The average total dry matter intake (DMI) amounts of GC, BR, CA and PS were 8.88 kg DM/d, 8.92 kg DM/d, 9.14 kg DM/d and 9.04 kg DM/d, respectively. The data indicated that the different starch sources did not significantly affect the feed intake and did not significantly affect the dry matter intake of concentrate diets and NS.

Rumen pH, NH_3-N, lactate, and short-chain fatty acid production concentration

The rumen fermentation characteristics are shown in Table 3. There were no significant differences in the ruminal pH, concentration of NH_3-N, propionate and butyrate for the different starch sources. However, the concentration of total SCFA was significantly higher in CA than in GC and BR, particularly the concentration of acetate. The different starch

Table 2 Effect of dietary treatments on dry matter intake (kg dry matter/d)

Item	Concentration (% dry matter)				SEM	*p*-Value
	GC	BR	CA	PS		
Total intake, kg DM/d	8.88±0.77	8.92±0.64	9.14±1.01	9.04±0.89	0.90	0.97
Concentrate intake, kg DM/d	4.98±0.68	5.05±0.50	5.02±0.89	5.19±0.42	0.72	0.98
Napier grass silage intake, kg DM/d	3.90±0.36	3.87±0.27	4.12±0.38	3.86±0.74	0.44	0.81
Concentrate: Napier grass silage	56.1:43.9	56.6:43.4	54.9:45.1	57.4:42.6	-	-

GC = ground corn; BR = ground broken rice; CA = ground cassava; PS = pineapple stem starch; SEM = standard error of the mean
Values shown as mean ± SD

sources did not affect the proportion of acetate, propionate, and butyrate to total SCFA but affected the concentration of lactate, with CA having the highest total lactate in both the L- and D-forms ($p < 0.01$).

Dry matter disappearance

The pattern of ruminal fermentation at 0 hr, 4 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr post-incubation and the overall means are given in Table 4. Among the concentrate diets, PS had the significantly highest at 0 hr and 4 hr DM disappearance followed by CA, while GC and BR had similar levels of DM disappearance at 0 hr and 4 hr. PS had the significantly highest DM disappearance at 4 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr. GC had the significantly lowest DM disappearance among the concentrate diets at 4 hr, 8 hr, 12 hr and 24 hr post-incubation. After 48 hr incubation in the rumen, the fermentability of CA was significantly lower than that of PS, GC and BR, respectively.

The degradation of Napier grass silage at 0 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr post-incubation was unchanged by the dietary treatments. However, at 4 hr post-incubation, DM disappearance of CA was significantly lower than that of GC, BR and PS, respectively.

Table 3 Effects of dietary treatments on ruminal fluid pH and concentration of NH_3-N, lactate, and short-chain fatty acid (SCFA)

