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Research Article
Effect of Fermentation Using Different Microorganisms on Nutritive Values of Fresh and Dry Cassava Root

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Abstract

Background and Objective: Cassava (*Manihot esculenta* Crantz) is widely grown in sub-tropical and tropical areas, producing roots as an energy source containing high soluble carbohydrate but low in crude protein. The process of protein enrichment of animal feed using microorganisms in a semi-solid culture to improve the nutritional value of ruminants feed has been considered. This study aimed to investigate the effect of microorganism fermentation on nutritional values of cassava products and *in vitro* rumen fermentation and digestibility. **Materials and Methods:** The experimental design was a 2×4 factorial arrangement in a completely randomized design. Factor A was two types of cassava root (fresh cassava root (FC) and cassava chip (CC)) and factor B was four sources of microorganism inclusion [no microorganism (No), Yeast (Y), effective microorganism (EM) and Yeast+EM (EMY)), respectively. **Results:** The results found that crude protein of cassava root was dramatically increased by Y and EM fermentation and the highest was found in CC ($p<0.05$). The gas kinetics, cumulative gas production (96 h) and *in vitro* dry matter and organic matter digestibility were enhanced by Y and EM fermentation ($p<0.05$), especially in CC group. Moreover, Y and EM could increase concentration of volatile fatty acids and ammonia-nitrogen while reduced methane production ($p<0.05$). Ruminal bacteria and fungi were increased whereas protozoa population was reduced by Y and EM fermentation. **Conclusion:** In conclusion, Y and EM fermentation could improve nutritional values of cassava products and enhance nutritional digestibility, rumen fermentation efficiency while decrease protozoa and methane production. However, further researches in feeding trial could be conducted.

Key words: Cassava products, effective microorganisms, yeast, rumen fermentation, digestibility, *in vitro* gas production

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feed quantity and quality is becoming the critical issue which affected on the productivity of livestock. Researchers have been trying to find alternative protein sources which could help to increase livestock productivity and efficiency[1]. Cassava (*Manihot esculenta*, Crantz) is widely grown in the tropical region[2] producing root as an energy source for ruminant. On the other hand, beside high fermentable carbohydrate, the root contains low crude protein (2-3%). Protein enrichment of animal feed such as a culture of *Saccharomyces cerevisiae* has become common practice in ruminant nutrition. According to Polyorach *et al.*[3,4] and Boonnop *et al.*[5], crude protein of cassava chip was increased from 2-30.4 or 47% by yeast fermented treatment.

Moreover, effective microorganisms (EM) are a product characterized by a mix of aerobic and anaerobic microorganisms consisting of three major groups: i.e., photosynthetic bacteria, lactobacillus bacteria and yeasts and/or fungi. It is produced in vats from cultivations of over 80 varieties of microorganisms. The microorganisms are drawn from 10 genera belonging to 5 different families. The use of EM in animal husbandry is also clearly identified in many parts of the world. Syomiti *et al.*[6] reported that supplement EM in drinking water at 0.2% has beneficial effects on cell wall constituents' degradability and thus utilization of high fiber diets. Inclusion of a protein-rich feed ingredient in the formulation of ruminant rations enhances feed utilization. Furthermore, Kassu *et al.*[7] studies the effect of EM on nutritive quality of coffee husk silages, it was found that EM could improve nutritional quality of coffee husk. However, study of improving nutritional value of cassava products using microorganism still lack of data. Therefore, the present study was conducted to investigate the effect of microorganism on nutritional values of cassava products and rumen fermentation and digestibility of beef cattle using *in vitro* gas production technique.

MATERIALS AND METHODS

Preparation of yeast fermented cassava products: Cassava products preparation were adapted from the method of Polyorach *et al.*[3] and some important details were as follows: Activated yeast was prepared using 20 g of microorganism (yeast, EM, EMY) and 20 g cane sugar mixed with 100 mL distilled water, then mixed well and incubated at room temperature for 1 h (A). Liquid media was prepared using 16 g molasses and 100 mL distilled water, followed by addition of 56 g urea (B). Mixed (A) and (B) at 1:1. After 66 h, the yeast medium solution was mixed with cassava chips at a ratio of 1 mL: 1.3 g and then fermented in solid state under shade for 3 days, followed by sun-drying for 48 h. The final product is stored in plastic bag for later analysis.

