Songklanakarin J. Sci. Technol.
40 (4), 896-903, Jul - Aug. 2018

http://www.sjst.psu.ac.th

Original Article

Effects of ensiled *Aspergillus oryzae* and *Saccharomyces cerevisiae* cassava pulp as replacement for concentrate on ruminal fermentation in rumen-fistulated cows

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Received: 11 June 2016; Revised: 1 May 2017; Accepted: 16 May 2017

Abstract

Four experiments were conducted. I) The concentration of reducing sugar was determined following incubation with *Aspergillus oryzae* and an 8x11 factorial arrangement with 8 formulas of cassava components and 0 to 10 d of incubation. The reducing sugar increased from day 3 and the highest was found for 100% cassava pulp. II) Crude protein (CP) and urea content were determined after incubating with *A. oryzae* and *Saccharomyces cerreviseae* and a 4x6 factorial arrangement with 4 formulas of cassava components and 6 urea levels. The highest CP was observed in 37.5% cassava pulp, 25% cassava chip, and 37.5% cassava peel. III) The design was a 3x4 factorial arrangement with 3 formulas of cassava components and 4 urea levels. CP was unaffected by cassava components but increased with increasing urea levels. IV) The design was a 3x3 Latin squares with 3 fistulated cows and 3 periods. Treatments were 4 kg/cow/d concentrate, 3.2 kg/cow/d concentrate plus 0.8 kg/cow/d ensiled cassava pulp (ECP) and 2.4 kg/cow/d concentrate plus 1.6 kg/cow/d ECP. ECP increased pH, molar proportions of acetic and butyric acids but reduced molar proportion of propionic acid.

Keywords: *Aspergillus oryzae*, *Saccharomyces cerevisiae*, ensiled cassava products, cassava pulp, cassava peel

1. Introduction

In the processing of cassava flour and after removing the flour from the tuber, the rest is cassava pulp which can be used as animal feed. Unfortunately, the protein content of the peel and pulp is low and cannot be regarded as good quality feed for animals. However, it has been reported that fermentation of carbohydrate substrates by micro-organisms could bring about an increase in crude protein (CP) content (Antai & Mbongo, 1994). Fungal and yeast fermentations have been identified as inexpensive tools to increase the protein level of such by-products. The attractive characteristics in the use of microorganisms include their fast growth rate, the high level of protein, and the comparable good nutritional values.

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Fungal cellulase and amylase, particularly from *Aspergillus* species is widely used for commercial enzyme production. Recently, *Aspergillus oryzae* has been reported to yield cellulase activity (Begum *et al*., 2009), which is an important enzyme required for the catabolism of cellulose into smaller sugars. In addition, *A. oryzae* also has an excellent capacity of α-amylase production under solid state fermentation using spent brewing grains (Francis *et al*., 2002) and wheat bran (Sivaramakrishnan *et al*., 2007). Therefore, the highest reducing sugar content of cassava pulp fermentation with *A. oryzae* may indicate the capability of *A. oryzae* to produce enzymes, especially cellulase and amylase to hydrolyze glucosidic linkages in polysaccharides.

Utilization of local feed especially fermentation of cassava peels by pure culture *Saccharomyces cerevisiae* could increase its protein content from 2.4% in non-fermented cassava to 14.1% in fermented products (Antai & Mbongo, 1994). Fermentation of cassava flour with *S. cerevisiae* enhanced the protein level from 4.4% to 10.9% and decreased

the amount of cyanide content (Oboh & Akindahunsi, 2003). Furthermore, Boonnop *et al.* (2009) reported that cassava chip can be nutritionally improved with *S. cerevisiae* called yeast fermented-cassava chip and could be used for animal feed. Khampa *et al.* (2009) found that feeding diets containing yeast fermented cassava peel and pulp resulted in higher ruminal pH, ammonia-N, and blood urea nitrogen.

The aims of the present study were to investigate the use of fungus followed by the yeast to improve the protein value of cassava peel, cassava pulp, cassava chip, and their mixtures and to determine the effect of ensiled cassava peel (ECP) as a replacement for concentrate on ruminal fermentation in rumen fistulated cows.

2. Materials and Methods

Four experiments were conducted in the present study. They included I) determination of the concentration of reducing sugar in cassava products and mixtures of cassava products after incubating with *A. oryzae*, II determination of CP and urea content of cassava products or mixtures of cassava products after incubating with *A. oryzae* and *S. cerevisiae* plus urea on a small scale, III) the same as experiment 2 on a medium scale, and IV) the effect of ensiled cassava pulp as a replacement for concentrate on ruminal fermentation in rumen fistulated cows.