Item	Concentration (% dry matter)				SEM	*p*-Value
	GC	BR	CA	PS		
Rumen pH						
0 hr	6.82±0.18	6.84±0.25	6.71±0.17	6.79±0.18	0.14	0.60
4 hr	6.55±0.20	6.72±0.13	6.57±0.11	6.63±0.06	0.13	0.36
8 hr	6.53±0.13	6.60±0.22	6.46±0.22	6.51±0.08	0.13	0.51
NH_3-N, mgN/100 mL						
4 hr	13.53±1.90	13.52±2.66	14.74±2.41	15.10±2.46	0.92	0.30
8 hr	14.34±0.67	14.70±1.84	14.68±2.35	14.56±2.03	1.62	0.68
Lactate, µmol/mL (at 4 hr)						
Total lactate	1.57±0.01^{b}	1.63±0.14^{b}	2.14±0.10^{a}	1.33±0.19^{c}	0.09	<0.01
L–lactate	0.57±0.06^{b}	0.77±0.16^{ab}	0.92±0.01^{a}	0.60±0.16^{b}	0.12	<0.01
D–lactate	1.00±0.07^{ab}	0.86±0.16^{bc}	1.22±0.20^{a}	0.73±0.19^{c}	0.12	<0.01
Total SCFA, mmol/L						
4 hr	68.93±3.52^{b}	73.52±9.07^{ab}	78.27±6.79^{a}	77.44±6.03^{a}	3.79	0.03
8 hr	70.54±9.92^{b}	72.31±11.7^{b}	82.48±10.8^{a}	75.47±5.79^{ab}	3.68	<0.01
Acetate, mmol/L						
4 hr	47.93±3.42^{c}	52.81±7.65^{b}	56.17±4.86^{a}	55.36±6.07^{a}	3.24	0.02
8 hr	50.89±7.35^{b}	51.42±7.63^{b}	58.27±6.72^{a}	54.02±5.66^{ab}	2.68	0.01
Propionate, mmol/L						
4 hr	12.28±0.40	11.99±1.17	11.85±1.50	11.96±1.02	1.01	0.94
8 hr	11.62±1.19	11.61±2.50	12.65±1.89	11.24±1.04	1.10	0.52
Butyrate, mmol/L						
4 hr	8.61±1.03	8.72±1.00	10.26±0.70	10.12±1.00	1.08	0.11
8 hr	9.03±0.86	9.28±1.86	11.56±2.79	10.21±1.24	1.79	0.24
Acetate, mol/100 mol						
4 hr	69.53±2.13	71.83±1.39	71.76±0.56	71.49±2.22	1.47	0.31
8 hr	70.94±2.17	71.11±2.02	70.65±1.64	71.58±2.08	1.71	0.95
Propionate, mol/100 mol						
4 hr	17.81±0.53^{a}	16.31±1.03^{ab}	15.14±0.64^{b}	15.44±0.93^{b}	0.87	0.01
8 hr	16.31±1.20	16.06±1.08	15.34±1.61	14.89±1.87	0.78	0.17
Butyrate, mol/100 mol						
4 hr	12.49±1.71	11.86±0.82	13.14±0.95	13.07±2.08	1.35	0.52
8 hr	12.80±1.83	12.83±1.04	14.02±1.69	13.53±1.75	1.83	0.79
Acetate: Propionate						
4 hr	3.90±0.23^{b}	4.40±0.36^{ab}	4.74±0.20^{a}	4.63±0.34^{a}	0.30	0.02
8 hr	4.38±0.42	4.43±0.45	4.61±0.54	4.81±0.70	0.25	0.17

GC = ground corn; BR = ground broken rice; CA = ground cassava; PS = pineapple stem starch; SEM = standard error of the mean
Values (mean ± SD) within a row superscripted with different lowercase letters are significantly ($p < 0.05$) different

Table 4 *In situ* dry matter disappearance of concentrate and Napier grass silage with different starch sources

Period of incubation (hr)	Concentration (% dry matter)*				SEM	*p*-Value
	GC	BR	CA	PS		
Concentrate						
0 hr	39.23±2.32[b]	37.39±5.44[b]	53.92±4.00[a]	58.21±2.73[a]	2.24	<0.01
4 hr	48.94±2.32[b]	49.61±4.30[b]	57.19±5.85[a]	61.21±1.58[a]	2.37	<0.01
8 hr	54.74±3.19[b]	61.23±5.79[ab]	64.98±5.52[a]	67.28±1.89[a]	3.69	<0.01
12 hr	62.84±7.19[b]	73.66±5.32[a]	70.99±5.56[a]	70.99±0.64[a]	3.19	<0.01
24 hr	79.29±5.52[b]	86.23±2.26[a]	83.59±0.82[ab]	86.36±2.81[a]	3.10	0.04
48 hr	89.91±1.16[a]	90.58±0.58[a]	88.40±1.53[b]	90.70±0.42[a]	0.66	<0.01
72 hr	91.03±0.70[a]	91.72±0.85[a]	89.22±1.52[b]	90.92±0.52[a]	0.64	<0.01
Napier grass silage						
0 hr	21.85±1.37	19.90±2.20	21.84±2.95	20.88±1.48	1.20	0.13
4 hr	25.94±3.18[a]	24.75±2.96[ab]	22.09±0.53[b]	23.39±2.07[ab]	1.47	0.02
8 hr	28.64±4.98	32.55±3.91	28.49±5.73	30.04±3.32	2.54	0.16
12 hr	32.58±7.20	37.31±5.87	36.33±4.88	35.23±4.44	2.64	0.13
24 hr	42.36±0.76	46.86±4.36	43.25±4.20	43.78±6.08	2.83	0.55
48 hr	49.50±8.45	50.91±4.91	50.81±4.17	50.51±3.93	2.53	0.85
72 hr	53.20±6.27	54.25±4.99	54.26±3.69	53.27±4.68	2.08	0.81

GC = ground corn; BR = ground broken rice; CA = ground cassava; PS = pineapple stem starch; SEM = standard error of the mean
Values (mean ± SD) within a row superscripted with different lowercase letters are significantly ($p < 0.05$) different

Dry matter degradability kinetics

The effects of rumen incubation on the kinetics of degradation of the DM levels of the concentrate dietary treatments are presented in Table 5. The results suggested that PS had the significantly highest soluble fraction (a) and effective dry matter digestibility (ED) and the significantly lowest degradable rate (c).