Experimental design and dietary treatments: This study was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a 2×4 factorial arrangement in a completely randomized design with 3 replications per treatment. The treatments were two different cassava forms [fresh cassava root (FC) and cassava chip (CC)] and four types of microorganism sources (no microorganism (No), yeast, effective microorganism (EM) and mixed yeast and EM (EMY)). Rice straw was used as a roughage source. Samples of roughage and concentrates were dried at 60°C, then ground to pass a 1 mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC[8], neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al.*[9].

Animals and preparation of rumen inoculums: Animals rumen fluid was collected from animals fed with concentrate (14.0% CP and 80.6% TDN) at 0.5% of BW in to equal portions, at 07.00 and 16.00 h and rice straw was fed on *ad libitum* basis. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 20 days before the rumen fluid was collected. On 20 days, 1,000 mL rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through 4 layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

***In vitro* fermentation of substrates:** Samples of each total mixed substrate (500 mg), following respective treatments were weighed into 50 mL serum bottles. For each treatment, 3 replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass[10], in a proportion 2:1 (mL:mL) at 39°C under continuous flushing with CO_2. Thirty milliliters of rumen inoculum mixture were added into each bottle under CO_2

flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39°C (96 h) for *in vitro* gas test. Thirty min after starting the incubation, the bottles were gently mixed and then mixed 3 times every 3 h. For each sampling time, 3 bottles containing only the rumen inocula were included within each run and the mean gas production values of these bottles were used as blanks. The blank values were subtracted from each measured value to give the net gas production.

Sample and analysis: During the incubation, data of gas production was measured immediately after incubation at 0, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Orskov and McDonald[11], as follows:

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

Where:

- a = Gas production from the immediately soluble fraction
- b = Gas production from the insoluble fraction
- c = Gas production rate constant for the insoluble fraction (b)
- t = Incubation time
- (a+b) = Potential extent of gas production
- y = Gas produced at time "t"

Inoculum ruminal fluid was sampled at 0, 4, 6, 12 and 24 h post inoculations. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into 2 portions, the first portion was centrifuged at 16,000 rpm for 15 min and the supernatant was stored at -20°C before NH_3-N analysis using the micro-Kjeldahl methods[8] and VFA analysis using HPLC[12]. The second portion was fixed with 10% formalin solution in a sterilized 0.9% saline solution for a total direct count of bacteria, protozoa and fungi made by the methods of Galyean[13], based on the use of a hemocytometer (Boeco, Hamburg, Germany).

In vitro degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left from above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD)[14]. Calculation of ruminal methane (CH_4) production using VFA proportions was made according to Moss *et al.*[15] and as follows:

$$CH_4 \text{ production} = 0.45 \text{ (acetate)}-0.275 \text{ (propionate)}+0.4 \text{ (butyrate)}$$

Statistical analysis: Data used for statistical analysis consisted of 2 levels of cassava form, 4 levels of microorganism sources, 3 replications. All data were analyzed as a 2×4 factorial arrangement in a completely randomized design (CRD) using the PROC GLM of SAS[16]. Data were analyzed using the model:

$$Y_{ij} = \mu+A_i+B_j+AB_{ij}+\varepsilon_{ij}$$

Where:

- Y = Observations
- μ = Overall mean
- A_i = Effect of factor A (protein sources, i = 1-2)
- B_j = Effect of factor B (level of roughage to concentrate (R:C) ratio, j = 1-5)
- Ab_{ij} = Interaction between factor A and B
- ε_{ij} = Residual effect

Multiple comparisons among treatment means were performed by Duncan's new multiple range test (DMRT)[17]. Differences among means with $p<0.05$ were accepted as representing statistically significant differences.

