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Using spontaneously fermented cassava pulp (SFCP) neglected in an open-pit as the energy source of fermented total mixed ration (FTMR) on the in vitro gas production technique

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Abstract The nutritional value of spontaneously fermented cassava pulp (SFCP) was investigated. Results showed that SFCP is rich in organic matter and low in crude protein. More importantly, SFCP is easily perishable because of its high moisture content. After digging it up, the SFCP from the pit must be mixed with the feed ingredients as a fermented total mixed ration (FTMR) to prevent perishment. The pH of FTMR remained around 3.37–4.12, whereas that of 1.0% of urea in the diet gradually increased to exceed 4.00. In vitro gas production has been conducted to determine the optimum level of the SFCP in the dietary feed. The fermentation of the insoluble FTMR was decreased, with a high level of SFCP replacement compared to the cassava chips ($P < 0.01$), while the fractional rates of gas production were not significantly different ($P > 0.05$). However, using cassava chips in FTMR had the highest gas production at 96 h after incubation ($P < 0.01$). Notably, total gas production at 96 h was only 8–10% less with the SFCP than with the cassava chips. Based on this investigation, SFCP is considered a valuable carbohydrate source for ruminants to make lower price feed.

Keywords: Cassava pulp, FTMR, In vitro gas production, SFCP, Urea

Introduction

The cassava flour factory in Vientiane had produced the cassava pulp using approximately 10-15% of the cassava root. Over the years, the cassava pulp had been neglected in the large open-pit (250 meters long, 50 meters wide, and about 7 meters deep). It contains about 100,000 m^3, equivalent to about 100,000 tons, of cassava pulp, which would be equivalent to about 0.5-2.3 million USD base on the price of wet cassava pulp, which varies from 5-23 USD/ton. Although the surface had rotted due to aerobic conditions, cassava pulp from 1.0-7m from the surface revealed that these were acceptably spontaneously fermented cassava pulp (SFCP) with pH values in the range of

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3.2-3.5 based on the epiphytic lactic acid bacteria that are present on cassava pulp and convert water-soluble carbohydrates into lactic acid in the ensiling process. As a consequence, the pH is reduced, and the feed is preserved under anaerobic conditions (Napasirth *et al*., 2015). Importantly, when the SFCP is exposed to air, aerobic spoilage will commence. After digging, the SFCP from the open-pit must be mixed with the feed ingredients as a fermented total mixed ration (FTMR) and re-fermented immediately to prevent perishment. FTMR has been widely used for ruminants because it combines roughage, energy, and protein sources, minerals, vitamins, and feed additives formulated into a single feed mix to satisfy the nutrient requirement of animals (Schingoethe, 2017). Commonly, urea has been widely used as non-protein nitrogen (NPN) source in ruminant diets in Laos because of the lower cost per unit of nitrogen. However, the addition of urea in the ensiling process has impacted the fermentation pH and it acts by preventing rapid pH declines, thereby, causing the growth of undesirable microorganisms. Therefore, this experiment aimed to use different percentages of the SFCP as an energy source in the FTMR, with different urea levels on chemical composition, and gas production kinetics.

Materials and methods

Sample collection

Collecting undisturbed SFCP samples for this experiment was conducted by using the PVC pipe (eight meters long and ten centimeters in diameter). The pipe was pushed vertically from the surface of the cassava pulp till the bottom of the pit. Then removes the cassava pulp one meter from the top of the pipe to eliminate the infected cassava pulp. The samples were collected according to the following procedure: Undisturbed SFCP samples were taken at 2, 3, 4, 5, 6 and 7 meters below the surface meters and then mixed. Seventy samples were collected at different sites around the open-pit. Three kilograms of the SFCP were prepared in this way and were taken to be examined further. The pH of the SFCP was immediately measured using a pH meter.

FTMR preparation and chemical analysis

The FTMRs were formulated (Table 1) and mixed using a small-scale system of silage fermentation (Cai *et al*., 1999) for the in vitro gas production technique to analyze the chemical composition of raw materials and the main changes that occur during the ensiling process. The samples of SFCP and FTMR were dried in a forced-air oven at 50 ℃ for 96 h and then ground to pass through a 1 mm screen. Dry matter (DM), crude protein (CP), and crude ash were analyzed according to methods of AOAC (1990). Cell wall components were analyzed by the methods of Goering and Van Soest (1970). Fermentation products of the SFCP silages and fermented feed were analyzed from cold water extracts. Ten grams of SFCP silage were homogenized with 90 ml of sterilized distilled water (Cai *et al*., 1999). The silage pH was measured with a glass electrode pH meter (MP230; Mettler Toledo, Greifensee, Switzerland).