Napier grass silage did not affect the different starch sources of the a, b and a + b fractions of DM degradability and ED (Table 5). The fractional rate of degradation of the c fraction was significantly higher in BR compared to GC and CA.

Discussion

For starch utilization in ruminants, the primary focus should be on intake, ruminal degradation and the fermentation of the starch. The ruminal NH_3-N concentration was not influenced by the treatment diets, indicating a synchronism between the amounts of nitrogen and energy available in the rumen (Alves

Table 5 DM degradability kinetics of concentrate diets with different starch sources

Degradation kinetics	Concentration (% dry matter)				SEM	*p*-Value
	GC	BR	CA	PS		
Concentrate						
a, %	38.63±3.21[c]	35.75±5.40[c]	51.38±4.12[b]	55.88±2.66[a]	1.74	<0.01
b, %	55.97±4.62[a]	56.87±5.57[a]	39.80±5.92[b]	37.66±2.52[b]	2.28	<0.01
a + b, %	94.58±2.82	92.63±0.87	91.18±3.21	93.54±0.06	1.55	0.07
c, %/hr	0.05±0.01[b]	0.08±0.02[a]	0.06±0.02[ab]	0.05±0.01[b]	0.01	<0.01
ED, %	66.28±3.17[c]	71.09±2.95[b]	72.65±2.67[ab]	74.91±0.28[a]	1.66	<0.01
Napier grass silage‡						
a, %	20.19±2.27	18.91±2.39	19.60±2.11	19.47±1.57	1.17	0.52
b, %	38.84±5.39	35.27±3.75	36.85±3.43	35.34±3.56	3.14	0.39
a + b, %	52.03±2.91	54.18±4.60	56.45±3.71	54.81±3.51	2.44	0.08
c, %/hr	0.04±0.01[b]	0.06±0.01[a]	0.04±0.01[b]	0.05±0.01[ab]	0.01	0.02
ED, %	37.94±1.82	38.17±4.11	36.23±3.73	36.40±3.66	1.68	0.30

GC = ground corn; BR = ground broken rice; CA = ground cassava; PS = pineapple stem starch; SEM = standard error of the mean; a = a soluble fraction; b = potentially degradable fraction (%); c = degradation rate constant b per hour; ED = effective dry matter digestibility (assuming rate of passage of 0.05 hr^{-1})
Values (mean ± SD) within a row superscripted with different lowercase letters are significantly ($p < 0.05$) different

et al., 2010). The feed intake was not affected by the starch source (Table 2), in agreement with other research where it was found that 40% of the starch in concentrate was used for fattening steers (Khongpradit et al., 2020). Various external factors also affected feed intake (Forbes, 2000). NDF has been proposed as a reliable predictor of consumption under the buffering capacity of the rumen (Wannapat et al., 2014). A low-fiber or high soluble carbohydrate diet results in low rumen pH and can reduce the digestibility of NDF that influences the ruminal passage rate, for which physical filling effects limit feed intake (Tripathi et al., 2004; Sung et al., 2007; Linton and Allen, 2007). In the present study, ruminal pH was not altered by the diets containing corn, broken rice, cassava or pineapple stem-based diets. It is also known that rumen buffering could avert pH depression which leads to the enhancement of rumen ecology (Kang et al., 2014). Therefore, the different starch sources in the diet did not affect the feed intake.

SCFA and lactic acid can build up in the rumen and reduce ruminal pH following a meal (Dijkstra et al., 2012). The ruminal SCFA concentration is related negatively to ruminal pH (Allen, 1997), which in turn is related to large variation between diets in removal. In addition, the buffering and neutralizing of acids in the rumen increases the ruminal pH (Dijkstra et al., 2012). During the experimental periods, the animals consumed 54.9–57.4% (DM basis) of concentrate diet. This ratio of diet may not induce a decrease in the ruminal pH. Furthermore, lactate is quickly absorbed and then metabolized in the liver (González et al., 2012). Although D-lactate is the main cause of the decline in ruminal pH, in the present study, the concentration of total lactate in the rumen did not alter the ruminal pH.