RESULTS

Chemical composition of cassava products: The chemical composition of cassava products are presented in Table 1. It was found that DM and CP have interaction ($p<0.05$) between cassava form (CF) and microorganism source (MS) by dry group with EM and dry cassava form group with EMY were highest ($p<0.01$) of CP (44.2 and 45.3% CP, respectively). Moreover, NDF was reduced by EM both in fresh and cassava chip ($p<0.05$) while there was no difference among treatments on ADF content ($p>0.05$).

Gas production kinetics and *in vitro* digestibility: Cumulative gas production for each of the substrate treatments presented as gas production and the values for estimated parameters obtained from the kinetics of gas production models for substrates studied are given in Table 2 and Fig. 1. This studied revealed that the intercept value (a)

Table 1: Chemical composition of cassava products (dry matter %)

Cassava forms	Microorganism sources	Dry matter	Organic matter	Crude protein	Ether extract	Neutral detergent fiber	Acid detergent fiber
Fresh	Non	65.3^{c}	95.0^{b}	3.1^{f}	2.1^{d}	7.8^{a}	6.2
	Y	66.4^{c}	96.3^{b}	28.7^{e}	3.2^{bc}	7.2^{abc}	5.8
	EM	66.7^{c}	98.2^{a}	30.4^{d}	3.5^{bc}	6.9^{bcd}	5.3
	EMY	66.1^{c}	98.5^{a}	31.8^{c}	3.7^{b}	6.8^{cd}	5.0
Dry	Non	86.2^{a}	96.0^{b}	3.5^{f}	2.5^{cd}	7.7^{ab}	6.1
	Y	84.3^{b}	98.1^{a}	42.1^{b}	5.3^{a}	6.8^{cd}	5.3
	EM	84.7^{ab}	98.5^{a}	44.2^{a}	5.6^{a}	6.5^{cd}	4.9
	EMY	85.0^{ab}	98.8^{a}	45.3^{a}	5.8^{a}	6.3^{d}	4.8
SEM		00.54	00.43	00.38	0.34	0.26	0.45
Interaction							
Cassava		**	*	**	**	ns	ns
Microorganism		ns	**	**	**	**	ns
Cassava, Microorganism		*	ns	**	ns	ns	ns

$^{a-f}$Values on the same row with different superscripts differ (p<0.05), *p<0.05, **p<0.01, ns: Non-significant different, SEM: Standard error of the mean, Non: Unused microorganism, Y: Yeast, EM: Effective microorganism, EMY: Effective microorganism with yeast

Table 2: Gas kinetics and degradability affected by dietary cassava productions

		Gas kinetics[1]				Gas (96 h)/ 0.5 g DM	*In vitro* degradability (%)	
Cassava forms	Microorganism sources	a	b	c	a+b	substrate	IVDMD	IVOMD
Fresh	Non	2.6^{e}	81.2^{f}	0.50	83.8^{d}	84.5^{d}	55.4^{f}	60.6^{f}
	Y	3.2^{cd}	86.3^{de}	0.49	89.5^{b}	90.1^{bc}	67.4^{d}	71.9^{d}
	EM	2.4^{e}	87.3^{dc}	0.49	89.7^{b}	91.0^{b}	69.7^{c}	75.0^{c}
	EMY	4.4^{b}	85.5^{de}	0.49	89.9^{b}	91.1^{b}	71.6^{bc}	76.2^{bc}
Dry	Non	2.6^{e}	84.3^{e}	0.44	86.9^{c}	87.6^{c}	60.9^{e}	66.2^{e}
	Y	3.1^{d}	88.9^{bc}	0.44	92.1^{b}	92.6^{b}	72.9^{b}	77.8^{b}
	EM	3.6^{c}	91.6^{a}	0.45	95.3^{a}	96.2^{a}	75.5^{a}	80.6^{a}
	EMY	5.5^{a}	91.1^{ab}	0.44	96.5^{a}	97.8^{a}	76.1^{a}	81.1^{a}
SEM		0.156	0.793	0.008	0.805	00.880	00.660	00.769
Interaction								
Cassava		**	**	**	**	**	**	**
Microorganism		**	**	ns	**	**	**	**
Cassava, Microorganism		**	ns	ns	ns	ns	ns	ns