2.1 Experiment I

This experiment aimed to determine the concentration of reducing sugar in the cassava products and mixtures of cassava products after incubation with *A. oryzae.* Ten kg each of wet cassava chip, cassava pulp, and cassava peel were taken from a cassava flour factory and sun-dried for 72 h. They were then ground through 1.0 mm screens and kept in plastic containers with caps. To obtain good cassava products or mixtures of cassava products suited for the growth of fungi, a completely randomized 8x11 factorial design was used. Eight formulas of feedstuffs were tested: (1) 100% cassava pulp (CSPu), (2) 100% cassava peel (CSPe), (3) 100% cassava chip (CSC), (4) 75% CSPu+25% CSC, (5) 25% CSC+ 75% CSPe, (6) 50% CSPu+50% CSC, (7) 50% CSC+50% CSPe, and (8) 25% CSC+37.5% CSPu+37.5% CSPe (Factor A). Three replicates of each 40 g for each formula were placed into 8 oz glass bottles and were incubated at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days (Factor B), giving a total of 264 bottles. Water was added until the moisture content was 70%. All bottles were autoclaved at 121 °C for 15 min and then left to cool down at room temperature. After cooling down, 4 ml of $3.25x10^6$ cells/ml of *A. oryzae* were inoculated and then left at room temperature.

After each incubation period, the content of each tube was removed from the bottle, placed into a plastic bag, tied, and kept at -20 °C. After day 10 of incubation, all samples were taken from the freezer and then plastic bags, placed on Petri dishes and put into 60 °C hot air oven for 72 h. After drying, samples were ground through a 1.0 mm screen and then put into plastic bottles with caps and kept at room temperature until analysis for sugar. One gram of each sample was put into 20x150 mm screw-cap test tube, followed by 9 ml of distilled water. The tubes were centrifuged at 5000 rpm for 15 min. Then 0.1 ml of the supernatants was put into 20x150 mm screw-cap test tubes, followed by 0.9 ml of distilled water to dilute the solution. Samples were then measured for absorbance using a spectrophotometer at 540 nm wave length and the absorbance was recorded. The concentration of sugar in the sample was then calculated.

2.2 Experiment II (small scale)

The objective of this experiment was to determine the content of CP and urea in the cassava products or mixtures of cassava products after incubating with *A. oryzae* and *S. cerreviseae* plus urea on a small scale. The experiment was a completely randomized 4x6 factorial design with Factor A and 4 formulas (1) 100% cassava pulp (CSPu), (2) 100% cassava peel (CSPe), (3) 75% CSPu+25% cassava chip (CSC), and (4) 37.5% CSPu+25% CSC+37.5% CSPe, while Factor B was the addition of urea at 0, 1.25, 2.50, 5.00, 7.50, and 10.00% of dry matter (DM). The selected cassava products and their mixtures were based on the results obtained from Experiment I which gave the top four reducing sugar content after fermentation with *A. oryzae*. Three replicates of each 40 g of each formula were placed into 8 oz glass bottles. Water was added until the moisture content was 70%. All bottles were autoclaved at 121 °C for 15 min and then left to cool to room temperature. After cooling, 4 ml of $3.25x10^6$ cells/ml of *A. oryzae* were inoculated and then left at room temperature. All bottles were incubated for 3 days. After 3 days, the bottles were inoculated with *S. cerreviseae* and the levels of urea were added according to the treatment imposed. Samples were left at room temperature for 7 days. After 7 days of incubation, samples were taken and dried at 60 °C for 72 h to stop the microbial activity. The samples were then taken for grinding through 1.0 mm screen and further analyzed for CP and urea.

2.3 Experiment III (medium scale)

The objective of the third experiment was to determine CP and urea content of the cassava products or mixtures of cassava products after incubation with *A. oryzae* and *S. cerevisiae* plus urea on a medium scale. The experiment was a completely randomized 3x4 factorial design, with Factor A and 3 formulas: (1) 100% cassava pulp (CSPu), (2) 100% cassava peel (CSPe), and (3) 37.5% CSPu+25% Cassava chip (CSC)+37.5% CSPe while Factor B was 4 levels of added urea at 0, 4.0, 5.0, and 6.0% of DM. The selected cassava products and their mixtures are based on the results obtained from Experiment II that gave the 3 best results. The addition of urea at 5% was considered to be the best level from Experiment II, thus 4.0 and 6.0% urea was set in this experiment to determine whether these levels could be used. Three replicates of each 1 kg of each formula were placed into 5 liter plastic bottles. All procedures were done as in Experiment I and then Experiment II.

