Ruminal Degradability and Bypass Nutrients of Alkaline or Steam-Treated Cassava Chip and Corn Grain

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ABSTRACT

Modification of starches in high-energy feeds using various treatment methods led to the diverse effects on ruminal degradation and bypass protein and starch. Thus, the objectives of the present experiment were to investigate the effects of modification methods applied to high-starch energy feed sources on ruminal degradability and bypass nutrients determined using *in situ* and *in vitro* gas production techniques. This experiment was designed using a 2×4 factorial arrangement in a completely randomized design (CRD). The first factor was high-starch energy feed consisted of two levels, i.e., corn grain and cassava chips. The second factor was the treating method consisted of four levels, i.e., no treatment, steam treatment, NaOH treatment, and steam with NaOH treatment. Chemical composition, ruminal in situ disappearance, bypass of crude protein (CP) and non-fiber carbohydrate (NFC), and gas production characteristics were measured in pre-treated and post-treated samples. The results showed that significant interactions (p<0.01) between high-starch energy feed sources and treating methods were observed with respect to CP, ether extract (EE), and NFC, dry matter degradability (DMD), effective degradation (ED), and effective gas production (EP). Cassava chips had lower CP and EE but higher NFC, DMD, ED, and EP (p<0.01) compared with corn grain. Treating methods have no influence on CP and EE (p>0.05) in cassava chips and corn grain. DMD, ED, and EP of treated cassava chips were lower, while bypass CP and bypass NFC of treated cassava chips were higher than untreated cassava chips (p<0.05). On the contrary, treated corn grain led to higher DMD, ED, and EP, but lower bypass CP and bypass NFC than untreated corn grain did (p<0.01). DMD and gas production characteristics in cassava chips and corn grain showed significant correlations between *in situ* nylon bags and *in vitro* gas production techniques within each high-starch energy feed source. In conclusion, steam and NaOH treatments are two alternative methods that can modify the starches of high-starch energy feeds to alter ruminal degradation by decreasing solubility and degradability in cassava chips but increasing degradability in corn grain.

Keywords: cassava chips; corn grain; bypass starch; steam treatment; NaOH treatment

INTRODUCTION

Starch is an important non-fiber carbohydrate (NFC) component that must be balanced between degradation in the rumen and digestion in the small intestine to provide energy to ruminal microorganisms and ruminants. In Thailand, high-starch energy feeds, including ground corn and cassava chips, are typically used in beef and dairy cattle diets (Pilachai *et al.*, 2012). Cassava chips and corn grain starches have inherent differences in terms of ruminal solubility and fermentability. Their different starch degradation capacities depend on grain kernel structure, pericarp, amylose to amylopectin ratio, and other nutrients such as protein, fat, and fibers found in cassava chips or corn grain (Singh *et al.*, 2013). The starches in cassava chips are high in amylopectin, low in protein and fat, and without pericarp, whereas the

starches in corn grain are high in amylose, protein, and fat contents and contain pericarp and cuticle protein (Sarocha *et al.*, 2018). Kanjanapruthipong *et al.* (2001) report that cassava chips contain 80.16% NFC are easily degraded within the rumen and 2-3 times more degraded than cornmeal (65.41% NFC). Cassava starch is highly soluble and rapidly degradable in the rumen at over 90%, whereas corn starch is only 40%-60% degraded in the rumen (Sommart *et al.*, 2000). The strong pericarp and cuticle protein around the seed with starch-protein or starch-fat bonding in corn grain may limit starch degradation in the rumen. As for cassava chips, the absence of protein cuticle and high amylopectin content results in high solubility and rapid degradation in the rumen. This affects rumen ecology when using high amounts of cassava chips in ruminal diets, as the rapidly fermentable starch increases lactic acid production in

the rumen, resulting in a low pH value, and may predispose animals to rumen acidosis (Aschenbach *et al.,* 2011; Metzler-Zebeli *et al.,* 2013). Recently, Pilachai *et al.* (2014) noted that the treatment of cassava chips with 1% lactic acid (LA) decreased the degradation rate of DM and starch under *in vitro* studies. This result is in line with those reported by Pilachai *et al.* (2017), who indicated that the use of LA-treated cassava versus untreated cassava in rations with a high proportion of cassava could prevent rumen acidosis. In addition, differences in suitable levels of ruminal degradability between corn grain and cassava chips are the main reason for researching a treatment to break down the strong cuticle structure in corn grain, thereby reducing the solubility of starch in cassava chips to optimize degradability in the rumen. Many previous studies have shown that the chemical structure of grain undergoes various changes during processing. For example, the ratios of amylose and amylopectin contents affect the chemical and physical morphology of the grain when treated by heating or alkali treatment (Silva *et al.,* 2016). The amylopectin content in starch is easily dispersed in water to form a gel and undergo retrogradation, whereas high amylose content promotes gel formation with a strong retrogradation (Silva *et al.,* 2016). Changes in the morphology of starch granules after processing indicate that alkaline and heat treatments affect the granular structure of the starch. Moreover, high temperatures and alkali treatment can also break down corn grain's strong pericarp and cuticle protein, resulting in increased solubility and increased ruminal degradation (Gonzalez-Rivas *et al.,* 2017). However, data on high-starch energy feeds such as cassava chips and corn grain after heat or alkaline treatments include limited information pertaining to cassava chips. Therefore, this matter remains unknown regarding cassava chips. We hypothesized that various treatment of starch modifications would result in changes in the physical and chemical structures of cassava chips and corn grain and would have diverse effects on ruminal degradation and bypass nutrients. Therefore, our objectives were to investigate the effects of steam, sodium hydroxide (NaOH), and a combination of steam and NaOH treatments on ruminal degradation and the bypassing of nutrients in two high-starch energy feeds (i.e., corn grain and cassava chips) using an *in situ* nylon bag technique and an *in vitro* gas production technique.

