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Improvement of Cassava Pulp Nutrients by Yeast Fermentation with Chicken Manure

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Abstract

The aim of this study was to investigate the use of chicken manure as a nitrogen source for the improvement of cassava pulp by fermentation with yeast *Saccharomyces cerevisiae*. The experiment was arranged as 9 x 4 factorial in complete randomized design (CRD). Nine treatments with different compositions of cassava pulp, yeast, ammonium sulfate (AS), or chicken manure (CM) were fermented for 0, 10, 20, and 30 days. The results showed a significant interaction between treatment composition and fermentation time ($p<0.01$). The highest level of crude protein at 6.28% was observed when cassava pulp was fermented with yeast and CM for 30 days, increased from 1 – 2% of plain cassava pulp. The crude fibre was reduced to 11.4% from 15.6% of plain cassava pulp when it was fermented with yeast and AS for 20 days while CM did not noticeably help in the reduction of crude fibre. Cassava pulp mixed with yeast at 0 days showed the highest gross energy (3484.89 kcal/kg). We found that CM and increase of fermentation time significantly caused the reduction of the gross energy. In addition, AS led to the most reduction of pH at 20 and 30 days but an increase of pH value was observed when CM was used ($p<0.01$). In conclusion, CM can be used as a nitrogen source in yeast fermented cassava pulp to increase protein content; however, it could lessen the gross energy and interfere with the pH value of an acidic condition in fermentation.

Keywords: Cassava pulp, chicken manure, fermentation, *Saccharomyces cerevisiae*

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1. Introduction

The increases of world population require abundant sustainable foods to feed them, animal food sources have importance contribution in global human food demand. One of the constraints of animal production especially monogastric animals is feed availability and cost. Monogastric animals compete for feeds with human such as cereals and soybeans. Rice and maize are preferred for human consumption and exceed cassava, such that cannot be spared for feeding animals when are scarce, [1]. Therefore, it is important to find a diverse feedstuff which can replace the conventional animal feeds.

Since 2000 production of cassava (*Manihot esculent* Crantz) in the world has increased for 60%, possibly because cassava plant does not require comparative higher inputs, drought resistance, can be grown in mountainous slope of low fertility and acid soil tolerant [2, 3].

Cassava pulp is a solid moist fibrous material remaining after starch extraction from cassava root. This residue is sold at low-cost, and may reach up to 30% of the whole original cassava root depends on the efficiency of cassava starch processing factory [4]. The nutritional problems of cassava pulp are higher fibre content (up to 27.75%), low crude protein (less than 2.37%) and ether extract 0.6%. Several efforts have been done by scientists to solve these problems. Appreciation to scientists now cassava pulp can be improved and used as feed in livestock with comparable higher nutritional value than before [5-7].

Yeast fermented cassava pulp (YFCP) is one of improved cassava pulp which can help animal producers to supplement energy and protein source to their animals. Also, it is a way of environmental conservation because the cassava pulp as a waste if not treated will produce unpleasant smell and putrefaction hence environment pollution [8]. Moreover, presence of yeast and higher fibre may act as probiotic and prebiotic and bring the nutraceuticals effect to animals [9-11].

For better improvement, Cassava pulp requires source of nitrogen to the microorganisms. In most cases inorganic commercial fertilizers such as ammonium sulfate, diammonium sulfate, and urea are used [12]. However, these inorganic fertilizers sometime are not common available at rural areas to small holder poor resource farmers.

The objective of this study was to use organic fertilizer (dried layer chicken manure) as a source of nitrogen to yeast in cassava pulp fermentation in order to increase protein and decrease crude fibre for animal feed. Possibly this study is a first work to provide an alternative for commercial inorganic source of nitrogen.