2.4 Experiment IV

The last experiment was to evaluate the effect of ensiled cassava pulp as a replacement for concentrate on ruminal fermentation in rumen fistulated cows.

2.4.1 Preparation of ensiled cassava pulp

Ten drums each of 80 kg of wet cassava pulp taken from a cassava flour factory. The contents of each drum were placed into 10 plastic 150 liter drums. Water was added for a final moisture content of 70%. Each drum was then fitted with a hose at the bottom of the drum to introduce steam. The hose was attached to a steam pipe connected to a boiler. The cassava pulp was allowed to stream for 4 h and then left to cool to room temperature. After cooling, 320 ml of $3.25x10^6$ cells/ml of *A. oryzae* were inoculated into each drum and the contents were thoroughly mixed and then left at room temperature. All drums were incubated for 3 days. After 3 days, *S. cerevisiae* was inoculated into each drum and 4.0% urea was added. The 4.0% urea was selected because this level gave the best results from Experiment III. The drums were left at room temperature for 7 days. After 7 days, the ensiled cassava pulp was removed, sun-dried for 4 days, and stored for further use.

2.4.2 Management of rumen fistulated cows

Three Holstein Friesian Crossbred (>75% HF) fistulated dairy cows housed in individual pens (3x3 m) were assigned to one of 3 treatments in a 3x3 Latin squares design. The experiment was comprised of 3 periods with 21 d in each period: 14 d for adaptation to the diets followed by 7 d for ruminal sample collection. The individual dietary treatments were 1) 4 kg/cow/d of pelleted concentrate (control), 2) 3.2 kg/cow/d of pelleted concentrate plus 0.8 kg/cow/d (20% replacement) of ECP, and 3) 2.4 kg/cow/d of pelleted concentrate plus 1.6 kg/cow/d (40% replacement) of ECP.

Diets offered as 4 kg/cow/d of concentrate containing 21% CP plus ECP according to treatments and 6 kg/cow/d of rice straw, divided into 2 equal meals at 08:00 and 16:00 h together with *ad libitum* clean water. Feed offered and feed refused were measured and recorded daily during the experimental periods. The DM content (48 h at 60 °C) of the rice straw for individual cows was determined daily to calculate the dry matter intake (DMI). The samples were ground through a 1 mm screen for chemical analysis. The DM of rice straw and concentrate were determined by oven drying at 105 °C to a constant weight. The samples were analyzed for CP, ether extract, ash (Association of Official Analytical Chemists [AOAC], 1995), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest *et al.,* 1991).

2.4.3 Ruminal fermentation

To evaluate ruminal fermentation, on the last day of each experimental period (d 21), ruminal fluid samples were collected from each fistulated non-lactating cow at 0, 3, and 6 h after the morning feeding. The pH of the rumen fluid was immediately determined at the time of sampling by a pH meter. To determine the levels of volatile fatty acids (VFAs) and ammonia-N, 36 ml samples of rumen fluid were put into 50 ml centrifuge tubes containing 4 ml of 1M H_2SO_4 and then centrifuged at 1895 rpm for 15 min. The supernatants were collected and put into 25 ml test tubes and then capped and stored at -20 °C until analysis. The analyses of acetic acid, propionic acid, and butyric acid used gas chromatography (Hewlett Packard GC system HP6890, USA, 19091N-113 INNOWAX, Length (meters) 30, I.D. (mm) 0.32 WIDE-BORE, Film (um) 0.25). Ammonia-N concentration was determined by the Kjeldahl analysis (AOAC, 1995).

2.5 Statistical analysis

All data in Experiment I-III were subjected to analysis of variance according to factorial design using the Statistical Analysis System (1996). Significant differences between treatment means were tested by Duncan's Multiple Range Test (Steel & Torrie, 1980).

In Experiment IV, measurements of intake, pH, ammonia-N, and VFAs were analyzed by ANOVA using SAS (19 96). Significant differences between treatment means were tested by Duncan's Multiple Range Test (Steel & Torrie, 1980).