$^{a-f}$Values on the same row with different superscripts differ (p<0.05), **p<0.01, ns: Non-significant different, SEM: Standard error of the mean, Non: Unused microorganism, Y: Yeast, EM: Effective microorganism, EMY: Effective microorganism with yeast, [1]a: Gas production from the immediately soluble fraction, b: Gas production from the insoluble fraction, c: Gas production rate constant for the insoluble fraction, a+b: Gas potential extent of gas production, Gas (96 h)/0.5 g DM substrate: Cumulative gas production at 96 h (mL/0.5 g DM substrate), IVDMD: *In vitro* dry matter digestibility, IVOMD: *In vitro* organic matter digestibility

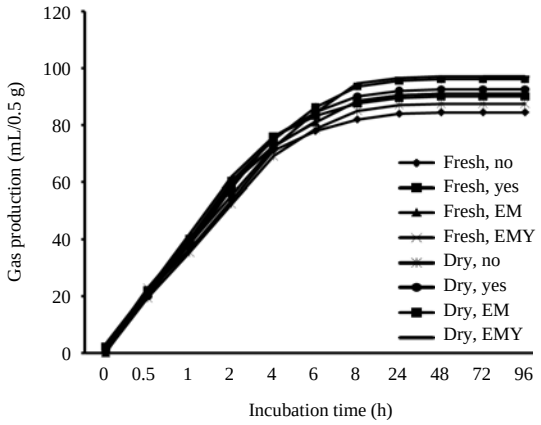

Fig. 1: Cumulative gas production affected by dietary cassava productions

has interaction (p<0.01) between cassava forms and microorganism sources. When considerate effects of factors, It was found that the gas production from insoluble fraction (b), the gas production rat constant for the insoluble fraction

Table 3: Ammonia nitrogen, volatile fatty acids and methane production affected by dietary cassava productions

Cassava forms	Microorganism sources	NH_3N (mg dL^{-1})	TVFA (mM L^{-1})	C_2 (%)	C_3 (%)	C_4 (%)	C_2:C_3	CH_4[1]
Fresh	Non	20.5^{e}	77.7^{d}	64.1^{a}	24.8^{e}	11.1	2.6^{a}	26.4^{a}
	Y	21.7^{d}	83.1^{cd}	59.4^{b}	31.4^{c}	9.2	1.9^{c}	21.8^{c}
	EM	22.4^{cd}	87.3^{bc}	56.5^{c}	33.4^{b}	10.1	1.7^{d}	20.2^{d}
	EMY	22.8^{c}	88.27^{abc}	55.7^{c}	33.3^{b}	11.0	1.7^{d}	20.3^{d}
Dry	Non	20.4^{e}	88.3^{abc}	62.2^{a}	27.5^{d}	10.4	2.3^{b}	24.5^{b}
	Y	24.1^{b}	92.3^{ab}	56.7^{c}	33.8^{b}	9.5	1.7^{d}	20.0^{d}
	EM	26.3^{a}	96.7^{a}	53.4^{d}	36.5^{a}	10.1	1.5^{e}	18.0^{e}
	EMY	26.6^{a}	96.9^{a}	52.4^{d}	35.9^{a}	11.7	1.5^{e}	18.4^{e}
SEM		0.24	2.69	0.73	0.56	0.73	0.04	0.39
Interaction								
Cassava		**	**	**	**	ns	**	**
Microorganism		**	**	**	**	ns	**	**
Cassava, Microorganism		**	ns	ns	ns	ns	ns	ns