MATERIALS AND METHODS

Animal Ethics

The experimental procedure was approved by the Animal Ethics Committee of Khon Kaen University based on the Ethics of Animal Experimentation of the National Research Council of Thailand. Record no. ACUC-KKU-64/60.

Experimental Design

The experiment was designed in a 2×4 factorial arrangement in a completely randomized design (CRD).

The first factor was high-starch energy feed consisted of two levels, i.e., corn grain and cassava chips, while the second factor was the treating method consisted of 4 levels, i.e., no treatment, steam treatment, NaOH treatment, and steam with NaOH treatment. The eight treatment combinations were 1) cassava chips (CV), 2) steamed cassava chips (CVS) 3), NaOH treated cassava chips (CVN), 4) steamed and NaOH treated cassava chips (CVSN), 5) corn grain (C), 6) steamed corn grain (CS), 7) NaOH treated corn grain (CN), and 8) steamed and NaOH treated corn grain (CSN).

Sample Preparations

All corn grain and cassava chips samples were delivered to our laboratory from the animal feed plant, Khon Kaen University, in which the same lot. The method for steam treatment was modified by Domby (2014). The samples of corn grain and dried cassava chips were soaked in water at a ratio of 1:3 (sample: water) for 6 hours (h) to increase moisture content and then steamed at 150°C for approximately 45 min in a steam pot. After that, the steamed corn grain and cassava chips were spread to a carpet for 48 h in room condition before drying in the oven at 60°C for 48 h and grinding through a 2-mm screen for use as a sample test. The method for NaOH treatment was prepared by a modified method of Srakaew *et al.* (2021). The samples of corn grain, dried cassava chips, steamed corn grain, and steamed cassava chips were treated with 3.5% NaOH (35 g/kg). The NaOH was applied as a 35% (wt/vol) solution with water and mixed with the samples for 15 min. Then treated samples and residual NaOH were loosely packed into one jar per treatment and remained uncovered for 24 h. After a reopened time, the mixed samples were spread to a carpet for 48 h in room condition before drying in the oven at 60°C for 48 h and then grinding through a 2-mm screen before the test.

Sample Collection and Chemical Analysis

The samples of corn grain, cassava chips, and all treated samples were dried at 60°C for 72 h. The dried samples were grounded over a 1-mm screen for chemical composition analysis. The dry matter (DM) was determined by oven drying at 95°C-100°C under pressure < 100 Hg for 5 h (934.01; AOAC, 2005). Ash content was determined by combustion at 550 °C for 3 h in a muffle furnace (942.05; AOAC, 2005). The concentration of nitrogen (N) content was measured using Kjeldahl method (2001.11; AOAC, 2005), and crude protein (CP) content was calculated as N×6.25. To determine the ether extract (EE), samples were extracted with petroleum ether using FOSS extractor (2003.06; AOAC, 2005). Gross energy (GE) was determined with an adiabatic calorimeter bomb (AC500, LECO Corporation, Michigan-USA). The fiber components were determined by detergent methods, and samples were boiled in neutral detergent plus amylase to determine the neutral detergent fiber (NDF), and boiled in acid detergent to determine the acid detergent fiber (ADF) according to the procedures

as described by Van Soest *et al.* (1991). NFC was estimated by different chemical groups (Mertens, 1997) using the following formula:

NFC (%)= 100 - (CP (%) + EE (%) + NDF (%) + Ash (%))

DM Disappearance by *In Situ* Nylon Bag Technique

Three ruminal fistulated non-lactating Holstein dairy cows (BW = 525 ± 36.7 kg) were used in this experiment. Before the start of the experiment, animals were fed twice daily with a similar diet containing rice straw (60%) and commercial concentrate (40%) (16.0% CP, 10.46 MJ ME/kgDM) and stayed in 2×5 m^2 individual cage inside the evaporative barn. Free water was delivered to the cows by individual nipple cups for 14 days. DM disappearance was determined by using the *in situ* nylon bag technique according to Ørskov & McDonald (1979). All samples were oven-dried at 60°C for 48 h and grinding through a 2-mm screen. The whole non-modified or modified samples approximately 4 g feed sample was taken in pre-weighed nylon bags (l2×6 cm, 38 µm average pore size). All nylon bags were tied with nylon threads, fitted to an iron chain, and then suspended in the rumen of three fistulated cows for 2, 4, 8, 12, 16, 24, 36, and 48 h. The samples of each treatment were incubated simultaneously in three animals using triplicates base at each incubation time point. After each incubation time, all bags of each sample test were withdrawn from the rumen and hand-washed in lukewarm water for 10 min or until wash water was clear. Nylon bags containing mixed test feed residues were then oven-dried at 60°C for 48 h in a forced-air oven. All bags feed samples were collected for their corresponding blanks. The samples at 0 h of incubation were washed and dried in the oven with a similar condition. After that, DM loss was determined as a different weight before and after incubation.

Bypass of CP and NFC were determined at 16 h of incubation times (Calsamiglia *et al.*, 1995). Triplicate of mixed residues in nylon bags of each treatment were collected and dried at 60°C for 72 h then analyzed for chemical composition including DM, CP, and NFC. Different CP and NFC contents before and after incubation in the same samples were calculated and used for determining the percentage of CP and NFC degradability and their by-pass from the rumen (Ørskov & McDonald, 1979).

% Degradability of nutrients=
(Nutrient in the sample at 16 h incubation / Nutrient in the sample before incubation) × 100

% Bypass of nutrients=100 - (% of degradability of nutrient)

Data for ruminal disappearance characteristics of DM was fitted to the exponential equation following the procedure described by Ørskov & McDonald (1979) and using the NEWAY program.

P = a + b (1-e^{-ct})

where P was disappearance rate at time t (%), a was the intercept of the degradation curve at time zero (%), b was the fraction of DM which degraded in the rumen (%), c was a rate constant of the disappearance of fraction b (h^{-1}), and t was time of incubation (h).

