INTESTINAL DIGESTIBILITY OF THE RESIDUAL COMPONENTS OF CASSAVA PULP SOLID STATE 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 Holstein cows were used to determine the *in situ* degradability of cassava pulp solid state fermentation by *Saccharomyces cerevisiae*. Thirty-six sample bags of fermented cassava pulp were incubated in the rumen for 16 h to determine the *in vitro* intestinal digestibility. 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 *Saccharomyces cerevisiae* in cassava pulp. The present results indicate that fermented cassava pulp can improved, protein content and ruminal undegradable protein content.

Keywords: cassava pulp, intestinal digestibility, three-step procedure, residual *Saccharomyces cerevisiae*,

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

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(*Saccharomyces cerevisiae*) for protein enrichment before it is used as the high quality animal feed material (Oboh and Akindahunsi, 2005; Srinorakutara *et al*., 2006; Ubalua, 2007). Estimations of intestinal digestibility of rumen undegraded protein of feeds are critical in the application of protein evaluation systems for ruminants (Faría-Mármol *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, into peptides, amino acids and NH_3-N derived from proteolysis to be used in microbial protein synthesis and to impove 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 (Ørskov 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 contribute to the knowledge of degradation characteristics and lower-gut digestibility of different protein sources from cassava pulp fermentation by *S.cerevisiae* in ruminants fed by low quality roughages, since most of ruminant production in the tropics was based on low quality roughages.

Materials and Methods

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* species, strain SC-47 (NCYC) in the minimum amount of 1×10^{13} CFU/1 g.

About 1 kg of cassava pulp is used for fermentation. The moisture content of cassava pulp was adjusted to 50% by adding 10% urea, 1.25% molasses. Three samples were prepared by mixing cassava pulp with 0, 0.5, 2.5, and 5% of *S. cerevisiae*. A control sample contained no *S. cerevisiae*. The samples were incubated for 1, 3 and 5 days, dried, grounded and subsequently analyzed.

Animals and Ruminal Degradability Study

Three, ruminally fistulated Holstein Frisian dairy cows with an average weigh of 320 ±15 kg and 4-5 years old were used to determine ruminally degradability and intestinal digestibility of cassava pulp fermented yeast.

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 72 h, after determining N content, were put into a 50 ml centrifugation tube in quantities equivalent to 15 mg of N. 10 ml of a 0.1 N HCl solution (pH 1.9), containing 1 g/litre 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/litre 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) 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 $10000 \times g$ 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 (AOAC, 1990). The supernatant was analysed for soluble N by Kjeldahl method (AOAC, 1990).

Data Analysis

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:

%CP degradability = [(initial CP - post incubate CP)/initial CP] × 100.

Statistical Analysis

Data of *in vitro* three-step procedure was calculated as described by McNiven *et al*. (2002). Data were analyzed by SAS (1998). The statistical analysis of data three-step *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

The chemical composition of the cassava pulp fermented yeast studied is presented in Table 1. The CP content of the cassava pulp fermented by yeast products showed the concentrations of true protein and NPN were numerically lower than in soybean meal (Borucki Castro *et al*., 2007). Cassava waste from starch industry utilized as

Table 1. The chemical composition of cassava pulp of fermented by *S. cerevisiae*

Yeast (%)	Fermentation time (days)	Crude protein (%DM)	True protein (%DM)	Non protein nitrogen (%DM)
0	1	14.64 [a]	13.01	1.63
	3	19.39 [b]	17.80	1.59
	5	13.13 [a]	11.11	2.02
0.5	1	16.07 [a]	14.06	2.01
	3	20.38 [bc]	18.41	1.97
	5	21.50 [c]	19.55	1.95
2.5	1	18.14 [b]	16.49	1.65
	3	19.98 [bc]	18.32	1.66
	5	21.66 [c]	19.88	1.78
5.0	1	21.46 [c]	19.72	1.74
	3	21.20 [c]	19.57	1.63
	5	26.34 [d]	24.74	1.60
SEM		0.716	0.726	0.048
Main effect	Yeast	*	ns	ns
	Day	*	ns	ns
	Yeast × day	*	ns	ns

Different letters within the same column mean values with different significance.
**p<0.05, ns: non significant difference.*

animal feed fermentation with *Rhizopus and Rhizopus sp*. 26R. The protein was enriched to 24% after the fermentation (Putipipatkajon and Srinophakun, 1999).

The protein content gradually augmented with time because the yeast converts nitrogen source to protein. The highest of crude protein and true protein were 26.34 and 24.74%DM respectively in the fermented cassava pulp with 5% *S. cerevisiae* at 5 day period.

The rumen undegradable protein (RUDP) was determined by the *in vitro* nylon bag technique. Table 2 showed the *in vitro* intestinal digestion of feeds. Rumen undegradable protein increased with the increased the incubation time for all treatments.

Incubation of bags in the rumen before insertion into the duodenum increased intestinal undegradable CP (de Boer *et al*., 1987; Stern *et al*., 1997). 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 (Licitra *et al*., 1999).

Conclusions

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. The further investigations are now undertaken in the same laboratory to confirm the above postulation. This method could be more useful for routine feed evaluation without the need for a rumen fistulated animal.

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Table 2. *In vitro* intestinal digestion of feeds

Yeast (%)	Fermentation time (days)	Intestinal digestibility (%)	Rumen undegradable protein (%)
0	1	43.55 ^{ab}	76.45 ^{a}
	3	43.72 ^{ab}	78.35 ^{a}
	5	44.01 ^{ab}	79.45 ^{ab}
0.5	1	43.26 ^{ab}	80.13 ^{ab}
	3	42.10 ^{a}	78.78 ^{a}
	5	42.50 ^{a}	77.92 ^{a}
2.5	1	42.95 ^{a}	79.67 ^{ab}
	3	44.18 ^{b}	82.34 ^{b}
	5	44.88 ^{b}	80.98 ^{ab}
5.0	1	42.52 ^{a}	82.55 ^{b}
	3	41.94 ^{a}	83.89 ^{bc}
	5	45.97 ^{b}	84.78 ^{c}
SEM		0.208	0.142
Main effect	Yeast	ns	*
	Day	*	*
	Yeast × day	ns	*

Different letters within the same column mean indicate significant difference ($p<0.05$)
** $p<0.05$, ns: non significant difference.*

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