3. Results and Discussion

3.1 Experiment I

The content of reducing sugars was low before fermenting the cassava products (day 0) but increased markedly at day 3 of fermentation (Table 1). The average highest reducing sugar content was found for 100% CaPu (181.1 mg/g) followed by 37.5% CSPu, 37.5% CSPe, 25% CSC (124.3 mg/g), and 75% CSPu+25% CSC (119.2) while the average lowest reducing sugar content was for 100% CSC (35.10 mg/g). When comparisons were made among the cassava products and their mixtures, the highest average reducing sugar content was also found in 100% CSPu (139.0 mg/g) followed by 37.5% CSPu, 37.5% CSPe, 25% CSC (119.0 mg/g) and 75% CSPu+25% CSC (91.4 mg/g), while the average lowest reducing sugar content was found in 100% CSC (32.4 mg/g). Although 100% CSC contained higher starch than other cassava products and their mixtures, the content of reducing sugar measured was the lowest. This can be explained by gelatinization of the starch during autoclaving of the substrate, subsequently *A. oryzae* only grew on the outer surface of the substrate exposed to air and could not penetrate into the inner substrate. Similar results were also obtained previously by Raimbault (1998), Iyayi and Losel (2001a), and Pothiraj and Eyini (2007) Solid state fermentation of cassava products using various fungi including *A. oryzae* was reported to increase reducing sugar content.

3.2 Experiment II and III

The CP contents of the cassava products are given in Table 2 and Table 3. In Experiment II, the highest crude protein content following *A. oryzae* and *S. cerevisiae* fermentation was observed in 37.5% CSPu, 25% CSC, 37.5% CSPe followed by 100% CSPu, while the lowest was found in 100% CSC and 75% CSPu+25% CSC. In Experiment III, there was no significant difference in CP content between the cassava products. As expected, the CP content increased significantly as the added amount of urea increased in both experiments. The increase in CP content could be attributed to the possible secretion of some extracellular enzymes into the fermented products by fermenting microorganisms and to the addition of urea. Apart from this, the increase in the growth and

Table 1. Reducing sugar content of cassava products and their mixtures fermented with *A. oryzae* at various times (0-10 days) of fermentation.

Day	Formula								Mean
	1	2	3	4	5	6	7	8	
0	6.48^{c,w}	3.72^{d,y}	13.42^{a,w}	9.37^{b,x}	4.75^{cd,y}	9.54^{b,y}	5.70^{cd,z}	5.86^{c,y}	7.35^{z}
1	66.00^{a,v}	25.69^{b,x}	28.75^{b,v}	63.65^{a,w}	29.28^{b,x}	34.32^{b,x}	34.90^{b,y}	58.93^{a,x}	41.52^{y}
2	85.57^{b,v}	61.58^{cd,w}	33.70^{f,uv}	63.65^{cd,w}	69.02^{c,w}	44.40^{ef,x}	53.61^{de,x}	106.10^{a,w}	64.70^{x}
3	181.14^{a,u}	75.33^{d,v}	35.10^{e,uv}	119.24^{b,u}	88.92^{cd,v}	89.91^{c,uv}	80.25^{cd,u}	124.27^{b,vw}	99.27^{uv}
4	172.80^{a,u}	91.81^{d,u}	35.72^{e,uv}	116.26^{c,u}	89.50^{d,v}	76.28^{d,vw}	76.86^{d,uv}	136.58^{b,v}	99.47^{uv}
5	167.18^{a,u}	87.06^{b,uv}	40.02^{c,u}	106.84^{b,uv}	103.91^{b,uv}	94.25^{b,u}	80.49^{b,u}	144.67^{a,uv}	103.05^{u}
6	178.91^{a,u}	81.40^{e,uv}	36.39^{f,uv}	112.71^{c,u}	93.59^{d,v}	74.59^{e,vw}	75.58^{e,uvw}	145.66^{b,uv}	99.85^{uv}
7	168.51^{a,u}	76.57^{c,v}	31.72^{d,uv}	106.92^{b,uv}	104.49^{b,uv}	68.39^{c,w}	67.90^{c,uvwx}	158.80^{a,u}	97.91^{uvw}
8	169.21^{a,u}	53.85^{de,w}	31.02^{f,v}	108.33^{c,uv}	102.26^{c,uv}	70.46^{d,w}	63.06^{de,vwx}	145.33^{b,uv}	92.94^{w}
9	172.30^{a,u}	58.57^{de,w}	33.82^{e,uv}	97.92^{c,v}	104.36^{c,uv}	65.58^{d,w}	70.34^{d,uvw}	142.73^{b,uv}	93.20^{w}
10	160.95^{a,u}	62.24^{d,w}	36.88^{e,uv}	110.03^{c,uv}	114.69^{c,u}	72.31^{d,w}	60.67^{d,wx}	139.68^{b,uv}	94.68^{vw}
Mean	139.00^{a}	61.62^{e}	32.41^{f}	91.41^{c}	82.25^{d}	63.64^{e}	60.85^{e}	118.96^{b}	