The effective degradability (ED) of DM was, therefore, calculated using the following equation.
ED = a+[bc/(c+k)]

where, k assuming the rate of particulate outflow from the rumen, k, is 0.05 h^{-1} by the equation of Ørskov & McDonald (1979).

In Vitro Gas Production

The *in vitro* gas production technique was used according to Menke & Steingass (1988). Rumen fluid was obtained from the same three fistulated non-lactating Holstein dairy cows. Preparation of artificial saliva was conducted by mixing 200 mL of macro-mineral solution, 0.1 mL of micro-mineral solution, 200 mL of buffer solution, and 1 mL of resazurin solution with 500 mL of distilled water. CO_2 gas was bubbled through the solution until the color change to pink per purple. The 0.4 g of the air-dried samples were weighed into 50 mL glass bottle as design at 20 replicate/treatments (8 bottles for gas production measurement and 12 bottles for DM degradability test). The bottles were warmed at 39°C before the injection of 30 mL of rumen fluid-buffer mixture (20 mL of artificial saliva and 10 mL of rumen fluid) into each bottle then incubated in a controlling incubator at 39°C. During the incubation, the gas production was measured by injecting non-pressure glass syringes into each incubated bottle before reading and recording gas volume showed in the syringes at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 30, 36, 42, and 48 h. Cumulative gas production data were fitted to the NEWAY program's model of Ørskov & McDonald (1979).

GP = a + b (1-exp$^{(-ct)}$)

where a was the gas production from the immediately soluble fraction (mL), b was the gas production from the insoluble fraction (mL), c was the gas production rate constant for the insoluble fraction (b) (% h^{-1}), t was incubation time (h), (a+b) was the potential extent of gas production (mL), and GP was gas produced at time "t" (mL).

The effective gas production (EP) was, therefore, calculated using the following equation.
EP = a+[bc/(c+k)]

where, k assuming the rate of particulate outflow from the rumen, k, is 0.05 h^{-1} by the equation of Ørskov & McDonald (1979).

Statistical Analysis

All obtained data were subjected to the analysis of variance (ANOVA) procedures of SAS (1998) according to a 2×4 factorial arrangement in CRD. Treatment means were compared using Duncan's New Multiple Range Test (Steel & Torrie, 1980). Mean differences were considered significant at $p<0.05$. Standard errors of means were calculated from the residual mean square in the

analysis of variance. The relationships between *in situ* and *in vitro* gas production parameters were obtained by simple linear regression analysis. The statistical model was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_k + e_{ijk,}$$

where Y_{ijk} was observation in treatment combination ij and replicated j, μ was over-all sample means, $\alpha_{i\ was}$ effect of factor A, β_j was effect of factor B, $\alpha\beta_i$ was effect of interaction AB, and e_{ijk} was error associated with observation.

RESULTS

Chemical Composition

The chemical compositions of untreated and treated corn grain and cassava chips are presented in Table 1. Gross energy and ash were not significantly different among treatment combinations, high-starch energy feeds, and treating methods. For CP, EE, and NFC, a significant interaction (p<0.01) was detected between high-starch energy feed sources and treating methods. The NDF and ADF contents were influenced by high-starch energy feeds, which were higher (p<0.01) in untreated and treated corn grain than in untreated and treated cassava chips but were not influenced by treatment methods. The results showed that untreated and treated corn grains had higher CP and EE contents (p<0.01) than untreated and treated cassava chips. Although treating methods reduced CP and EE in both high-starch energy feeds, the reduction was not significant. CP and EE contents in corn grain and cassava chips were decreased (p<0.05) after steam and NaOH treatments. Untreated corn grain had a lower NFC content than steam cassava chip (p<0.01). On the other hand, differences in treatment methods had no effect on NFC content.

In Situ DM Degradability

Degradability patterns are presented in Figure 1. The results indicated that the overall mean of untreated and treated cassava chips underwent higher degradation (p<0.01) than untreated and treated corn grain (Figure 1B). At 0 to 48 h of incubation, the DM disappearance of cassava chips decreased for all treatment methods (p<0.01) (Figure 1C). By contrast, the results for corn grain showed that all treating methods increased DM degradation (p<0.01) (Figure 1D).

In Situ Degradation Characteristics

The effect of treatment methods and high-starch energy sources on the rapidly soluble fraction (a_{is} fraction), potentially degradable fraction (b_{is} fraction), rate of degradation of *b* fraction (c_{is}), and effective degradability potential (ED_{is}) are presented in Table 2. A significant interaction of high-starch energy feed sources and treating methods were observed for b_{is}, c_{is}, and ED_{is} (p<0.01) and a_{is} (p<0.05). Treatment's main effects on *in situ* degradation characteristics were largely explained by differences of a_{is}, b_{is}, c_{is}, and ED_{is}. Fractions of a_{is}, c_{is}, and ED_{is} for cassava chips were higher (p<0.01), but the b_{is} fraction was lower (p=0.07) than that of corn grain. In terms of treatment methods, all treated cassava chips (CVS, CVN, and CVSN) showed a decrease (p<0.01) in a_{is} and ED_{is} fractions compared with untreated cassava chips (CV), whereas the fraction of b_{is} increased significantly (p<0.01) after treatment. Treatment methods of corn grain in the CS, CN, and CSN groups did not significantly affect (p>0.05) the a_{is} and c_{is} fractions. However, treating methods led to the increased b_{is} and ED_{is} fractions.