In vitro gas production

The objective of the in vitro gas production technique was to determine the optimum inclusion level of the SFCP in dietary feed to maximize the total gas production and fermentation characteristics. This experimental design is completely randomized in a 3 × 3 factorial design, combined with 0, 50, and 100% of the SFCP and 0, 0.5, and 1% of urea (dry material basis) in the FTMR, with three replicates. Rumen fluid was collected from two Lao-native beef cattle fitted with permanent rumen cannulas. Ruminal contents were collected immediately before the morning feeding, mixed in equal volumes, and strained through two layers of cheesecloth into prewarmed thermos flasks flushed with CO_2 before using. Filtered rumen fluid was pooled and used as the source of inoculum. A total of 200 mg of samples were weighed and transferred to a 50 ml glass bottle sealed with a pharmaceutical butyl rubber stopper and aluminum cap, with seven replications per treatment plus seven empty bottles containing only the buffered rumen fluid, for a total of 64 bottles. The samples were each incubated with 30 ml buffered rumen inoculum in a sample glass bottle placed in a hot air oven maintained at 39 ℃. Total gas production was determined according to the modified procedure described by Makkar *et al*. (1995). During the incubation, gas production was recorded 14 times, at intervals between 2 and96 h during the period of fermentation. Accumulative gas production data were fitted to the model of Orskov and McDonal (1979) as follows:

$$y = a + b\,[1\text{-Exp}\,(-ct)]$$

where: a = the intercept, ideally reflecting the fermentation of the soluble fraction (ml); b = the fermentation of the insoluble (but with time fermentation, ml); c = the rate of gas production (ml/h.); d = |a| + b, t = incubation time; and y = the gas produced at time “*t*”.

Table 1. The chemical composition of the SFCP and FTMRs

	SFCP	SFCP 0%			SFCP 50%			SFCP 100%		
		0.0%	**0.5%**	**1.0%**	**0.0%**	**0.5%**	**1.0%**	**0.0%**	**0.5%**	**1.0%**
Feed ingredients, %										
Chopped Napier grass		30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wet brewers grains		26.00	25.50	25.00	26.00	25.50	25.00	26.00	25.50	25.00
Cassava chip		42.50	42.50	42.50	21.25	21.25	21.25	-	-	-
SFCP		-	-	-	21.25	21.25	21.25	42.50	42.50	42.50
Urea		-	0.5	1.0	-	0.5	1.0	-	0.5	1.0
Mineral mixed		0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt		0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Molasses		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sulfur		0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
pH	3.30	3.48	3.83	4.00	3.37	3.64	4.12	3.39	3.64	4.04
Chemical composition, % of DM										
DM (% as fed)	18.35	23.14	22.94	23.34	23.44	22.87	23.74	23.24	22.63	22.79
OM (% of DM)	97.64	98.78	98.47	98.55	98.43	98.61	98.86	98.65	98.79	98.83
CP (% of DM)	2.78	12.09	12.96	13.78	11.86	12.46	13.62	11.86	12.46	13.30
NDF (% of DM)	26.07	50.87	51.11	51.72	50.33	51.97	51.56	51.29	51.82	51.23
ADF (% of DM)	17.76	34.37	35.42	35.35	34.12	35.51	35.36	34.24	35.85	35.80
EE (% of DM)	1.82	3.89	4.32	3.96	4.01	4.17	4.05	3.97	4.23	4.05

SFCP = Spontaneously fermented cassava pulp.

Table 2. In vitro gas production kinetics and gas accumulation of the SFCP and FTMRs

Items	Gas production kinetics (0.2 g DM)				Gas volume at 24 h, ml	Gas volume at 48 h, ml	Gas volume at 96 h, ml
	a, ml	b, ml	c, ml/h	d, ml			
SFCP levels							
0.0%	3.37	103.18^{A}	0.04	107.57^{A}	69.52	92.34^{A}	103.80^{A}
50.0%	2.68	95.14^{B}	0.04	100.40^{B}	61.81	83.99^{B}	96.09^{B}
100.0%	1.69	94.69^{B}	0.03	98.67^{B}	59.45	81.18^{B}	93.64^{B}
Urea levels							
0.0%	1.25	97.12	0.04	101.69	61.38	83.82	96.95
0.5%	2.80	97.51	0.04	102.32	62.88	85.21	97.24
1.0%	3.68	98.38	0.04	102.63	66.53	88.49	99.34
SEM	1.11	1.48	0.16	1.32	2.10	1.66	1.25
P-value							
SFCP levels	0.78	0.01	0.59	<0.01	0.07	<0.01	<0.01
Urea levels	0.60	0.90	0.92	0.90	0.47	0.25	0.33
Interaction	0.34	0.36	0.29	0.15	0.18	0.20	0.36

^{ABC}Mean within columns with different superscript letters differ ($P < 0.01$).