[a-e]Values on the same row with different superscripts differ (p<0.05), **p<0.01, ns: Non-significant different, SEM: Standard error of the mean, NH_3-N, ammonia nitrogen, TVFA: Total volatile fatty acid, C_2: Acetic acid, C_3: Propionic acid, C_4: Butyric acid, C_2: C_3, acetic acid: Propionic acid ratio, Non: Unused microorganism, Y: Yeast, EM: Effective microorganism, EMY: Effective microorganism with yeast, [1]Methane production (mmol L^{-1}) calculated by Moss *et al.*[15] = 0.45 (C_2)-0.275 (C_3)+0.4(C_4)

Table 4: Microorganisms affected by dietary cassava productions

Cassava forms	Microorganism sources	Bacteria ($\times 10^8$ cell mL^{-1})	Protozoa ($\times 10^5$ cell mL^{-1})	Fungi ($\times 10^5$ cell mL^{-1})
Fresh	Non	3.8^{e}	4.0^{a}	2.1^{e}
	Yeast	5.3^{d}	2.3^{c}	3.3^{c}
	EM	7.3^{c}	1.9^{d}	4.1^{b}
	EMY	7.4^{c}	1.9^{d}	4.3^{b}
Dry	Non	5.5^{d}	2.8^{b}	2.7^{d}
	Yeast	8.1^{b}	1.7^{de}	4.0^{b}
	EM	10.8^{a}	1.6^{de}	5.8^{a}
	EMY	10.8^{a}	1.4^{e}	5.9^{a}
SEM		00.232	0.118	0.147
Interaction				
Cassava		**	**	**
Microorganism		**	**	**
Cassava, Microorganism		**	**	**

[a-e]Values on the same row with different superscripts differ (p<0.05), **p<0.01, ns: Non-significant different, SEM: Standard error of the mean, Non: Unused microorganism, Y: Yeast, EM: Effective microorganism, EMY: Effective microorganism with yeast

(c), potential extent of gas production (a+b), cumulative gas production at 96 h, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) of dry cassava form were significantly higher (p<0.01) than fresh cassava form. Moreover, microorganism sources affected (p<0.01) on b, a+b, cumulative gas production at 96 h, IVDMD and IVOMD by EM and EMY group were highest (p<0.01) (96.2 and 97.6/0.5 g DM substrate, respectively) followed by Y and No group, respectively.

Rumen fermentation: The volatile fatty acid (VFA), ammonia-nitrogen (NH_3-N) and methane production (CH_4) are presented in Table 3. The results revealed that have an interaction between CF and MS group by the highest (p<0.01) were cassava dry form with EM and EMY (26.3 and 26.9 mg dL^{-1}) and the lowest (p<0.01) were fresh and dry cassava with No group (20.5 and 20.4 mg dL^{-1}). Microorganism sources affected on total VFA, C_2, C_3, C_2:C_3 and CH_4 production by total VFA and C_3 of EMY and EM group were highest (p<0.01) follow by Y and No group while C_2, C_2:C_3 and CH_4 production of No group were highest (p<0.01) follow by Y, EM and EMY group. These result probably due to some effects of yeast and EM which contained in cassava products.

Rumen microbes: Table 4 presents the effect of EM treatment of cassava product on microorganism. Bacterial and fungal population were increased in treatment with cassava product treated with EM (p<0.05). On the other hand, EM treated cassava product reduced protozoa population especially in cassava chip rather than fresh cassava (p<0.05).

DISCUSSION

Chemical composition of cassava products: Fermentation of EM could increase the CP content of the cassava product. This increase could be due to the increase in growth and

proliferation of the fungi or bacterial complex in the form of single cell proteins may possibly account for the apparent increase in the protein content. It could be also due to NPN (urea) level addition which is a good N source use for synchronized soluble carbohydrates in the rumen of ruminants. Crude protein of cassava products in this experiment were similar to those reported earlier by Polyorach *et al.*[3,4]. Moreover, Kassu *et al.*[7] reported study the effect of EM on the nutritive quality of coffee husk silages, it was found that significant improvement in the total ash, EE and CP content of pure coffee husk ensiled with the use of EM. Samsudin *et al.*[18], who studied on the improving the nutritive value of rice straw treated with biological treatments showed that fungal treated and with EM could reduce in lignocellulosic contents as shown by decreased value of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of treated rice straw may increase the nutrients availability to animals. This high protein content could be attributed to the ability of the EM (*S. cerevisiae*) to secrete some extracellular enzymes such as amylases, linamarase and cellulase into the cassava mash during their metabolic activities, which would lead to yeast growth.