(1) 100% cassava pulp (CSPu), (2) 100% cassava peel (CSPe), (3) 100% cassava chip (CSC), (4) 75% CSPu+25% CSC, (5) 25% CSC+75% CSPe, (6) 50% CSPu+50% CSC, (7) 50% CSC+50% CSPe and (8) 25% CSC+37.5% CSPu+37.5% CSPe

^{abcdef} means without same letters on the same row are significantly different (P<0.0001)

^{uvwxyz} means without same letters on the same column are significantly different (P<0.0001)

Table 2. Crude protein content (%) and urea residue (%) in the fermented cassava products with increasing urea addition. *(Experiment II)*

	% Urea addition	% Crude protein	% Urea residue
100%CSPu	0.00	6.02^{f}	0.25^{f}
	1.25	11.14^{e}	0.79^{e}
	2.50	17.99^{d}	1.67^{d}
	5.00	27.41^{c}	3.13^{c}
	7.50	38.29^{b}	4.66^{b}
	10.0	49.85^{a}	5.77^{a}
100%CSPe	0.00	6.70^{f}	0.28^{f}
	1.25	10.68^{e}	0.85^{e}
	2.50	16.53^{d}	1.77^{d}
	5.00	27.05^{c}	3.29^{c}
	7.50	37.64^{b}	4.66^{b}
	10.0	47.63^{a}	6.20^{a}
75%CSPu, 25%CSC	0.00	6.66^{f}	0.17^{f}
	1.25	11.53^{e}	1.06^{e}
	2.50	16.53^{d}	1.93^{d}
	5.00	25.35^{c}	3.61^{c}
	7.50	37.25^{b}	4.78^{b}
	10.0	45.54^{a}	5.54^{a}
37.5%CSPu, 25%CSC, 37.5%CSPe	0.00	6.81^{f}	0.29^{f}
	1.25	12.30^{e}	0.79^{e}
	2.50	20.86^{d}	1.73^{d}
	5.00	28.30^{c}	2.61^{c}
	7.50	41.86^{b}	4.81^{b}
	10.0	51.34^{a}	5.55^{a}
Formula	100% CSPu	25.11^{b}	2.71^{b}
	100% CSPe	24.37^{c}	2.84^{a}
	75% CSPu+25% CSC	23.81^{c}	2.85^{a}
	37.5% CSPu+25% CSC, 37.5% CSPe	26.91^{a}	2.63^{c}
% Urea addition	0.00	6.54^{f}	0.25^{f}
	1.25	11.41^{e}	0.88^{e}
	2.50	17.98^{d}	1.78^{d}
	5.00	27.03^{c}	3.16^{c}
	7.50	38.76^{b}	4.73^{b}
	10.0	48.59^{a}	5.76^{a}
P-value			
Formula		<0.0001	<0.0001
% Urea addition		<0.0001	<0.0001
Formula*% Urea addition		ns	Ns

CSPu, cassava pulp; CSPe, cassava peel; CSC, cassava chip

^{a,b,c,d,e,f} Means within the same column within formula having different letters are different at P<0.05.

Table 3. Crude protein content (%) and urea residue (%) in the fermented cassava products with increasing urea addition. *(Experiment III)*

	% Urea addition	% Crude protein	% Urea residue
100%CSPu	0.0	5.02[d]	0.07[d]
	4.0	22.78[c]	2.52[c]
	5.0	27.51[b]	2.93[b]
	6.0	30.88[a]	3.32[a]
100%CSPe	0.0	7.51[d]	0.17[d]
	4.0	23.97[c]	2.20[c]
	5.0	26.38[b]	3.05[b]
	6.0	28.76[a]	3.84[a]
37.5%CSPu, 25%CSC, 37.5%CSPe	0.0	5.24[c]	0.18[d]
	4.0	23.39[b]	2.26[c]
	5.0	29.28[a]	2.72[b]
	6.0	29.42[a]	3.76[a]
Formula	100% CSPu	21.55	2.21
	100% CSPe	21.65	2.31
	37.5% CSPu+25% CSC, 37.5% CSPe	21.83	2.23
% Urea addition	0.0	5.92[d]	0.14[d]
	4.0	23.38[c]	2.32[c]
	5.0	27.72[b]	2.90[b]
	6.0	29.69[a]	3.64[a]
P-value			
Formula		ns	ns
% Urea addition		<0.0001	<0.0001
Formula*% Urea addition		ns	ns