Table 1. Chemical composition of high-starch energy feeds (cassava chips and corn grain) with treating methods

Items	DM %	CP	EE	Ash	NDF	ADF	NFC	GE kcal/kgDM
		% on DM basis						
Treatments								
CV	90.40	2.27±0.30[b]	1.02±0.03[b]	5.58±0.00	14.410.086[a]	6.50±0.49[a]	76.71±0.41[ab]	3,713.02±10.59
CVS	90.10	1.71±0.03[b]	0.73±0.04[b]	4.99±0.01	14.89±0.62[a]	6.30±0.35[a]	77.62±0.51[a]	3,746.04± 0.03
CVN	89.70	1.61±0.04[b]	0.67±0.10[b]	6.64±0.04	14.62±0.11[a]	6.44±0.16[a]	76.38±0.28[ab]	3,543.02± 0.40
CVSN	90.20	1.51±0.04[b]	0.78±0.05[b]	6.26±0.08	15.81±0.04[a]	6.28±0.04[a]	75.57±0.25[ab]	3,527.01± 2.88
C	90.60	8.44±0.35[a]	4.95±0.17[a]	4.62±0.07	10.98±0.07[b]	3.74±0.06[b]	70.89±0.03[b]	3,700.03±12.95
CS	89.90	7.85±0.36[a]	4.35±0.07[a]	4.74±0.06	10.80±0.19[b]	4.06±0.19[b]	72.28±0.80[ab]	3,911.04± 5.90
CN	90.10	7.61±0.27[a]	3.68±0.03[a]	5.61±0.99	10.11±0.65[b]	4.54±0.25[b]	73.02±0.64[ab]	3,884.01± 4.02
CSN	90.00	7.67±0.47[a]	4.45±0.22[a]	5.07±0.00	10.39±0.55[b]	4.15±0.22[b]	72.42±0.60[ab]	3,767.02±14.40
SEM	-	1.203	0.779	1.203	1.628	0.816	2.028	152.004
Comparison, P-value								
High-starch energy feeds		<0.01	<0.01	0.26	<0.01	<0.01	<0.01	0.42
Treating methods		0.05	0.01	0.10	0.24	0.77	0.69	0.53
Interaction		<0.01	<0.01	0.18	<0.01	<0.01	<0.01	0.28

Note: Means in the same column with different superscripts differ significantly (p<0.05). SEM= standard error of means; DM= dry matter; CP= crude protein; EE= ether extract; NDF=neutral detergent fiber; ADF= acid detergent fiber; NFC= non-fiber carbohydrate; GE= gross energi; CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.
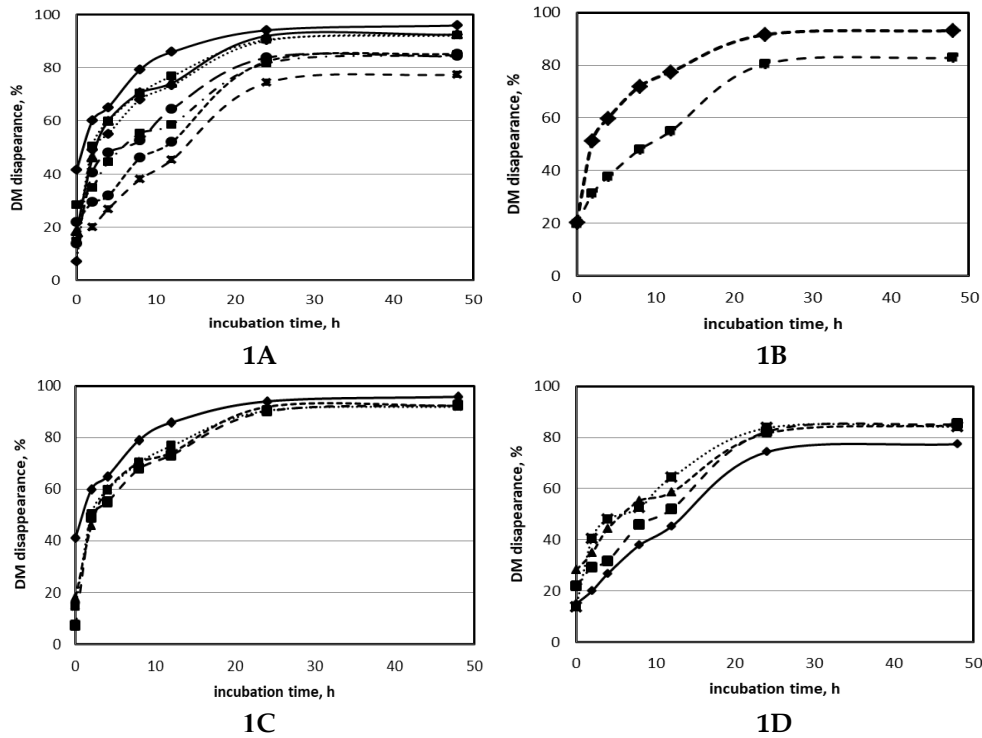
Figure 1. *In situ* DM disappearance of treatment combinations between the high-starch energy feeds and treating methods (1A; ◆= CV, ■= CVS, ▲= CVN, ■= CVSN, ✱= C, ●= CS, ■= CN, ●= CSN), different high-starch energy feeds (1B; ◆= cassava, ■= corn), different treating methods within cassava chips (1C; ◆= CV, ■= CVS, ▲= CVN, ■= CVSN) and different treating methods within corn grain (1D; ◆= C, ■= CS, ▲= CN, ■= CSN). CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.