2. Materials and Methods

2.1. Location, material collection and preparation

This experiment was conducted at faculty of Animal Science and Agricultural Technology, Silpakorn university, Phetchaburi, Thailand. Cassava pulp was bought from EK animal feed store - Tha yang Phetchaburi province in Thailand. *Saccharomyces cerevisiae* yeast (Lesaffre Saf-Instant®, France), ammonium sulfate (Crown brand, Terragro, Thailand) and table sugar were bought at nearby store. Chicken manure was collected from layer pens at university farm, removed trashes, and dried in hot air oven (60 °C) for 3 days, then grinded into fine using pestle and mortar, and blander.

2.2 Experimental design

This experiment was performed in 9(A) × 4(B) factorial arrangement in complete randomized design (CRD) with three replications. Factor A was arranged into 9 treatments and factor B was set as 4 different fermentation time (0, 10, 20 and 30 days) [13]. Detail of treatments were shown in Table 1. All treatments with nitrogen source supplementation were hypothetical calculated approximately as 5% crude protein.

2.3. Preparation of cassava pulp fermentation

Cassava pulp fermentation prepared by a method of Huu & Khammeng (2014) [14] with modification. Cassava pulp and chicken manure were weighted according to each treatment composition. Yeast solution (10% w/v) was prepared by mixing 24 grams of yeast with 20% sterile sugar solution, stirred well for 30 minutes. Ammonium sulfate solution was prepared as a 20% solution in sterile water. Then 10 millilitres of yeast solution were added into treatment 2, 5, 6, 7, 8, and 9 each to obtained 1% yeast inoculation. Ammonium sulfate solution was added to obtain a desired concentration of ammonium sulfate in each treatment. All treatments were adjusted the moisture to 20% by addition of sterile water and then thoroughly mixed. Finally, the treatments were packed anaerobically each in a plastic bag and incubated at room temperature for 0, 10, 20, and 30 days. When reaching the specific fermentation time, the samples were stored in the freezer until analysis.

Table 1: Description of preparing fermentation in each treatment (factor A)

Treatment	Composition								
	% Cassava pulp	% Yeast		% Ammonium sulfate		% Chicken manure	Sugar	Water	Total %
	Gram	Gram	mL	Gram	mL	Gram	Gram	mL	Gram
T1	100.00	0.00	0.00	0.00	0.00	0.00	0.00	20.00	100.00
T2	99.00	1.00	10.00	0.00	0.00	0.00	2.00	10.00	100.00
T3	98.00	0.00	0.00	2.00	10.00	0.00	0.00	10.00	100.00
T4	71.00	0.00	0.00	0.00	0.00	29.00	0.00	20.00	100.00
T5	97.00	1.00	10.00	2.00	10.00	0.00	2.00	0.00	100.00
T6	90.00	1.00	10.00	1.50	7.50	7.50	2.00	2.50	100.00
T7	83.50	1.00	10.00	1.00	5.00	14.50	2.00	5.00	100.00
T8	76.50	1.00	10.00	0.50	2.50	22.00	2.00	7.50	100.00
T9	70.00	1.00	10.00	0.00	0.00	29.00	2.00	10.00	100.00
Total	785.00	6.00	60.00	7.00	35.00	102.00	6.00	85.00	900
× 4	3140.00	24.00	240.00	28.00	140.00	408.00	24.00	340.00	3,600.00

Note: The light-coloured columns are inoculant solution

2.4. Data collection

Cassava pulp, chicken manure and treatments were analyzed for dry matter, crude protein, crude fibre, and gross energy using proximate analysis, following the AOAC [15]. The pH value was determined by dissolved sample in distilled water (1: 10 ratio) and pH was measured by pH meter (Adwa AD12, Hungary).

2.5. Statistical Analysis

The mean ± standard deviation of dry matter, gross energy, crude protein, crude fibre, and pH were statistically analysed using analysis of variance (ANOVA) and compared by Duncan's New Multiple Range Test (DMRT) in R program (Significance was defined by $p < 0.05$).

3. Results

Before fermentation, the chemical composition (dry matter, crude protein, crude fibre and gross energy) of cassava pulp and chicken manure used in this study was evaluated (Table 2). Cassava pulp had higher dry matter, crude fibre and gross energy compared to chicken manure which had higher crude protein. The low protein of 1.99% in cassava pulp reduces its nutritive value as an ingredient of animal feed. Therefore, improvement of cassava pulp nutritive values has been interested nowadays.

