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Protein Enrichment of Cassava Pulp Fermentation by *Saccharomyces cerevisiae*

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Abstract: The purpose of this study was to determine intestinal digestibility of residual components of cassava pulp solid state fermentation by *Saccharomyces cerevisiae* for animal feed. Three ruminally cannulated animal were used to measure *in situ* rumen Dry Matter (DM) and Crude Protein (CP) degradability characteristics of cassava pulp solid state fermentation by *S. cerevisiae*. Nylon bags containing 3 g (as fed basis) of each feed was immersed in duplicate at each time point in the ventral rumen of each goat for 2, 4, 8, 12, 24, 48 and 72 h. Rumen feed residues from bags of 16 h incubation were used for estimation of lower gut digestibility by the technique of *in vitro* pepsin-pancreatin digestion. The results of the chemical analysis indicated that fermentation was slightly improved Ruminal Undegradable Protein (RUP) of cassava pulp. The highest value of RUP was significantly differ ($p<0.05$) after 5 days of fermentation period. Ruminal undegradable protein content increased ($p<0.05$) with the addition of *S. cerevisiae* in cassava pulp. The present results indicate that fermented cassava pulp can improve protein content and ruminal undegradable protein content.

Key words: Cassava pulp, enrichment, fermentation, *Saccharomyces cerevisiae*, digostion, Thailand

INTRODUCTION

One of the most important problems in animal husbandry is that the animals could not be fed adequately (Saricicek and Kilic, 2004). Cassava or tapioca (*Manihot esculenta*, Crantz), a root crop is one of the major crop grown, especially in northeast of Thailand. In Thailand, cassava pulp is always sold as a cheap animal feed material in substitution of urea, yeast and other. Therefore, through the solid state fermentation, protein content in the cassava pulp can be increased that can lower the cost of animal feed. Cassava pulp is fermented with yeast (*S. cerevisiae*) for protein enrichment before it is used as the high quality animal feed material (Srinorakutara *et al*., 2006; Oboh and Akindahunsi, 2003; Ubalua, 2007). Estimations of intestinal digestibility of rumen undegraded protein of feeds are critical in the application of protein evaluation systems for ruminants (Faria-Marmol *et al*., 2002).

The rate and extent of protein degradation in the rumen is very crucial as it determines the availability of nitrogen to microorganisms and amino acids in the small intestine to the host animals. The protein consumed by the ruminant should be partly degradable in the rumen in to peptides, amino acids and NH_3-N derived from proteolysis to be used in microbial protein synthesis and to improved rumen ecology. It is therefore, very important to determine the degradability and digestion of different feed ingredients which are grown and used in different locations. Incubation of feeds in nylon bags in the rumen of cannulated ruminants have been used to determine the extent of rumen degradation of the feed protein (Orskov and McDonald, 1979; Rao and Prasad, 1989; Islam *et al*., 2002). The feed N which escapes rumen degradation and digestibility can be further measured by a three-step *in vitro* procedure (Calsamiglia and Stern, 1995, Kamalak *et al*., 2005).

Therefore, the objective of this study was to investigate the effect of the inoculants, *S. cerevisiae* separately and in combined application on changes in fermentation quality and nutrient composition with duration of storage of dry cassava pulp.

MATERIALS AND METHODS

Experimental design and treatments: The experiment was taken according to 4×4 factorial arrangements in Complete Randomized Design (CRD). Factor A were the level of

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S. cerevisiae (0, 0.5, 2.5, 5 g) and factor B were the times of fermentation (0, 1, 3 and 5 days). The dietary treatments were as follows:

T1 = *S. cerevisiae* at 0% fermented cassava pulp at time 0 day
T2 = *S. cerevisiae* at 0% fermented cassava pulp at time 1st day
T3 = *S. cerevisiae* at 0% fermented cassava pulp at time 3rd day
T4 = *S. cerevisiae* at 0% fermented cassava pulp at time 5th day
T5 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 0 day
T6 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 1st day
T7 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 3rd day
T8 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 5th day
T9 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 0 day
T10 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 1st day
T11 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 3rd day
T12 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 5th day
T13 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 0 day
T14 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 1st day
T15 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 3rd day
T16 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 5th day

Sample preparation: Cassava pulp was collected from the factory of cassava starch production in Nakhon Ratchasima province, Thailand. It was dried in hot air oven at 60°C for 48 h or until it was dried completely before performing the experiment. The yeast culture used in this experiment contains as the effective agent living non-pathogenic yeast of the *S. cerevisiae* in the minimum amount of 1×10^{13} cfu g^{-1}.