Table 2. *In situ* degradation characteristics of high-starch energy feeds (cassava chips and corn grain) with treating methods

Items	*In situ* degradation characteristics			Effective DM degradability (ED$_{is}$), %
	a_{is}, %	b_{is}, %	c_{is}, %h^{-1}	
Treatments				
CV	42.00±2.00[a]	52.60±1.14[c]	0.140±0.01[b]	52.10±3.55[a]
CVS	13.70±1.70[b]	69.60±8.32[b]	0.210±0.01[a]	31.40±8.66[bc]
CVN	21.10±2.43[b]	67.00±4.00[b]	0.260±0.01[a]	41.50±6.17[b]
CVSN	17.70±0.17[b]	66.70±6.66[b]	0.250±0.02[a]	37.30±6.44[b]
C	18.50±2.00[b]	64.10±1.14[b]	0.040±0.01[c]	22.30±6.73[c]
CS	19.90±1.70[b]	79.40±8.32[a]	0.030±0.01[c]	34.60±7.88[b]
CN	28.90±2.43[b]	76.70±4.00[a]	0.050±0.01[c]	30.10±8.88[bc]
CSN	20.20±0.17[b]	65.90±6.66[b]	0.080±0.02[c]	26.20±7.38[bc]
SEM	2.438	2.747	0.010	2.143
Comparison, P-value				
High-starch energy feeds	0.74	0.07	<0.01	0.01
Treating methods	0.03	0.98	<0.01	<0.01
Interaction	0.02	0.01	<0.01	<0.01

Note: Means in the same column with different superscripts differ significantly (p<0.05). SEM= standard error of means; a_{is}= the intercept of the degradation curve at time zero; b_{is}= the fraction of DM which degraded in the rumen; c_{is}= a rate constant of the disappearance of fraction b; CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.

In Situ Ruminal Bypass CP and NFC

Degradable CP and NFC, bypass CP, and bypass NFC were calculated and are presented in Table 3. There was a significant interaction between main effects (p<0.01) in ruminal degradability and bypass of CP and NFC. The main effects were originally evaluated. The data showed that cassava chips underwent higher CP and NFC degradation in the rumen than corn grain did, leading to lower (p<0.01) bypass CP and NFC flow

from the rumen to the lower gut. Steam and NaOH treatments changed the physical form of cassava chips and corn grain. Enhanced degradability of CP and NFC was found in treated corn grain (CS, CN, and CSN), but treated cassava chips (CVS, CVN, and CVSN) showed significant decreases (p<0.01) when compared with untreated high-starch energy feeds. Bypass CP and NFC increased significantly in treated cassava chips (CVS, CVN, and CVSN), whereas no differences were shown in treated corn. Treated corn grain (CS, CN, and CSN) had lower (p<0.05) bypass NFC than did untreated corn grain (C).

In Vitro Gas Production Characteristics

Patterns of *in vitro* gas production characteristics for various treating methods and high-starch energy feeds are presented in Table 4 and Figure 2. A significant interaction of main effects was observed for c_{gas}, and ED_{gas} (p<0.01) but a_{is} and b_{is} were not influenced (p>0.05) by high-starch energy feeds and treating methods interaction. For high-starch energy feeds, cumulative gas production over 48 h, rate of gas production (c_{gas}), and effective gas production potential (EP_{gas}) of cassava chips were higher (p<0.01) than those of corn grain (Table

Table 3. Ruminal bypass crude protein (CP) and non-fiber carbohydrate (NFC) of treatment combinations between the high-starch energy feeds (cassava chips and corn grain) and treating methods

Items	Ruminal degradability, %		Ruminal bypass, %	
	CP	NFC	CP	NFC
Treatments				
CV	82.72±0.57[a]	95.40±0.42[a]	17.32±0.57[c]	4.60±0.42[c]
CVS	73.74±0.85[b]	88.59±0.85[b]	26.31±0.85[bc]	11.41±0.85[b]
CVN	70.41±0.57[b]	88.12±1.13[b]	29.63±0.57[b]	11.88±1.13[b]
CVSN	70.66±0.28[b]	88.35±1.84[b]	29.45±0.28[b]	11.65±1.84[b]
C	55.24±0.99[d]	84.40±1.13[b]	44.80±0.99[a]	15.60±1.13[a]
CS	61.19±0.99[c]	86.20±0.71[b]	38.95±0.99[a]	13.80±0.71[ab]
CN	60.72±1.70[c]	87.36±0.03[b]	39.36±1.70[a]	12.44±0.03[b]
CSN	61.33±0.42[c]	87.72±0.28[b]	38.74±0.42[a]	12.28±0.28[b]
SEM	1.760	1.892	2.950	1.728
Comparison, P-value				
High-starch energy feeds	<0.01	0.19	<001	<001
Treating methods	2.33	3.58	0.05	0.23
Interaction	0.01	0.01	0.01	0.01

Note: Means in the same column with different superscripts differ significantly (p<0.05). SEM= standard error of means; CP= crude protein; NFC= non-fiber carbohydrate; CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.

Table 4. *In vitro* gas production characteristics of high-starch energy feeds (cassava chips and corn grain) with treating methods

Items	*In vitro* gas production characteristics			Effective gas production (EP$_{gas}$), %
	a_{gas}, %	b_{gas}, %	c_{gas}, %h^{-1}	
Treatments				
CV	-7.85±0.35	72.60±2.51	0.120±0.023[a]	48.50±4.62
CVS	-5.35±0.11	64.00±2.79	0.080±0.002[b]	40.10±2.05
CVN	-5.14±0.06	64.90±2.53	0.110±0.006[b]	44.40±2.34
CVSN	-5.38±0.24	67.20±1.54	0.100±0.014[b]	44.20±2.67
C	-5.87±0.34	54.60±0.39	0.070±0.001[b]	34.20±0.03
CS	-6.02±0.07	64.30±1.83	0.070±0.002[b]	39.20±0.98
CN	-6.05±0.07	70.70±1.13	0.070±0.000[b]	39.40±0.65
CSN	-5.95±0.11	63.40±0.87	0.100±0.005[b]	42.20±0.79
SEM	0.36	0.26	0.010	4.323
Comparison, P-value				
High-starch energy feeds	0.62	0.68	0.01	<0.01
Treating methods	0.42	0.42	0.53	0.21
Interaction	0.36	0.26	0.01	0.11