Table 2: Crude protein, crude fibre and gross energy of cassava pulp and chicken manure (as feed basis)

Parameters	Cassava pulp	Chicken manure
Dry matter (%)	87.03 ± 0.25	25.39 ± 0.68*
Crude protein (%)	1.99 ± 0.19	13.93 ± 0.29
Crude fibre (%)	15.63 ± 0.73	13.32 ± 1.48
Gross energy (kcal/kg)	3456.667 ± 50.38	2386.467 ± 24.31
pH value	4.67 ± 0.11	7.89 ± 0.05

Note: Data are expressed as mean ± standard deviation (n = 3). * Chicken manure dry matter is in fresh basis.

Improvement of cassava pulp nutritive values by fermentation with yeast and nitrogen source either ammonium sulfate or chicken manure were investigated in this study. The crude protein, crude fibre, gross energy, and pH of fermented cassava pulp in each treatment at different fermentation time were determined. Significant interaction between treatment composition and fermentation time were observed in all parameters.

Table 3: Crude protein of fermented cassava pulp in each treatment at different fermentation time

Treatment (A)	Fermentation Day (B)				Mean (A)
	0	10	20	30	
T1	1.51 ± 0.43 ^{i}	1.63 ± 0.20 ^{i}	1.49 ± 0.01 ^{i}	1.74 ± 0.36 ^{i}	1.59 ± 0.28 ^{E}
T2	1.79 ± 0.04 ^{i}	1.93 ± 0.10 ^{i}	2.32 ± 0.20 ^{hi}	2.41 ± 0.79 ^{hi}	2.11 ± 0.44 ^{D}
T3	3.55 ± 0.14 ^{efg}	3.82 ± 0.27 ^{cdefg}	3.68 ± 0.12 ^{cdefg}	3.15 ± 0.21 ^{gh}	3.55 ± 0.31 ^{C}
T4	3.88 ± 0.16 ^{cdefg}	4.11 ± 0.10 ^{bcdefg}	4.24 ± 0.21 ^{bcdef}	4.68 ± 0.03 ^{bc}	4.23 ± 0.33 ^{B}
T5	3.53 ± 0.15 ^{efg}	4.58 ± 1.02 ^{bcd}	4.19 ± 0.39 ^{bcdef}	3.87 ± 0.22 ^{cdefg}	4.04 ± 0.63 ^{B}
T6	3.62 ± 0.02 ^{defg}	3.44 ± 0.18 ^{fg}	3.63 ± 0.27 ^{defg}	3.72 ± 0.25 ^{cdefg}	3.60 ± 0.21 ^{C}
T7	3.58 ± 0.37 ^{defg}	4.40 ± 0.48 ^{bcdef}	4.22 ± 0.17 ^{bcdef}	4.41 ± 0.30 ^{bcdef}	4.15 ± 0.46 ^{B}
T8	3.90 ± 0.31 ^{cdefg}	4.98 ± 1.02 ^{b}	4.48 ± 0.25 ^{bcde}	4.45 ± 0.64 ^{bcdef}	4.45 ± 0.67 ^{AB}
T9	4.30 ± 0.51 ^{bcdef}	4.35 ± 0.24 ^{bcdef}	4.43 ± 0.34 ^{bcdef}	6.28 ± 0.38 ^{a}	4.84 ± 0.93 ^{A}
Mean (B)	3.30 ± 0.96 ^{b}	3.74 ± 1.15 ^{a}	3.59 ± 1.08 ^{a}	3.86 ± 1.33 ^{a}	
% CV	10.67				
P-value					
A	***				
B	***				
A x B	***				

Note: Results are in dry matter basis and are expressed as mean ± standard deviation (n = 3).