CSPu, cassava pulp; CSPe, cassava peel; CSC, cassava chip
[a,b,c,d] Means within the same column within formula having different letters are different at P<0.05.

proliferation of the fungi/yeast complex in the form of single cell proteins may possibly account for the apparent increase in the CP content of the cassava products. Similarly, Iyayi and Losel (2001b) found increases in CP content of the cassava products due to fermentation by *A. niger* or *S. cerevisiae*. The yeast demonstrated the better ability to enrich the products than the fungi. According to Wainright (1992), fermentation of cereals leads to improvement in protein content and reported that fermenting corn meals with the yeast *S. cerevisiae* and *Candida tropicalis* increased the protein content of the products and that the protein content could be further increased by adding malt extract to the meals. Similar studies by Essers (1994) showed the ability of fungi and yeast to enrich the protein of cassava products. Although good growth of fungi and yeast can be obtained without adding nitrogen, protein production was markedly increased with the addition of nitrogen sources. This means that the medium itself does not contain enough assimilated nitrogen for the microorganisms to be able to grow and consume the abundant carbohydrate. The present study has revealed that urea worked as the nitrogen source for the growth of fungi and yeast. Results obtained from this study show that supplementing cassava products with urea before fermenting enhances the growth of the microorganisms and leads to greater cell mass production and consequently more crude protein formation. Antai and Mbongo (1994) reported similar results when they worked on cassava peel.

Urea residues after fermentation of cassava products by *A. oryzae* and *S. cerevisiae* are shown in Table 2 and Table 3. Urea residues significantly increased with increased addition of urea in the products in both experiments. In addition, in Experiment II, urea residues were the highest in 100% CSPe and 75% CSPu+25% CSC, followed by 100% CSPe while 37.5% CSPu, 25% CSC, and 37.5% CSPe showed the lowest urea residue. In Experiment III, there were no significant differences in urea addition between the cassava products. The reason for lower urea residue is that the microorganisms use up the urea for their growth which resulted in a decreased amount of urea in the products. In contrast, the higher urea residue can be attributable to lower rate of utilization of urea by the microorganisms probably due to a lower carbon source for them to grow.

The increases in protein content were due to the effects of microbial cell growth process (Belewu & Babalola, 2009) and N source from urea. Although the research literature on cassava pulp or peel fermentation with microorganisms are not sufficiently available, a lot of information of fermented cassava has been widely reported. Chumkhunthod *et al*. (2001) reported that cassava root fermented with *C. utilis* could increase CP up to 18.3%. In the present study, the highest average level of CP produced from *A. oryzae* and *S. cerevesiae* was 48.59% at 10.0% urea (Experiment II) and 29.69% at 6.0% urea (Experiment III). However, this protein enhancement was included with a part of N from urea which is considered as a non-protein N. When consideration has been made from several factors (e.g., level of CP and urea residue) the optimum level of urea addition was at 4.0% from Experiment III because the average urea residue was at 2.32% and the average CP was 23.38%. This level of urea residue is considered to be safe for ruminant animals.

A. oryzae and *S. cerevisiae* appeared to be efficient by improving nutrient content of cassava pulp and this could be attributed to the ability of *A. oryzae* to secrete cellulase and amylase enzymes into the cassava pulp during the fermentation process in an attempt to make use of the cassava starch as a carbon source. Apart from this, the increase in the amount of the microbial biomass in the form of single-cell proteins may possibly account for the increase in the protein content of the *A. oryzae* and *S. cerevisiae* fermented cassava products (Akindahunsi *et al*., 1999).

3.3 Experiment IV

The chemical composition of ECP, concentrate, and rice straw used in this experiment are shown in Table 4. The percentages of CP content of the ECP and concentrate were 23.0 and 21.8%, respectively, which were comparable to replace each other. Apart from the CP content, the NDF, ADF, and ADL were higher in the ECP than in the concentrate while the concentrate contained higher crude fat than the ECP. The rice straw is considered to be low quality roughage containing high crude fiber, NDF, ADF, and ADL but low in CP and energy. The present experiment was designed to allow the cows to consume all of the feeds offered; therefore, the DM, CP, and net energy intakes were similar in all treatments (Table 5).