Note: Means in the same column with different superscripts differ significantly (p<0.05). SEM= standard error of means; a_{gas}= the gas production from the immediately soluble fraction; b_{gas}= the gas production from the insoluble fraction; c_{gas}= the gas production rate constant for the insoluble fraction (b); CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.
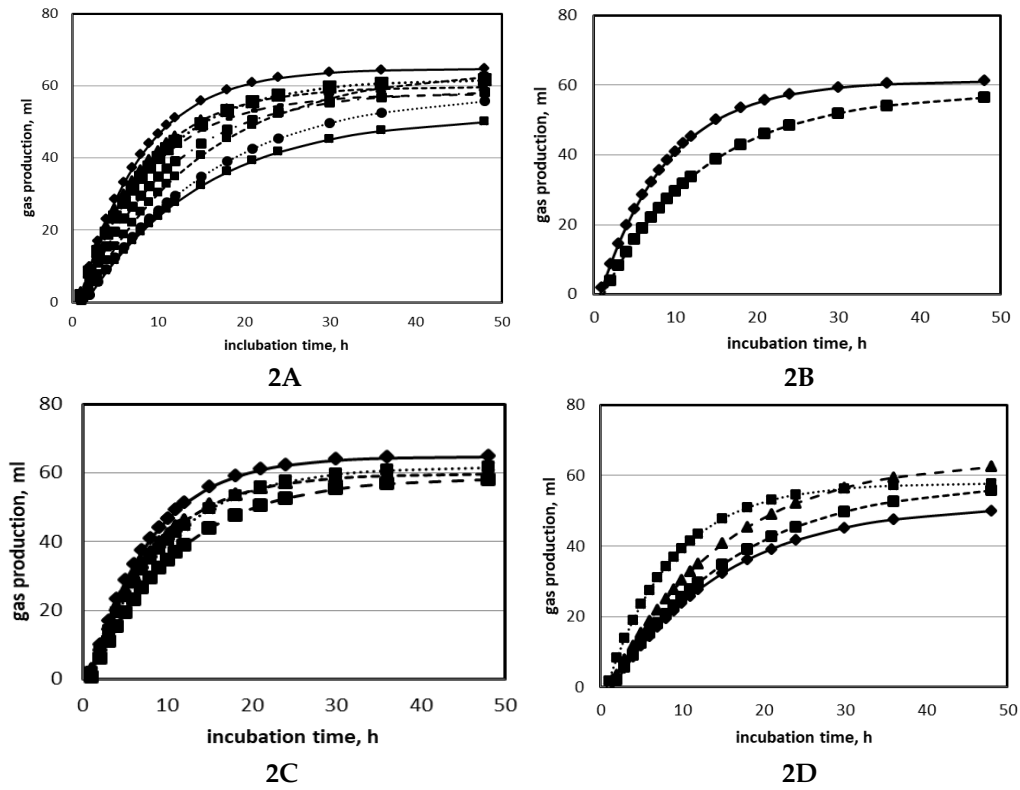
Figure 2. Cumulative gas production incubated with rumen fluid for treatment combinations between the treating methods and high-starch energy feeds (2A; ◆ = CV, – ■ – = CVS, --▲-- = CVN, ⋯■⋯ = CVSN, ■ = C, ⋯●⋯= CS, --■-- = CN, – ● – = CSN), different high-starch energy feeds (2B; ◆ = cassava, – ■ – = corn), different treating methods within cassava chips (2C; ◆ = CV, – ■ – = CVS, --▲-- = CVN, ⋯■⋯ = CVSN) and different treating methods within corn grain (2D; ◆ = C, – ■ – = CS, --▲-- = CN, ⋯■⋯ = CSN).
CV= untreated cassava chips; CVS= steamed cassava chips; CVN= NaOH treated cassava chips; CVSN= steamed and NaOH treated cassava chips; C= untreated corn grain; CS= steamed corn grain; CN= NaOH treated corn grain; CSN= steamed and NaOH treated corn grain.

4). The treated cassava chips (CVS, CVN, and CVSN) produced less gas during early incubation until after 12 h. Gas productions in the treated cassava chips (CVS, CVN, and CVSN) increased in the same manner, and the extent of gas production was similar but remained low (p<0.01) compared with the untreated cassava chips (CV). The EP_{gas} fraction decreased (p<0.05) in the CVS, CVN, and CVSN groups compared with CV. This result concurred with the degradability trial using the *in situ* nylon bag technique, which showed significantly decreased ED_{is} in treated cassava chips than untreated cassava chips. In terms of corn grain, treating methods affected (p<0.05) accumulated gas production, with higher gas production found in treated corn grain (CS, CN, and CSN groups) as compared with the untreated corn grain (C group). EP_{gas} fractions were higher (p<0.01) for the CS, CN, and CSN groups than for the C group.

Regression Equation

Apparent DMD using *in situ* nylon bag technique (Figure 1) and gas production using *in vitro* gas production technique (Figure 2) produced similar results. As can be seen from Table 5, *in vitro* gas production characteristics from the fraction of a_{gas}, b_{gas}, and EP_{gas} explained 90.61%, 93.83%, and 91.99% of the variations of a_{is}, b_{is},

and ED_{is} in cassava chips. *In situ* nylon bag characteristics from the fraction of a_{gas}, b_{gas}, c_{gas} and EP_{gas} explained 23.36%, 50.92%, 98.45%, and 89.74% of the variations of a_{is}, c_{is}, and ED_{is} in corn grain.