Statistical analyses

The various data were subjected to the analyses of variance (ANOVA) procedure for a completely randomized in a 3 × 3 factorial design using SAS software. Means were compared using Duncan's New Multiple Range test (Steel and Torrie, 1980).

Results

Chemical composition

The results of the chemical composition of the SFCP before re-fermenting and the FTMRs after 30 days of fermentation are shown in Table 1. During fermentation, mold was not found visually in any of the treatments. The SFCP contained 18.55, 2.78, and 26.07% of DM, CP, and NDF, respectively. Moreover, this result demonstrated that the SFCP from the open-pit had consistent pH around 3.30 and appeared to be perfectly stable as spontaneously fermented cassava pulp. After mixing the SFCP with feed ingredients, the pH of each treatment increased.

Gas accumulation and gas kinetics

The gas accumulation curves of all treatments are given in Figures 1 and 2. The parameters of the gas production kinetics are presented in Table 2. The interaction between the SFCP and urea on gas produced from a soluble fraction (a) and rate of gas production (c) was found to be non-significant ($P > 0.05$). However, there was a difference ($P < 0.01$) in gas production at 48 and 96 h after incubation and potential gas production (b) among the SFCP levels. There were no differences ($P > 0.05$) among urea levels. The cassava chip has more potential gas production than the SFCP (Table 2). Increasing the level of the SFCP decreased the total gas production ($P < 0.01$). However, the results showed that total gas production at 96 h was only 8-10% less with the SFCP than with the cassava chip. Additionally, the result showed no difference between the SFCP and the cassava chip for total gas production at 24 h after incubation. In this study, each treatment had a high rate of gas production, which may have been due to its low NDF content.

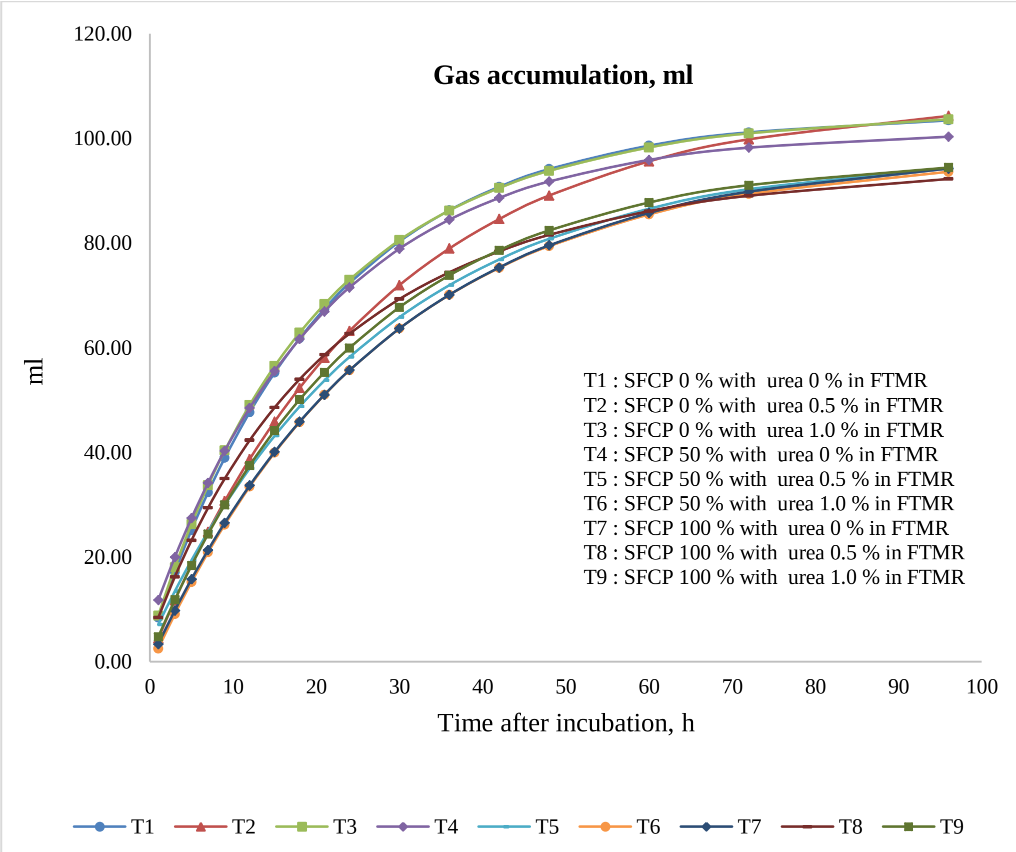

Figure 1. Pattern of in vitro gas accumulation on incubation of FTMRs in buffered rumen fluid