Starch is composed of two types of molecules: amylose, and amylopectin (Svihus et al., 2005). The amylose is slowly digestible in the rumen due to its molecular structure, while the amylopectin is easily digestible, thus leading to a rapid increase in SCFA and lactate after a meal (Stevnebø et al., 2009). The increase in total SCFA production was promoted by the production of acetate in PS and CA, with CA having the higher lactate production compared to PS. Lactate is an intermediate product of starch fermentation that was explained by the increase in the amylopectin content (especially in cassava) of easily fermentable carbohydrates contributing to higher SCFA and lactate levels (Wang et al., 2016). Thus, easily fermentable carbohydrates may provoke acidosis (Krause and Oetzel, 2006). PS has a high amylose content (Nakthong et al., 2017), which has a positive effect on starch gelatinization for the processed ruminant feed. Gelatinization is the breaking process of the hydrogen bonds of starch molecules when heat and water are present. There is resistance to gelatinization in high amylose-rich feedstuffs. In addition, the ruminal digestion rate of amylose is generally lower than that of amylopectin, which is desirable to prevent ruminal acidosis (Svihus et al., 2005; Stevnebø et al., 2009 Gómez et al., 2016).

The present study produced noticeable differences in DM degradation among the different starch sources. The *in situ* starch degradability levels of PS and CA were higher than those of GC and BR at 0 hr post-incubation. These variations in starch degradability between the diets were due to variations in the percentage of particles escaping through the bag pores and not being degraded by microorganisms (Cerneau and Michalet-Doreau, 1991). The DM disappearance for PS was greater than that for the other diets during 4–48 hr post-incubation. The tested starch sources contained different ADF contents (Khongpradit et al., 2020; Kotupan and Sommart, 2021), with the range from the highest to the lowest being cassava, pineapple stem starch, corn, and broken rice. This indicated that beef cattle fed with CA (with the highest ADF content) may have decreased digestibility in terms of DM disappearance at 48 hr and 72 hr.

PS had the highest soluble fraction (a) and higher ED than GC and BR. Nutrient digestibility was influenced by the composition of feedstuff in the diets. The diet containing PS provided the greatest effective degradability. PS had greater degradation of DM in the rumen because the fermentation activity of the ruminal amylolytic bacteria especially *Ruminococcus bromii* was higher in cattle receiving a diet rich in PS, as reported by Khongpradit et al. (2020). In other studies, corn and broken rice were reported to have a slower rate of degradation than other cereal grains because of their structure (presence or lack of pericarp, protein and endosperm), and starch protein complexes (Svihus et al., 2005; Stevnebø et al., 2009). Therefore, corn has the potential to modify starch granules by increasing degradation in the rumen (Owens et al., 1986). Consistent with the present study, the immediately soluble starch component of PS, CA, GC and BR observed were 55.88%, 51.38%, 39.23% and 37.39%, respectively. Degradability of PS and CA was related to the decreased composition of starch-protein complexes (Khongpradit et al., 2020), as the starch granules of corn and broken rice are embedded in a protein matrix (Philippeau et al., 2000; Monteils et al., 2002; Yang et al., 2020). ED, rumen fermentation and the utilization of nutrients are influenced by starch degradability in the rumen (Biricik et al., 2006). The ED of dry matter was higher for PS than for GC and BR, where the high ED was mainly due to a high soluble fraction (Maxin et al., 2013). The soluble fraction in PS was also high for dry matter. The high soluble fraction in PS could be explained by the difference in crude protein content in the starch source due to the breakdown of protein during fermentation (Maxin et al., 2013). Therefore, the differences in degradation according to different starch sources were improved by PS.

The present study indicated small differences in DM degradation of NS, whereas CA had the lowest DM degradation at 4 hr incubation. However, the ED values and potential degradability (a + b) were similar among the different starch sources, suggesting that the high starch content in the concentrate diet (40%) was not a limiting factor and that the inclusion of PS did not affect the efficiency of degradation of NS. The higher acetate-to-propionate ratio suggested fiber degradation was not influenced in high-starch diets (Xu et al., 2019).

In conclusion, this study suggested that PS could be used as a starch source in the ruminant diet without any negative effects. The different starch sources did not alter the DMI, ruminal pH and NH_3-N concentration. PS and CA had higher amounts of total short-chain fatty acid concentrations than in GC and BR. Ruminal digestibility of the PS concentrate diet as a starch source was greater than that of GC at 4–24 hr post-incubation. CA produced more lactate and influenced the rate of DM disappearance more so than NS. PS had a higher ruminal digestibility of the concentrate diet than BR and GC. PS had the highest ED and did not alter the lactate production or the digestibility.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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