DISCUSSION

Chemical compositions of cassava chips and corn grain have been altered by treatment methods. Crude protein and EE contents in untreated corn grain and treated corn grain were higher than in untreated cassava chips and treated cassava chips. The average range of CP and EE in corn grain (7.61-8.44, 3.68-4.95) and cassava chips (1.51-2.27, 0.67-1.02) were similar to many previous studies that reported cassava chips and corn grain as high-starch energy source feeds, with cassava chips containing 2.3% CP and 0.3% EE and corn grain containing 8.8% CP and 4.7% EE (Suksombat *et al.*, 2007). The reduction of CP and EE contents in all treated cassava chips and corn grain in this experiment were similar to the findings of Ma *et al.* (2019), who found that, after steaming, the bulk density of normal corn grain and the CP and EE concentrations decreased. This occurred because a higher-temperature treatment at 100 °C reduced the CP and EE concentrations. Similar results were reported by Bhuiyan *et al.* (2012), who observed a reduc-

Table 5. Prediction of *in situ* nylon bag characteristics and estimated parameters from *in vitro* gas production

Items	Equation and factors use	R^2	RSD	Sig.
Cassava chips (CV)				
a_{iscv}	$Y = -9.368_{agascv} - 31.971$	90.61	4.743	*
b_{iscv}	$Y = -1.935_{bgascv} + 193.92$	93.83	2.345	*
c_{iscv}	$Y = -0.960_{cgascv} + 0.331$	9.94	0.063	ns
ED_{iscv}	$Y = 1.385EP_{gascv} + 16.190$	91.99	0.569	*
Corn grain (C)				
a_{isc}	$Y = -10.482_{agasc} - 39.706$	23.36	5.122	ns
b_{isc}	$Y = 1.018_{bgasc} + 6.339$	50.92	6.568	ns
c_{isc}	$Y = 1.298_{cgasc} - 0.052$	98.45	0.005	**
ED_{isc}	$Y = 1.869EP_{gasc} - 8.488$	89.74	3.885	*
Combined high energy starch sources				
ED_{is}	$Y = 2.108EP_{gas} - 15.108$	93.24	3.541	**

Note: ns= non-significant different (p>0.05); *= p<0.05; **= p<0.01; RSD= residual standard deviation; Sig.= significant level; a_{is}= the intercept of the degradation curve at time zero; b_{is}= the fraction of DM which degraded in the rumen; c_{is}= a rate constant of the disappearance of fraction b; a_{gas}= the gas production from the immediately soluble fraction; b_{gas}= the gas production from the insoluble fraction; c_{gas}= the gas production rate constant for the insoluble fraction (b); EP_{gas}= effective gas production potential.

tion in EE and energy contents in corn grain because of high temperatures. The thermo-alkaline process of the NaOH treatment drastically changed the appearance in some areas of the grain kernels (e.g., protein, lipids, and the principal component as starch). Therefore, the alkaline steeping method produced low protein, lipid, and starch (Palacios-Fonseca *et al.*, 2013).

Because NFC is composed of starch, simple sugars, and soluble fiber, NRC (2001) reported that the starch content of certain feeds such as corn grain and barley ranged between 80.0% and 81.7% of NFC. Moreover, other researchers reported that whole corn contains high starch content (79.0%) (Perez-Carrillo *et al.*, 2011), whereas cassava chips contain less starch (69.7%) (Nitayavardhana *et al.*, 2010). It can therefore be assumed that the NFC in corn grain and cassava chips consists mainly of starch. Our experimental results showed that the NFC content in cassava chips ranged from 75.6% to 77.6%, higher than corn grain, which ranged from 70.9% to 73.0%. However, previous studies reported similar NFC content in ground corn (73.0%-73.4%) (Fernandes *et al.*, 2015) but lower NFC content in cassava chips (69.7%) (Suksombat *et al.*, 2007).

The DMD of cassava chips was higher than that of corn grain. Sommart *et al.* (2000) reported that the rate of ruminal DM degradation from cassava chips was higher than corn grain, whereas Huntington (1997) mentioned that ruminal degradable starch values following the *in situ* method for corn grain, wheat, and cassava chips were 49.5%, 88.3%, and 94.0%, respectively. The ruminal degradability of starch varies depending on the grain source. For example, barley, cassava, and wheat starch have high solubility with a slower rate of degradable protein matrix in their endosperms than corn starch (Moharrery *et al.*, 2014). Dry and ground corn tend to have a lower digestibility than other grains because of the resistance of their seed coat to digestion and less surface area per unit weight in grains with larger kernels (Gonzalez-Rivas *et al.*, 2017).

The DMD of cassava chips decreased for all treatment methods, whereas corn grain's disappearance increased in all treatment methods. These results indicate that steam and NaOH treatment methods negatively affected ruminal degradation in cassava chips but positively affected corn grain. These differences may depend on grain kernel structure, the pericarp of cereal grains, the structure of starch (amylose to amylopectin ratio), and the presence of other nutrient contents in the grain (e.g., protein, fat, and fiber) that can bond with starch molecules. The grain's physical structure and outer layers affect the digestibility of starch in the gastrointestinal tract and are often impermeable to water and bacterial degradation (Moharrery *et al.*, 2014). Moreover, the different characteristics of starch granules could considerably influence the ability of isolated amylolytic enzymes to digest starch and, thus, affect starch degradability in the rumen (Zhang *et al.*, 2005). Several previous studies found that the amylose/amylopectin ratio correlates negatively with starch digestion in animals. Zhang *et al.* (2005) found that high amylopectin and type B starch (long-chain amylopectin) content in cassava starch resulted in high solubility in water, similar to our results at 0 h of incubation when cassava showed higher solubility than corn. Temperature and NaOH treatment led to the decreased solubility and degradability of cassava starch. This was explained by gelatinization and debranching of amylopectin, which caused the increased levels of amylose in the starch (Shi *et al.*, 2011). Gelatinization at high temperature and with NaOH treatment and retrogradation when re-cooling led to the reduced solubility and degradability of starch in the rumen; however, the degradability of corn in the rumen increased when steam and NaOH treatment were applied. The results observed in our trial can be explained by the disruption of the corn grain cuticular protein matrix. Shen *et al.* (2015) found that *in situ* digestion results showed the greater starch degradability of steam-flake corn than ground corn (66.9% *vs* 46.9%). Moharrery *et al.* (2014) reported that more severe processing methods aimed at modifying the physical structure, such as steam and flaking, disrupted the protein matrix of corn and sorghum and increased the rumen degradability of

starch within the vitreous endosperm. Kung *et al.* (1983) studied whole corn treated with NaOH in laboratory trials. DM disappearances from nylon bags in the rumen of whole corn treated with 2.5, 3.5, or 4.9% NaOH for 30 h were measured at 59.6%, 72.4%, and 93.0%, respectively, compared with 82.2% for untreated ground corn.