It was found that organic matter (OM), CP, ether extract (EE) in dry cassava form group affected were higher ($p<0.01$) than fresh cassava form group. Moreover, microorganism source affected on OM, EE and NDF by EMY were the highest ($p<0.01$) followed by EM, yeast (Y) and unused microorganism (No), respectively. These results might be due to fresh cassava form contained higher cyanide content lead to limited fermentation activities of yeast lead to low nutritional value in fresh cassava form than dry form (Table 1). Boonnop *et al.*[5] found that cassava fermented yeast could improve nutritional value of cassava chip (dry form) compared to cassava root (fresh form).

Gas production kinetics and *in vitro* digestibility: Gas production and *in vitro* digestibility of DM and OM were enhanced by EM treatment on cassava product. The present results were probably due to difference chemical composition of cassava products as showed in Table 1 and cassava products promoting growth of rumen ruminal microorganism, especially, cellulolytic bacteria and lactictate-utilizing bacteria. Moreover, the positive effect and mode of actions of yeast products are generally considered to involve changes in rumen fermentation rates and patterns by removal of oxygen that occurs in ruminal fluid and in that way can prevent toxicity to the ruminal anaerobes[19] and yeast was effective at raising and stabilizing ruminal pH by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and thus, effectively compete with amylolytic lactate-producing bacteria[20]. A less acidic ruminal environment has been shown to benefit the growth and fiber degrading activities of cellulolytic microorganisms[21]. These results were similar to the finding of Wanapat *et al.*[22] that yeast fermented cassava chip protein (YEFECAP) can fully replace SBM in concentrate for dairy cows and improved rumen fermentation, dry matter intake, nutrient digestibility, milk production and composition.

In additional, mixed microbes for ruminants also have mainly been selected to improve various ruminal digestion by increasing pH in the rumen, fiber digestion and the synthesis of microbial proteins. Probiotics enhance growth and/or cellulolytic activity by rumen bacteria and prevent ruminal acidosis by balancing the VFAs ratios in the rumen. Therefore, mixed microbes supplementation in the diet may result in improved nutrient digestibility[23].

Rumen fermentation: The concentration of NH_3-N was increased in fermented cassava production group. The increase of NH_3-N concentration in the present study were similar to Wanapat and Pimpa[24], who reported that the optimal ruminal ammonia concentration for microbial growth ranged from 15-30 g/100 mL when ruminants were fed on rice straw. Moreover, Polyorach *et al.*[4] reported that using yeast fermented cassava chip products (YEFECAP) as a protein source with different roughage to concentrate ratio, it was found that NH_3-N was increase when increasing concentrate levels by NH_3-N ranged form 17.1-26.6 g/100 mL.

Total VFA and propionic acid (C_3) in dry cassava form were higher ($p<0.01$) while acetic acid (C_2), acetic acid:propionic acid ratio (C_2:C_3) and CH_4 production were lower than fresh cassava group. This could be due to the present of higher cyanide content and low nutritive value in fresh cassava which could affect on higher TVFA and C_3 and lower C_2, C_2:C_3 and CH_4 production in dry cassava form than fresh cassava form as showed in Table 3. Boonnop *et al.*[5] reported that there was a decrease in the HCN content when compared with the unfermented cassava products. Levels of the residual cyanide present in both fresh cassava root (FCR) (47.3 mg kg^{-1}) and cassava chip (CC) (0.5 mg kg^{-1}) were remarkably low when compared with the normal cyanide content of the unfermented cassava.