Table 4. Chemical composition of cassava peel, concentrate and rice straw (mean±SD).

	Ensiled cassava peel	21% CP concentrate	Rice straw
% dry matter (DM)	94.32±0.01	92.17±0.01	92.08±0.01
		% of DM	
Crude protein (CP)	23.02±0.24	21.83±0.09	1.34±0.02
Crude fat	2.65±0.03	4.94±0.25	1.54±0.11
Ash	13.73±0.15	12.45±0.12	15.84±0.24
Crude fiber	18.95±0.23	15.72±0.12	34.92±0.21
Neutral detergent fiber	54.33±0.04	36.58±0.08	73.58±0.04
Acid detergent fiber	45.39±0.14	21.89±0.21	59.16±0.15
Acid detergent lignin	9.38±0.02	6.21±0.04	10.44±0.03
Neutral detergent insoluble nitrogen	0.35±0.02	0.96±0.01	0.15±0.01
Acid detergent insoluble nitrogen	0.18±0.01	0.21±0.01	0.16±0.01
TDN_{1X}; %			
DE_P; Mcal/kg DM			
ME_P; Mcal/kg	45.50	65.45	37.76
DM	2.36	2.67	1.89
NE_{LP}; Mcal/kg	1.94	2.25	1.46
DM	1.17	1.39	0.84

[1]TDN_{1x} (%) = tdNFC + tdCP = (tdFA x 25.25) + tdNDF – 7)
DE_{1x}= ((tdNFC/100) x 4.2) + ((tdNDF/100) x 4.2) x ((tdCP/100) x 5.6 + ((FA/100) x 9.4) – 0.3
[2]DE_P (Mcal/kg) = (((TDN$_{1x}$ – ((0.18 x TDN$_{1x}$) – 10.3)) x Intake)/ TDN$_{1x}$) x DE$_{1x}$
[3]ME_P (Mcal/kg) = (1.01 x (DE_p) – 0.45) + (0.0046 x (EE-3))
[4]NE_{LP} (Mcal/kg) = (0.703 x ME_P) – 0.19, (EE >3 %)
[4]NE_{LP} (Mcal/kg) = (0.703 x ME_P) – 0.19) + ((0.097 x ME_P)/97) x (EE-30), (EE >3%)

Table 5. Effect of ensiled cassava peel as replacement for concentrate on feed intake.

Intake	Control	20% ECP	40% ECP
DM (kg)			
Ensiled cassava peel	0.00	0.75	1.50
Concentrate	3.68	2.94	2.21
Rice straw	5.52	5.52	5.52
Total	9.20	9.22	9.23
CP (g/d)			
Ensiled cassava peel	0.00	173	346
Concentrate	803	643	482
Rice straw	74	74	74
Total	877	890	902
NE (Mcal/d)			
Ensiled cassava peel	0.00	0.82	1.64
Concentrate	5.35	4.28	3.21
Rice straw	5.05	5.05	5.05
Total	11.90	11.65	11.40

ECP, ensiled cassava peel

The pH in the rumen fluid at 0, 3, and 6 h after feeding (Table 6) ranged from 6.54 to 6.83 and was affected by diets at 3 and 6 h after feeding. At 0 h, the ruminal pH was similar; however, the ruminal pH of 40% ECP cows was significantly higher (P=0.01) than the control and 20% ECP cows at 3 h after feeding while the ruminal pH of 20% ECP cows was higher (P=0.01) than 40% ECP cows but did not differ from the control cows at 6 h after feeding. The higher pH in the ruminal fluid of ECP cows reflected the urea residue in ECP products. However, the ruminal pH in all treatments was within the range of 5.80-7.00 below which the rumen function might be negatively affected according to de Veth and Kolver (2001). Khampa *et al.* (2009) also observed higher ruminal pH when feeding diets contained yeast, fermented cassava peel, and pulp.

The concentration of ammonia-N in the rumen fluid was unaffected (P>0.05) by the dietary treatments (Table 6). Ammonia-N is the product of ruminal fermentation of dietary CP, microbial protein, and non-protein nitrogen. A variation in ammonia-N concentration is dependent on the level of feeding, solubility of dietary protein, available carbohydrate, and frequency of feeding Seo *et al.* (2012). Satter and Slyter (1974) suggested that optimum ammonia-N concentration promoting highest microbial growth and DM degradability was between 50 and 80 mg/l. The present study found lower values at 0 and 6 h after feeding and within the normal range at 3 h after feeding compared to Satter and Slyter (1974).