The different superscript letters are statistically different by Duncan's New Multiple Range Test ($p \leq 0.01$)

For crude protein analysis, the changes in level of crude protein were influenced by both ingredients and fermentation. The used nitrogen source either ammonium sulfate or chicken manure increased the level of crude protein along with the increase of fermentation time. The highest level of crude protein at 6.28% was observed in treatment of cassava pulp fermented with yeast and chicken manure (T9) at 30 days (Table 3). By comparing among treatments, cassava pulp and cassava pulp fermented with yeast without nitrogen source supplementation (T1 and T2) showed low crude protein approximately 1.6 – 2.4% while cassava pulp fermented with yeast and chicken manure (T9) showed the best improvement. In addition, the results showed that fermentation had positive effect on crude protein level but not different among 10, 20 and 30 days (Table 3).

The level of crude fibre significant varied within treatments and fermentation times. Low fibre is preferred for using as feed ingredient in monogastric animals. Data showed that the lowest crude fibre was 11.4% observed in cassava pulp fermented with ammonium sulfate (T3) at 20 days (Table 4). However, when consider the treatment effect, cassava pulp fermented with yeast and ammonium sulfate (T5) showed the lowest crude fibre (Table 4). It should be noted that fermentation time significantly lower the crude fibre when increasing time of fermentation (Table 4).

Table 4: Crude fibre of fermented cassava pulp in each treatment at different fermentation time.

Treatment (A)	Fermentation Day (B)				Mean (A)
	0	10	20	30	
T1	13.50 ± 0.61 abcde	13.37 ± 0.07 abcde	13.02 ± 0.59 bcdefg	11.54 ± 0.33 hi	12.86 ± 0.90 BCD
T2	12.83 ± 0.94 bcdefgh	14.18 ± 0.74 ab	13.73 ± 0.22 abcd	12.99 ± 0.10 bcdefg	13.43 ± 0.79 AB
T3	13.07 ± 0.26 bcdefg	14.19 ± 1.03 ab	11.40 ± 0.38 i	11.73 ± 0.75 ghi	12.60 ± 1.30 CD
T4	13.66 ± 1.28 abcd	14.19 ± 1.03 a	12.39 ± 0.33 defghi	12.13 ± 0.34 efghi	13.17 ± 1.23 ABC
T5	12.23 ± 0.69 efghi	13.23 ± 1.19 abcdef	12.61 ± 0.14 cdefghi	11.96 ± 0.59 fghi	12.50 ± 0.83 D
T6	12.96 ± 0.28 bcdefg	13.51 ± 0.36 abcde	13.27 ± 0.31 abcdef	12.63 ± 0.64 cdefghi	13.09 ± 0.51 ABCD
T7	13.45 ± 0.51 abcde	13.88 ± 0.68 abc	13.36 ± 0.18 abcdef	11.80 ± 1.23 ghi	13.12 ± 1.06 ABCD
T8	13.81 ± 0.28 abcd	14.13 ± 0.26 ab	13.27 ± 0.96 abcdef	13.27 ± 0.44 abcdef	13.62 ± 0.63 A
T9	13.55 ± 0.90 abcde	14.18 ± 0.36 ab	13.69 ± 0.33 abcd	13.53 ± 0.62 abcde	13.74 ± 0.60 A

Mean (B)	13.91 ± 0.75 [a]	13.23 ± 0.79 [b]	12.97 ± 0.81 [b]	12.40 ± 0.89 [c]	
% CV	4.86				
P-value					
A	***				
B	***				
A x B	***				

Note: Results are in dry matter basis and are expressed as mean ± standard deviation (n = 3).

The different superscript letters are statistically different by Duncan's New Multiple Range Test ($p \leq 0.01$).

Gross energy level significantly affected by treatments and fermentation times. Treatment of cassava with yeast (T2) at 0 day had the highest energy of 3484.89 compared to other treatments. In addition, consider the treatment factor, yeast fermented cassava pulp (T2) and yeast fermented cassava pulp with ammonium sulfate (T5) showed high energy among treatments (Table 5). It could be noted that treatment using chicken manure caused reduction of gross energy. As fermentation days increased, the gross energy decreased, but there is no significant decrease in day 10, 20 and 30 (Table 5).