About 1 kg of cassava pulp is used for fermentation. The moisture content of cassava pulp was adjusted to 50% by adding 10% urea and 1.25% molasses. Three samples were prepared by mixing cassava pulp with 0, 0.5, 2.5 and 5.0% of *S. cerevisiae*. A control sample contained no *S. cerevisiae*. The above samples were incubated for 0, 1, 3 and 5 days, dried, ground through 2 mm sieve stored pending chemical analysis and mobile bags studies.

Animals and ruminal degradability: Three, ruminally cannulated growing goats with an average weigh of 15±2.5 kg and 8-10 months of age were used to determine ruminal degradability and intestinal digestibility of fermented cassava pulp. Each animals fitted permanent rumen cannulae were kept individual pens (0.9×1.4 m). The animals were fed a maintenance concentration diet and roughage (rice straw). The daily feed was offered in two equal portions, one at 08.30 a.m. and the other at 04.30 p.m. Drinking water was freely available.

Dry Matter (DM), Organic Matter (OM) and Crude Protein (CP) degradation rate in the rumen determined in the three meat goats. Nylon bags (4×7 cm) made from polyester cloth with average pore sizes of 45 μm. Orskov and McDonald (1979) were each filled with approximately 5 g of a test sample. All samples were prepared in triplicates and incubated in the rumen of each animal for 2, 4, 8, 12, 24, 48 and 72 h.

After removal from the rumen, bags were doused with clean water to halt fermentation and were rinsed until wash water ran relatively clear. Bags were stored at <0°C to a wait further processing. Once removed from storage, bags were thawed to room temperature and washed in a domestic washing machine until the wash water ran completely clear.

Samples were air-dried in a 60°C convection oven to a constant mass then air-equilibrated and weighed to determine residue mass. Residues were then removed and composited by duplicates within goat. Bag residue and original forage samples were ground to pass through a 1 mm screen and subsequently analyzed for DM, OM and CP according to the procedure of AOAC (1990), analyzed NDF and ADF. The bags were weighed and measured according to Orskov and McDonald (1979).

***In vitro* pepsin-pancreatin digestion procedure:** Samples of the feed residue from nylon bags at 16 h incubation time, the bags were removed from the rumen and were immediately washed with cold tap water until clear and dried in a forced air oven at 60°C for 48 h after determining N content were put into a 50 mL centrifugation tube in quantities equivalent to 15 mg of N. About 10 mL of a 0.1 N HCl solution (pH 1.9), containing 1 g L^{-1} of pepsin (sigma P-7012, sigma) were added and the samples incubated for 1 h in a 38°C shaker water bath. After

incubation, 0.5 mL of a 1 N NaOH solution and 13.5 mL of a pancreatin solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g L^{-1} of pancreatin (sigma P-7545, sigma) were added. The samples were incubated at 38°C for 24 h in a shaker water bath and mixed (magnetic stirrer) every 8 h. After incubation, 3 mL of a 100% (wt. vol^{-1}) solution of TCA were added to the tubes to stop enzymatic action and to precipitate undigested proteins. All tubes were mixed and allowed to stand for 15 min. The samples were centrifuged at 10,000×g for 15 min and the supernatant was analyzed for soluble N by the Kjeldahl method (AOAC, 1990). Pepsin-pancreatin digestion of protein was calculated as TCA-soluble N divided by amount of sample N (nylon bag residue) used in the assay (Calsamiglia and Stern, 1995).

Sample analysis: The nutritional composition of *S. cerevisiae* fermented cassava pulp product was evaluated using the protein content of Crude Protein (CP), Non-Protein Nitrogen (NPN), true protein and feed residues from bags after the 16 h incubation using Kjeldahl method.

The supernatant was analyzed for soluble N by Kjeldahl method (AOAC, 1990). All samples were analyzed for DM, Ash and CP according to AOAC (1990). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were determined by using the method of Goering and Van Soest.