For *in situ* degradation, the results of this experiment indicated that treating with steam and NaOH treatments decreased solubility (a_{is}) and effective degradability (ED_{is}) of starch from cassava chips in the rumen. Our results showed an apparent DMD that significantly decreased DM loss in the rumen during treatment with cassava chips. Moreover, corn grain treated with steam and NaOH increased the ability of degradation in the rumen, as confirmed by the DM disappearance in all treated corn grain that showed a greater DM degradability in the rumen than untreated corn grain. Shen *et al.* (2015) conducted an *in situ* digestibility test and found that steamed corn grain had greater starch degradability, c_{is}, and ED_{is} than untreated corn grain. Ahmadijoo *et al.* (2020) found higher digestibility and rumen fermentation profiles when the cows fed steam flaked corn and barley grains. Karami *et al.* (2018) hypothesized that increased starch degradability of steamed corn grain might be caused by the disruption of the protein matrix surrounding the endosperm starch granules and disorganization in the starch granules. In our experiment, high steaming temperatures and alkalosis might have disrupted the strong physical covering around the corn grain, decreasing water solubility and increasing microbial digestion in the rumen.

Treatment methods that used steam and NaOH enhanced the degradability of NFC, resulting in the decreased bypass NFC in corn grain. Treatment methods increased the bypass CP and NFC of cassava chips to between 51.91% and 71.07% and 148.04% and 158.26%, respectively. In addition, the treatment methods reduced the bypass CP and NFC of corn grain by 12.14% to 13.53% and 11.54% to 21.28%, respectively. Savari *et al.* (2018) reported that corn grain was almost completely resistant to microbial fermentation in the rumen, whereas grinding and steam processing methods have been used to improve ruminal carbohydrate availability in dairy cattle (Shen *et al.,* 2015). Chemical and physical changes in corn starch granules might be induced by manipulative factors such as moisture, temperature, pressure, and thermal alkalinity to increase the proportion of starch digested in the rumen (Zhong *et al.,* 2008). Conversely, we found that the same methodology with treated corn grain had a different effect on treated cassava chips. Treated cassava chips showed the decreased ruminal digestion of CP and NFC and the increased bypass CP and NFC compared with untreated cassava chips. These different results can be explained by diverse physical and chemical starch structures in corn grain and cassava chips. The non-protein seed coat of corn grain was easily gelatinized with the occurrence of starch aging recrystallization (Israkarn *et al.,* 2014). The major source of starch can be gelatinized by heat or NaOH addition. Li & Lim (2016) determine that thermal alkalinity is an efficient catalyst for chemical modifica-

tion of starch by inflating the crystalline region and promoting starch gelatinization and amylose leaching from the granule (Wang & Copeland, 2012), thus reducing the proportion of amylopectin in cassava starch (Cai *et al.,* 2014). Reducing the amylopectin content in cassava starch resulted in limited water solubility and increased starch recrystallization after gelatinization to form a resistant starch molecule that prevented the risk of starch digestion by ruminal microorganisms.

In vitro gas production characteristics, the gas production rate of high-starch cassava chips was higher than that of corn grain. Nitipot & Sommart (2003) studied *in vitro* gas production techniques. They found that cassava chips' gas production rate and effective gas production potential were higher than that of cornmeal, broken rice, and other industrial by-products. In terms of treatment methods, the EP_{gas} fraction decreased in the treated group compared with the control group. This result concurred with the degradability trial by the *in situ* nylon bag technique, which showed significantly decreased ED_{is} in treated cassava chips compared to untreated cassava chips. However, gas production was higher in treated corn grain. Karami *et al.* (2018) found that the gas production of steamed corn grain was higher than that of untreated corn grain. Gas production increased after treatment because NaOH or steam treatment caused starch gelatinization, disrupted intermolecular hydrogen bonds, and enhanced the corn kernel surface that was available for rumen microbial attachment. Gases were produced from the degradation of carbohydrates into volatile fatty acids and carbon dioxide (CO_2). The greater ruminal digestion of starch is associated with the high gas production in the rumen (Ungerfeld, 2020).

The inclusion of gas production characteristics in the regression equation improved the prediction accuracy of *in situ* characteristics. Especially, the fraction of EP_{gas} in the regression equation increased the accuracy of predicting ED_{is} in *in situ* characteristics of combined high-starch energy feeds. Cone *et al.* (1999) presented a close relationship (R^2 = 88.0%) found in *in situ* and *in vitro* gas production characteristics for silage samples. Kamalak *et al.* (2005) also noted a significant correlation between gas production and *in situ* DM disappearance and suggested the equation to predict effective DM degradability (EDMD) of roughage using gas kinetics. Insoluble fraction (b) alone explained 98.3% of the EDMD variation. With these high-starch energy feeds, either the *in situ* DMD or the *in vitro* gas production technique could be used to assess starch fermentation in the rumen since there was a very good positive relationship between the two techniques. The highly significant relationship suggested that ED_{is} can be predicted from EP_{gas}. Significant observations in the experiment indicate that the *in vitro* gas production technique can be applied to study the extent of DM degradation of high-starch energy feeds, instead of the *in situ* technique, which may have advantages in many aspects such as being less dependent on animals, avoiding the error associated with the loss of small particles through the pores of the bag, and reducing the inputs and the cost of labor.

CONCLUSION

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Treating cassava chips with steam and NaOH decreased ruminal degradability, leading to the increased bypass protein and NFC, whereas treating corn grain increased ruminal degradability, resulting in the decreased bypass protein and NFC. It is noteworthy that the different physical and chemical structures of corn grain and cassava chips have varied effects on the capacity for starch degradation in the rumen. Nevertheless, the *in vitro* study results should be applied to *in vivo* studies to assess any responses in ruminants.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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