Table 5 Gross energy of fermented cassava pulp in each treatment at different fermentation time.

Treatment (A)	Fermentation Day (B)				Mean (A)
	0	10	20	30	
T1	3373.18 ± 118.32 [abc]	3329.66 ± 74.37 [abcd]	3291.40 ± 37.26 [abcde]	3191.94 ± 97.17 [bcdef]	3296.55 ± 102.04 [AB]
T2	3484.89 ± 47.92 [a]	3278.14 ± 37.91 [abcde]	3467.45 ± 26.69 [ab]	3349.05 ± 77.49 [abcd]	3394.88 ± 99.17 [A]
T3	3447.39 ± 87.29 [ab]	3027.34 ± 54.42 [efg]	3249.82 ± 23.98 [abcde]	3371.48 ± 84.58 [abc]	3274.01 ± 175.71 [AB]
T4	3021.83 ± 78.63 [efg]	2925.58 ± 65.45 [fg]	2917.09 ± 31.32 [g]	2928.13 ± 5.52 [fg]	2948.13 ± 86.10 [D]
T5	3330.51 ± 19.95 [abcd]	3346.50 ± 57.06 [abcd]	3342.34 ± 102.09 [abcd]	3330.48 ± 63.59 [abcd]	3337.46 ± 98.49 [A]
T6	3325.32 ± 224.55 [abcd]	3267.56 ± 42.89 [abcde]	3310.98 ± 86.16 [abcd]	3200.57 ± 51.37 [bcde]	3276.11 ± 117.90 [AB]
T7	3276.44 ± 160.04[abcde]	3232.31± 101.46[abcde]	3110.94 ± 11.95[cdefg]	3116.50 ± 38.29 [cdefg]	3184.05 ± 120.91 [BC]
T8	3205.85 ± 32.95 [bcde]	3144.66 ± 83.77 [cdefg]	3122.88 ± 39.20 [cdefg]	3113.10 ± 76.35 [cdefg]	3146.62 ± 65.04 [C]
T9	3254.61 ± 329.99[abcde]	3086.48 ± 33.91 [defg]	3133.08 ± 135.82 [cdefg]	3025.96 ± 55.78[efg]	3125.03 ± 18.78 [C]
Mean (B)	3302.23 ± 191.94 [b]	3182.03 ± 149.35 [a]	3216.22 ± 168.66 [b]	3180.79 ± 160.37 [b]	
% CV	3.28				

P-value	
A	***
B	***
A x B	***

Note: Results are in kcal/kg basis and are expressed as mean ± standard deviation (n = 3).

The different superscript letters are statistically different by Duncan's New Multiple Range Test ($p \leq 0.01$)

The pH condition is an important parameter for microbial activities. Yeast cells metabolism efficient at slightly acidic environment. Therefore, pH value in cassava fermentation were determined in this study. The results demonstrated that pH value significantly differ within the treatments and in fermentation days. The lowest pH value was observed in yeast fermented cassava pulp (T2) and yeast fermented cassava pulp with ammonium sulfate (T5) at day 20 and 30 of fermentation (pH of 3.8 – 4.1). It might be noted that the treatments with ammonium sulfate lower the pH values but all treatments with chicken manure increased in the pH values. The results also clearly showed that an increasing of fermentation time lower the pH value to 5.63 at 30 days (Table 6).

Table 6 The pH of fermented cassava pulp in each treatment at different fermentation time.