Data analysis: Data for ruminal and intestinal degradation of DM and CP were fitted to the exponential equation following procedure described by Orskov and McDonald (1979):

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

Where:

p = Disappearance rate at time t
a = As intercept representing the portion of DM or CP solulized at initiation of incubation (time 0)
b = The fraction of DM or CP potentially degradable in the rumen
c = A rate constant of disappearance of fraction b
t = Time of incubation

The non-linear parameters a-c are estimated by an iterative least squares procedure. The Effective Degradability of DM (EDDM) or of CP (EDCP) were calculated using the following equation:

$$EDDM \text{ or } EDCP = a+(bc/(c+k))$$

Where, k was the estimate rate of out flow from the rumen (0.05 h^{-1}). Calculations as described by Subuh *et al.* (1996) were used for ruminal, post-ruminal and total tract protein disappearance using the nylon bag technique. Data of three step procedure were calculated as described by Calsamiglia and Stern (1995). Crude protein degradability was calculated as a percent of total CP:

$$\text{CP (\%) degradability} = \left[\frac{\text{(Initial CP} - \text{Post incubate CP)}}{\text{Initial CP}}\right]\times 100$$

Statistical analysis: Data of *in vitro* three step procedure was calculated as described by McNiven *et al.* (2002). Data were analyzed by SAS (1985). The statistical analysis of all data *in vitro* procedure was made according to the following model:

$$Y_{ij} = \mu+\delta_{ij}+\varepsilon_{ij}$$

Where:

Y_{ij} = The criteria under study
μ = Overall mean
δ_{ij} = Feed source effect (or treatment of roughage diet)
ε_{ij} = Residual

RESULTS AND DISCUSSION

Chemical composition of feed sources: The chemical compositions of fermented cassava pulp with different level of yeast (*S. cerevisiae*) were shown in Table 1. Generally, wide variations existed in the chemical composition of investigated feedstuffs. The protein content gradually augmented with time because the yeast converts nitrogen source to protein. The highest of crude protein and true protein were 26.4 and 24.7% DM, respectively in the fermented cassava pulp with 5% *S. cerevisiae* at 5th day period.

The chemical composition of the fermented cassava pulp by yeast studied is shown in Table 1. The crude protein content of the fermented cassava pulp by yeast products showed the concentrations of true protein and NPN were numerically lower than in soybean meal (Borucki *et al*., 2007).

Cassava waste from starch industry utilized as animal feed fermentation with *Rhizopus and Rhizopus* sp., 26R. The protein was enriched to 24% after the fermentation (Putipipatkajon and Srinophakun, 1999).

Rumen disappearances: The Rumen Undegradable Protein (RUDP) was determined by the *in vitro* nylon bag technique. Table 2-5 showed the *in vitro* intestinal digestion of feeds. RUDP were increased with the

Table 1: Chemical composition of fermented cassava pulp with different level of yeast (*S. cerevisiae*) (dry matter basis (%))

	Chemical compositions (DM% basis)							
Day to fermentation	DM	CP	Ash	NDF	ADF	ADL	TCP	NPN
Yeast (0 g) (days)								
0	97.10	9.20	3.90	31.60	19.00	5.00	6.10	3.10
1	97.10	14.70	3.60	28.20	18.70	3.10	13.00	1.70
3	97.90	19.40	3.40	27.60	18.90	3.30	17.80	1.60
5	97.90	13.10	3.20	27.30	19.20	5.20	11.10	2.00
Yeast (0.5 g) (days)								
0	97.20	17.70	3.60	27.80	19.10	4.40	15.30	2.40
1	98.00	16.10	3.70	31.90	18.90	3.10	14.10	2.00
3	97.40	20.10	2.80	26.90	18.50	3.10	18.20	1.90
5	97.10	21.50	3.90	25.10	19.10	3.50	19.10	2.40
Yeast (2.5 g) (days)								
0	97.60	22.50	3.40	24.60	18.20	3.00	20.40	2.10
1	96.60	18.10	3.80	29.00	17.80	3.80	16.50	1.60
3	96.80	20.00	3.10	25.90	17.10	3.00	18.30	1.70
5	96.70	21.70	3.60	26.00	19.10	3.30	19.90	1.80
Yeast (5.0 g) (days)								
0	98.70	14.50	3.40	26.70	19.10	3.70	11.60	2.90
1	98.60	21.50	3.30	26.90	19.00	4.30	19.50	2.00
3	98.70	21.20	4.40	28.00	18.60	3.70	19.60	1.60
5	98.00	26.40	3.70	25.50	19.40	4.80	24.70	1.70
SEM	0.12	1.00	0.06	0.36	0.10	0.17	1.04	0.08
p-values								
Yeast	0.05	0.16	0.35	0.21	0.15	0.48	0.16	0.02
Day	0.97	0.85	0.93	0.99	0.41	0.56	0.88	0.23
Yeast x day	0.98	0.95	1.00	0.92	0.44	0.43	0.97	0.07