Discussion

Cassava pulp from factories with a CP content of 2.17% DM was reported by Keaokliang *et al*. (2018), which was comparable to the 2.78 found in this study. However, SFCP in this experiment has a CP lower than that reported by Pilajun and Wanapat (2018) (2.78 and 4.8%, respectively). Although increasing the urea level in FTMR, a reduction in CP content is probably due to losses in ammonia from urea addition occurring during the ensiling process. However, the composition of cassava chips depends on breeds, harvesting age, environmental conditions, and planting season (Saengchan *et al*., 2015). After mixing the SFCP with feed ingredients, the pH of each treatment increased because of the alkalizing effect of the added urea. In general, it expected that spoilage microorganisms would be inhibited at a lower pH than at higher pH, which is closer to the optimum pH range of the spoilage microorganisms. Napasirth *et al*. (2015) reported that silages would be well preserved with a low pH below 4.0. However, this result accords with the study of Kamphayae *et al*. (2016), which found that the pH of cassava pulp is 3.63, while ureated FTMR silages typically have a higher than usual pH (~4.0).

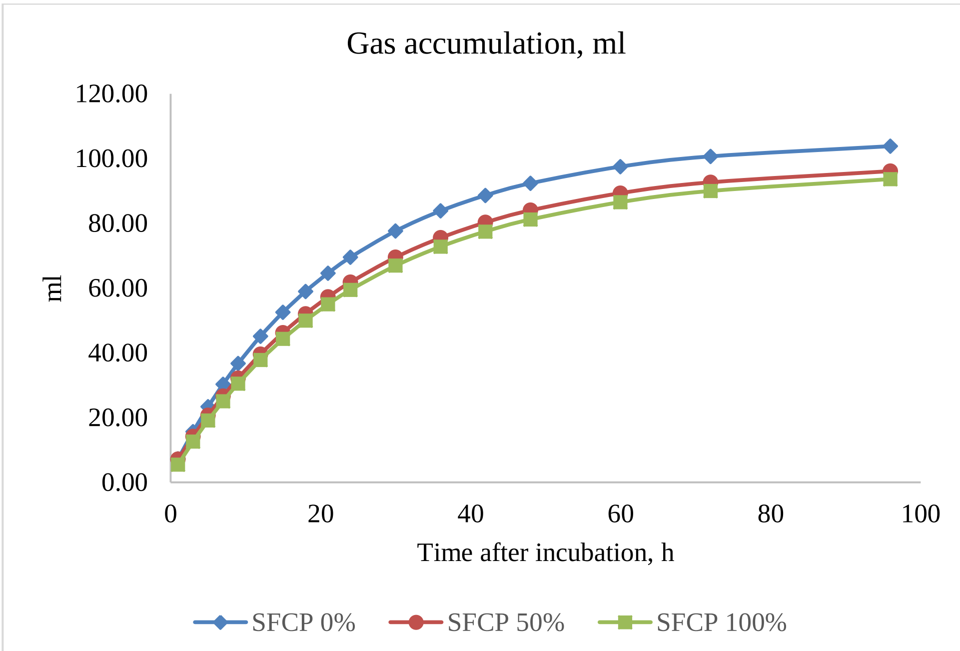

Figure 2. Effect of level of SFCP in FTMR on the gas accumulation

Supplementation of SFCP affected the total gas production (48 and 96 h), gas production for the fermentation of the insoluble (b), and the potential extent of gas production (d) ($P < 0.01$), but did not affect gas production from the immediately soluble fractions (a) and the rate of gas production ($P > 0.05$). The decrease in gas production kinetics in the present study could be due to the addition of SFCP in the FTMR, which could be from reduced starch content in the diets. Typically, cassava chip has a starch content of approximately 15%–32%, and starch could be lost with the pulp after starch extraction, up to 10% dry basis of starch input (Saengchan *et al*., 2015).

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