Treatment (A)	Fermentation Day (B)				Mean (A)
	0	10	20	30	
T1	4.61 ± 0.08 ^{n}	4.84 ± 0.06 ^{mn}	5.74 ± 0.11 ^{jk}	5.90 ± 0.13 ^{ij}	5.27 ± 0.59 ^{E}
T2	5.01 ± 0.08 ^{m}	4.85 ± 0.04 ^{mn}	5.66 ± 0.24 ^{jk}	5.31 ± 0.08 ^{l}	5.21 ± 0.34 ^{E}
T3	4.74 ± 0.06 ^{mn}	4.83 ± 0.08 ^{mn}	3.84 ± 0.04 ^{o}	4.12 ± 0.14 ^{o}	4.38 ± 0.44 ^{F}
T4	7.44 ± 0.05 ^{bc}	7.67 ± 0.16 ^{ab}	7.09 ± 0.10 ^{de}	6.59 ± 0.08 ^{fg}	7.20 ± 0.43 ^{A}
T5	4.94 ± 0.11 ^{m}	4.59 ± 0.04 ^{n}	3.91 ± 0.06 ^{o}	4.07 ± 0.08 ^{o}	4.38 ± 0.43 ^{F}
T6	6.98 ± 0.16 ^{e}	7.05 ± 0.02 ^{e}	5.51 ± 0.32 ^{kl}	5.70 ± 0.27 ^{jk}	6.31 ± 0.76 ^{D}
T7	7.35 ± 0.04 ^{cd}	7.39 ± 0.02 ^{bcd}	6.16 ± 0.21 ^{hi}	6.10 ± 0.05 ^{i}	6.75 ± 0.65 ^{C}
T8	7.38 ± 0.02 ^{bcd}	7.49 ± 0.05 ^{abc}	6.86 ± 0.08 ^{ef}	6.39 ± 0.05 ^{gh}	7.03 ± 0.46 ^{B}
T9	7.40 ± 0.08 ^{bcd}	7.76 ± 0.05 ^{a}	6.97 ± 0.38 ^{e}	6.47 ± 0.02 ^{g}	7.15 ± 0.53 ^{AB}
Mean (B)	6.21 ± 1.27 ^{a}	6.27 ± 1.38 ^{a}	5.75 ± 1.18 ^{b}	5.63 ± 0.92 ^{c}	
%CV	2.19				
P-value					
A	***				
B	***				
A x B	***				

Note: Results are in dry matter basis and are expressed as mean ± standard deviation (n = 3).

The different superscript letters are statistically different by Duncan's New Multiple Range Test ($p \leq 0.01$).

4. Discussion and conclusion

Feed cost especially protein ingredients contribute high production cost in animal industry. Cassava pulp can be one alternative to lower feed cost, but the low protein and higher fibre content limits its uses. The improvement of cassava pulp helps to increase nutrition value that will influence its uses. Nitrogen inorganic fertilizers had verified to improve cassava pulp nutrients when fermented with beneficial microorganisms [12]. In order to solve the use of inorganic fertilizers and promote the use of organic by-product, the present study efforted to investigate the use of chicken manure as organic nitrogen source of yeast in fermentation process of cassava pulp.

Organic ingredients analysis showed that cassava pulp had low crude protein of 1.99% (Table 2). This result related to [16], whereby samples from four different starch factories in northeast of Thailand, each season the mean CP was 2.17%. Cassava pulp used in this study also showed low average dry matter of 87.03% and gross energy 3556.67 kcal/kg compared to [17] which had average DM of 89.2% and GE of 3868.56 kcal/kg. Crude fibre of cassava ingredient in this study was 15.63% which lower than in [18] which had 19.3%. Crude fibre content is influenced by presence of cellulose in cassava root [19]. However, low crude fibre ingredient is preferred to monogastric animal feed.

The chicken manure had higher crude protein of 13.93% compared to cassava pulp 1.99%. But this percentage of CP in chicken manure is lower when compared to 15.4% CP of chicken manure from small holder farmers in Kenya reported by [20]. The crude fibre of chicken manure is 13.32% which is a result of undigestible materials, due to concentration of cell walls and ash [21]. Gross energy of chicken manure 2386.47 kcal/kg in this work was low compared to average of 2664 kcal/kg from 9 farms in Spain [22]. The chicken manure has comparable low gross energy due to low content of carbohydrate [20].