Table 6. Effect of ensiled cassava peel as replacement for concentrate on pH, ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA) at specified hours after feeding.

Post feeding	Control	20%ECP	40%ECP	SEM	P-value
pH					
Hour 0	6.83	6.82	6.74	0.031	0.15
Hour 3	6.54^{b}	6.56^{b}	6.65^{a}	0.008	0.01
Hour 6	6.78^{ab}	6.80^{a}	6.69^{b}	0.009	0.01
NH_3-N (mg/l)					
Hour 0	38.19	39.43	42.17	1.416	0.78
Hour 3	50.19	48.47	53.31	1.570	0.43
Hour 6	39.42	31.88	36.53	2.526	0.69
Acetate; C2 (mol/100 mol).					
Hour 0	66.27^{b}	73.18^{a}	68.93^{b}	0.868	0.04
Hour 3	65.68^{b}	70.59^{a}	68.91^{a}	1.020	0.04
Hour 6	64.80	69.27	69.89	1.622	0.49
Propionate; C3 (mol/100 mol)					
Hour 0	24.59^{a}	16.03^{c}	19.40^{b}	0.881	0.03
Hour 3	25.78^{a}	18.42^{b}	18.75^{b}	0.941	0.03
Hour 6	23.60^{a}	18.89^{b}	19.59^{b}	0.937	0.05
Butyrate; C4 (mol/100 mol)					
Hour 0	9.14^{b}	10.79^{a}	11.67^{a}	0.306	0.01
Hour 3	8.54^{c}	11.32^{b}	12.33^{a}	0.300	0.01
Hour 6	11.52	11.84	10.52	0.444	0.42
C2:C3					
Hour 0	2.7^{c}	4.6^{a}	3.6^{b}	0.102	0.01
Hour 3	2.6^{b}	3.9^{a}	3.7^{a}	0.156	0.03
Hour 6	2.8^{b}	3.7^{a}	3.6^{a}	0.105	0.02

SEM, standard error of the mean; ECP, ensiled cassava peel
[a,b] Means within a row with different superscripts are significantly different (P<0.05).

VFAs are the end-products of carbohydrate fermentation in the rumen. At 0 h before feeding, 20% ECP increased ($P<0.05$) the molar proportion of acetic acid compared to the control and 40% ECP, while at 3 h after feeding, both 20 and 40% ECP increased ($P<0.05$) the molar proportion of acetic acid compared to control. At 6 h after feeding, the molar proportion of acetic acid was unchanged (Table 6). The molar proportion of propionic acid decreased ($P<0.05$) by both 20 and 40% ECP at all times of sampling (Table 6). Both 20 and 40% ECP increased the molar proportion of butyric acid at 0 and 6 h post-feeding, whereas at 6 h post-feeding the molar proportion of butyric acid was similar (Table 6). The proportions of acetic acid to propionic acid increased ($P<0.05$) by dietary treatments at all times after feeding (Table 6).

The reduction in molar proportion of propionic and the increases in molar proportions of acetic and butyric acids can be explained by the fact that ECP diets contained higher fiber but lower amounts of carbohydrates than the control diet. Fermentation of fiber in the rumen yields acetic and butyric acids while fermentation of carbohydrate yields propionic acid (Allen, 1997) resulting in higher acetic and butyric acids but lower propionic acid in ECP dietary treatments.

4. Conclusions

Based on these experiments, it can be concluded that fermentation of cassava products using *A. oryzae* increased the amounts of reducing sugar and fermentation of cassava products using *A. oryzae* and *S. cerevisiae* increased CP content. In addition, replacing the concentrate with 20 and 40% ECP had no effect on ruminal concentrations of ammonia-N; however, the ECP increased the ruminal pH, and the levels of acetic and butyric acids increased while the level of propionic acid reduced which resulted in increases in the acetic to propionic acid ratio.

Acknowledgements

This work was supported by the National Research Council of Thailand. The authors would also like to express the sincere thanks to the University's Farm and the Center for Scientific and Technological Equipment, Suranaree University of Technology for providing the facilities. Financial support was provided by the Institute of Research and Development, Suranaree University of Technology.

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