DM = Dry Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin, TCP = True Protein and NPN = Non Protein Nitrogen

Table 2: Percentage of DM, disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats

	g yeast kg^{-1} fermented cassava pulp							
	Y (0 g)				Y (0.5 g)			
Items	D0	D1	D3	D5	D0	D1	D3	D5
CP disappearance (%)								
a	4.80	9.70	12.20	12.60	10.30	15.50	17.10	14.70
b	74.20	90.30	87.80	82.50	89.70	84.50	82.90	77.70
c	0.04	0.02	0.03	0.03	0.02	0.02	0.03	0.03
a+b	79.00	100.00	100.00	95.10	100.00	100.00	100.00	92.40
Effective degradability (%)								
0.05	39.20	35.40	42.70	42.00	38.90	38.20	46.10	44.30
0.08	30.80	27.70	34.10	33.80	30.60	31.30	37.90	36.30

	g yeast kg^{-1} fermented cassava pulp											
	Y (2.5 g)				Y (5 g)					p-values		
Items	D0	D1	D3	D5	D0	D1	D3	D5	SEM	Y	D	Y×D
CP disappearance (%)												
a	13.40	13.20	17.30	17.90	7.40	10.30	17.80	11.40	0.70	0.10	0.96	0.65
b	80.10	68.40	82.70	81.40	92.60	79.20	82.20	74.40	1.10	0.10	0.31	0.89
c	0.03	0.05	0.03	0.02	0.02	0.04	0.02	0.03	0.00	0.25	0.92	0.97
a+b	93.50	81.60	100.00	99.20	100.00	89.50	100.00	85.70	1.30	0.00	0.53	0.77
Effective degradability (%)												
0.05	41.30	46.40	45.40	44.80	35.90	47.70	43.40	36.30	0.70	0.40	1.00	0.79
0.08	33.50	38.60	37.50	37.10	27.50	38.70	35.90	29.10	0.70	0.30	0.98	0.70

a = as intercept representing the portion of DM or CP solulized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, DM = Dry Matter, Y = Yeast, D = Day

increased the incubation time for all treatments. In this experiment, cassava chip was a very fine dusty particle which would be lost easily from the bag in the rumen. The result was in agreement with Cone *et al.* (2002) who found that source of starch had effect on degradability in rumens. The values of CP intestinal digestibility of

Table 3: Percentage of OM disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats

	g yeast kg^{-1} fermented cassava pulp											
	Y (0 g)				Y (0.5 g)							
Items	D0	D1	D3	D5	D0	D1	D3	D5				
CP disappearance (%)												
a	14.90	19.60	23.40	18.00	19.70	23.30	22.70	18.80				
b	67.20	80.40	71.40	82.00	80.30	63.40	62.20	72.50				
c	0.04	0.02	0.03	0.02	0.02	0.03	0.05	0.05				
a+b	82.10	100.00	94.80	100.00	100.00	86.70	84.90	91.30				
Effective degradability (%)												
0.05	45.80	45.50	48.70	43.40	42.90	45.20	52.30	53.30				
0.08	38.20	38.10	41.60	36.00	36.00	39.10	45.20	45.10				
	g yeast kg^{-1} fermented cassava pulp											
	Y (2.5 g)				Y (5 g)					p-values		
Items	D0	D1	D3	D5	D0	D1	D3	D5	SEM	Y	D	Y×D
CP disappearance (%)												
a	22.00	26.80	27.30	26.80	22.10	23.80	28.00	21.50	0.60	0.50	1.00	0.50
b	78.00	73.20	72.70	73.20	73.40	69.00	71.80	78.50	1.00	0.40	0.80	0.20
c	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.01	0.37	0.84	0.97
a+b	100.00	100.00	100.00	100.00	95.60	92.80	99.80	100.00	1.10	0.40	0.90	0.70
Effective degradability (%)												
0.05	49.10	52.90	52.30	49.80	48.90	50.90	51.70	43.00	0.60	0.60	0.90	0.90
0.08	41.50	45.60	45.20	43.10	41.60	43.70	44.90	36.50	0.60	0.60	0.90	0.80