Fermentation of cassava pulp in this study revealed that crude protein in fermented cassava pulp significantly increased when chicken manure was used as nitrogen source. The highest increase was 6.28% observed in treatment of yeast fermented cassava pulp with chicken manure (T9) at day 30 of fermentation (Table 3). This increase may be due to the ability of yeast *Saccharomyces cerevisiae* to utilize carbon from carbohydrate of cassava pulp and nitrogen from chicken manure [23]. The *S. cerevisiae* cells may utilize organic and inorganic nitrogen in chicken manure because of presence of three permeases enzymes (MEP 1, MEP 2 and MEP 3), which facilitate the entrance of ammonia into the cell and linked with glutamate dehydrogenase then combine with glutamine by glutamine synthetase [24, 25]. Times of fermentation also affected the level of crude protein. As time increased also the crude protein level increased. The highest level was observed at 30 days but statistically not different between day 10, 20 and 30. This increase of crude protein is higher than those reported by [26], and [27] due to absence of nitrogen source, different time of fermentation and microorganism used in these studies.

Chicken manure showed possibility to be an alternative source of nitrogen to inorganic fertilizer in yeast fermentation of cassava pulp. The 2.4% increase of crude protein in this study is comparable related to [28] which reported 2.05% increment of crude protein in their study used a combination of 2% urea and 1.5% diammonium phosphate as a nitrogen source. However, the 2.4% increase in crude protein in this work was lower than 4.31% increase reported by [29], which used an easy availability of nitrogen source from 2% of peptone, and 8.9% [30], which utilized 4.05% urea together with 1% of diammonium phosphate as nitrogen supplementation to 2.02% yeast.

The lowest level of crude fibre of improved cassava pulp in this work was 11.4% in cassava pulp with ammonium sulfate (T3) at 20 days which was 2.79% decrease (Table 4). The 2.79% decrease is lower than 4.9% decrease in [28]. The lower decrease in the present study may be caused by less amount of ammonium sulphate (2%) used compared to combination of 2% urea and 1.5% diammonium phosphate. The crude fibre decrease because nitrogenous inorganic fertilizer decrease crude fibre [31]. The low crude fibre is important in feed because has higher digestibility compared to higher crude fibre which normally has low digestibility.

We noted that treatments without chicken manure had higher gross energy, due to high percentage of cassava pulp which contain carbohydrate [19] and low gross energy of chicken manure ingredient. Also, the gross energy decreased with increase of fermentation time. This may be due to the utilization of carbohydrate in cassava pulp to form other cellular components including protein in the yeast cells [28].

The pH condition is very important for yeast fermentation to provide proton gradient for uptake of nutrients and other metabolic processes [25]. *S. cerevisiae* yeast cells are efficiency at pH of 4 to 5 [32]. The lowest pH value in the present work was 3.91, observed in treatment contained cassava pulp with ammonium sulfate (T3) and cassava pulp with yeast and ammonium sulfate (T5) at 20 and 30 days. Longer period of fermentation decreased pH value. However, even though we found that use of chicken manure increased the pH of fermented cassava pulp, the best fermentation treatments in term of higher protein observed in yeast fermented cassava with chicken manure. This was possibly because *S. cerevisiae* can adapt up to 8.0 alkaline pH by the availability of special gene which utilizes iron and copper in chicken manure [21] for alkaline adaptation [33].

In conclusion, the results suggested that chicken manure can be used as a nitrogen source in yeast fermented cassava pulp to significantly increase protein content; however, it could lessen the gross energy and interfere with the pH value of an acidic condition in fermentation. The comparable low increase of crude protein observed may be caused by higher pH from chicken manure which led yeast to grow slowly for adaptation of slightly alkalinity environment. The best treatment in this work was treatment contained yeast fermented cassava pulp with chicken manure at 30 days. The next step is to investigate the nutritional and nutraceutical effect of this improved cassava pulp as an animal feed ingredient.

5. Acknowledgements

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