The increase of VFA profile in the present study was in the agreement with Polyorach *et al.*[4], who presented that the used of yeast fermented cassava chip protein (YEFECAP) as a protein source could increase total VFA and C_3 while decreased C_2:C_3 and CH_4 production when compare with soybean meal. In previous studies, feeding direct fed

microbials (*Enterococcus* sp. and *yeast* sp.) to feedlot cattle affected ruminal fermentation and nutrient digestion through the decrease of ruminal pH and butyrate and an increase of propionate[25]. Yeast could stimulated the growth and metabolism of rumen microorganisms especially lactate-utilizing bacteria, such as *Megasphaera elsdenii* or *Selenomonas ruminantium*[26] and supply different growth factors, such as amino acids, peptides, vitamins and organic acids, essential for the ruminal bacterial growth[19], hence, enhancing VFA concentration and reducing C_2:C_3 proportion[4]. Moreover, LAB was provided a constant lactic acid supply in the rumen, helps the overall microflora to adapt the lactic acid accumulation, stimulate lactate utilizing bacteria[27]. Various strains of LAB also activate macrophages to produce cytokines that stimulate immune response[28]. Yeast also has the potential to alter the fermentation process in the rumen in a manner that reduces the formation of CH_4 [21].

Rumen microbes: The result revealed that there were interactions between effects of cassava form and microorganism source on rumen microorganism especially, bacteria protozoa and fungi zoospores. Bacteria and fungi zoospores in cassava dry form with EMY (10.8×10^8 and 5.9×10^5 cell mL^{-1}, respectively) and EM (10.8×10^8 and 5.8×10^5 cell mL^{-1}, respectively) were the highest while protozoa in cassava fresh form with No was the highest ($p<0.01$) (4.0×10^5 cell mL^{-1}). This effect could be due to cassava dry form with EMY and EM was higher nutritional value as showed in Table 1 especially, CP content in cassava products. The additional protein provided by the cassava products would have increased availability of ammonia for rumen microflora, stimulating microbial growth and increasing rate of breakdown of forage. Moreover, it might be due to effects of yeast in cassava products. This result agrees with Polyorach *et al.*[4], who reported that using yeast fermented cassava chip products (YEFECAP) as a protein source, bacteria especially cellulolytic bacteria (*Fibrobactor succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus*)[26] and zoospores populations were significantly ($p<0.01$) higher than used soybean meal as a protein source. According to Newbold *et al.*[29] and Retta[27], there are two modes of action of yeasts in the rumen. Firstly, yeast remove oxygen in the rumen by yeast cells in the rumen used available oxygen on the surfaces of freshly ingested feed to maintain metabolic activity. This creates better conditions for the growth of strict anaerobic cellulolytic bacteria, stimulates their attachment to forage particles and increases the initial rate of cellulysis. Secondly, *Saccharomyces cerevisiae* is able to compete with other starch utilizing bacteria for fermentation of starch[26] leading to prevention of lactate accumulation in the rumen and had the ability to provide growth factors, such as organic acids or vitamins, thereby stimulating ruminal population of cellulolytic bacteria and lactate utilizing bacteria (e.g., *Megasphaera elsdenii* and *Selenomonas ruminantium*)[19,30].

CONCLUSION

Based on this study, it could be concluded that using microorganism could improve nutritional values of cassava products and improved *in vitro* nutrient digestibility and rumen fermentation while reducing CH_4 production and protozoa population. However, further research on the use of cassava product fermented with effective microorganisms as ruminant feeding should be conducted.

SIGNIFICANCE STATEMENT

This study discovers enrichment of cassava product quality by microorganism fermentation treatment that can be beneficial for improvement of ruminant feed and feeding to enhance productivity. This study help the researcher to uncover the critical area of the uses of microorganism fermentation to improve animal feed quality that many researchers were not able to explore. Thus, a new theory on microorganism fermentation treatment for the enrichment of cassava product quality may be arrived at.

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