Table 4: Percentage of CP disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats

	g yeast kg^{-1} fermented cassava pulp											
	Y (0 g)				Y (0.5 g)							
Items	D0	D1	D3	D5	D0	D1	D3	D5				
CP disappearance (%)												
a	43.80	32.10	39.00	4.40	49.40	41.50	-8.60	28.60				
b	40.30	28.80	35.00	1.00	47.10	38.70	-13.10	27.00				
c	0.02	0.02	0.02	0.04	0.02	0.02	0.01	0.02				
a+b	84.10	60.90	74.10	5.50	96.50	80.20	-21.70	55.60				
Effective degradability (%)												
0.05	45.30	33.20	40.40	4.90	51.20	43.00	-10.20	29.60				
0.08	44.80	32.80	39.90	4.80	50.60	42.40	-9.60	29.20				
	g yeast kg^{-1} fermented cassava pulp											
	Y (2.5 g)				Y (5 g)					p-values		
Items	D0	D1	D3	D5	D0	D1	D3	D5	SEM	Y	D	Y×D
CP disappearance (%)												
a	44.60	16.60	24.50	13.40	35.80	5.30	52.90	17.00	3.10	0.40	0.2	1.0
b	43.60	14.90	23.10	11.80	33.70	3.80	51.00	13.50	3.20	0.40	0.2	1.0
c	0.02	0.02	0.03	0.02	0.02	0.03	0.01	0.02	0.02	0.70	1.0	0.8
a+b	88.20	31.50	47.60	25.20	69.50	9.10	103.90	30.50	6.30	0.40	0.2	1.0
Effective degradability (%)												
0.05	46.30	17.40	25.40	14.00	37.10	5.50	53.90	17.50	4.00	0.30	0.3	1.0
0.08	45.70	17.10	25.00	13.80	36.60	5.50	53.50	17.30	3.80	0.30	0.3	1.0

a = as intercept representing the portion of DM or CP solulized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, CP = Crude Protein, Y = Yeast, D = Day

fermented cassava pulp with *S. cerevisiae* were somewhat higher, however the observed differences between treatments were not significant ($p<0.05$).

Incubation of bags in the rumen before insertion into the duodenum increased intestinal undegradable CP (Stern *et al*., 1997; De Boer *et al*., 1987). In the *in vitro* enzymatic technique or three step technique, there were also numerous factors influencing the value, e.g., the time of incubation (Cone *et al*., 2002), group of feedstuffs (Tomankova and Kopecny, 1995), variety of enzyme (Roe *et al*., 1991), pH of buffer and enzyme concentration (Licitraa *et al*., 1999).

Table 5: CP disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) in rumen and intestine of meat goats at 16 h incubated time

	CP disappearance (%)		
Day to fermentation	Rumen	Intestine	Total
Yeast (0 g)			
0	52.90	39.00	91.90
1	25.70	55.20	80.80
3	18.30	39.30	57.60
5	44.40	42.30	86.70
Yeast (0.5 g)			
0	24.40	40.00	64.40
1	20.60	51.20	71.80
3	12.80	32.40	45.20
5	15.50	43.90	59.40
Yeast (2.5 g)			
0	18.50	54.30	72.90
1	17.00	14.60	31.60
3	9.70	43.90	53.60
5	13.80	39.90	53.70
Yeast (5.0 g)			
0	31.50	30.50	62.10
1	8.60	19.90	28.50
3	10.80	39.90	50.70
5	14.80	54.50	69.30
SEM	2.16	2.18	3.59
p-values			
Yeast	0.16	0.49	0.31
Day	0.96	0.97	0.94
Yeast x day	0.57	0.64	0.51

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein

CONCLUSION

The results obtained from this experiment could have a great impact on animal feed especially using local resources-based diets. The present results indicate that fermentation of cassava pulp by yeast can improve CP content and rumen undegradable protein. This method could be more useful for routine feed evaluation without the need for a rumen cannulated animal.

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