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Efficacy of fermented cassava pulp with chicken manure on nutrients digestibility, haematological parameters, and growth performance of barrow pigs

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ABSTRACT

Cassava pulp is a by-product from the starch industry which was used as cheap alternative livestock feed. However, it has a limitation of low protein and high fibre to use in monogastric animals. Nutrient improvement of cassava pulp usually employs the use of inorganic nitrogen fertilizer. Here we demonstrated the use of chicken manure as an organic nitrogen source in compensation for the inorganic nitrogen. This research aimed to investigate the efficacy of cassava pulp fermented by dried chicken manure with yeast (CPCM) in a maize-soybean-based diets of pigs on nutrients digestibility, haematological parameters, and growth performance of barrow pigs as alternative energy feed. The results showed that the CPCM had crude protein increase from 1.99% of the plain cassava pulp to 8.54% ($p < 0.05$), while the crude fibre of CPCM decreased from 15.63 to 13.85%. Investigation of the diets containing CPCM as a replacement of maize at 4 levels (0%, 5%, 10%, and 15%) in twenty-four castrated male pigs (57.13 ± 3.29 kgs initial bodyweight) revealed that the control diet (0%CPCM) had the greatest digestibility percentages in all categories including dry matter, crude protein, crude fibre, gross energy, and total phosphorus ($p < 0.05$). Among CPCM replacement diets, the 15%CPCM generally verified a greater digestibility. The results of the feeding trial showed that there was no significant difference ($p > 0.05$) in feed intake, weight gain, feed conversion ratio, and haematological parameters among the four treatment diets. The haematological results showed that all parameters fall under the normal ranges of haematological pigs' references. In conclusion, the results confirmed that CPCM can be used in the replacement of up to 15% in maize-soybean pig diets without any harmful effects.

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INTRODUCTION

Pig production is one of the important economic activities which contribute income and food to the increasing population. One of the constraints of pig production especially in poor resource farmers is the high price and inaccessibility of feed in which the feed cost may be higher up to 70% (Manyelo et al., 2020). In addition, pigs compete for food with humans. Rice and maize are used widely as pig feed but they are also preferred for human consumption. Thus, they cannot be spared enough for feeding pigs (Muhanguzi et al., 2012). Therefore, it is important to find a diverse feedstuff that can be an alternative to conventional pig feeds.

Cassava pulp is a solid fibrous by-product from the cassava starch industry. It is a good choice to use as a raw material for animal feed because, it has low cost and accounts for up to 30% of the whole original cassava root depending on the efficiency of the factory (Ghimire et al., 2015). However, the nutritional compositions are the problem for using it as an animal feed ingredient. Compared to maize, cassava pulp is high in fiber content up to 26% but low in crude protein content which is less than 2% (Heuzé et al., 2016). The common way to improve cassava pulp nutrients is the fermentation in the presence of effective microorganisms such as yeast and the inorganic nitrogen fertilizers. Several research on cassava pulp fermentation has been performed (Sugiharto, 2019). For examples; the study of Fermentation of cassava pulp with yeast, urea and di-ammonium phosphate, increased the crude protein of cassava pulp from 9.5% to 18.4% (Sengxayalth, 2017). Also, the study of effects of cassava pulp fermented with *Aspergillus oryzae* as a feed ingredient substitution in laying hen diets, where by urea was used as nitrogen supplement in the fermentation. The results showed that the crude protein of cassava pulp increased from 1.98% to 13.3%, while crude fibre decreased from 13.6% to 10.7% (Okrathok et al., 2018). However, the use of inorganic nitrogen commercial fertilizers is not sustainable in rural areas, especially for resource-poor farmers. Chicken manure and poultry litter have been used as pig feed by some livestock keepers in different places (Flachowsky, 1997). Dried chicken manure has a nitrogen of 3.5% and high protein of 422 g/kg which exceeds all forages (Trevino et al., 2002). Its availability, inexpensive, and high nitrogen inspire to use it as an alternative to inorganic fertilizers in cassava pulp improvement.

Therefore, to explore the new procedure to improve cassava pulp without the use of inorganic fertilizer, an investigation of chicken manure as a replacement for inorganic fertilizer in the fermentation of cassava pulp is of interest. Cassava pulp fermented with chicken manure and yeast was produced and used in replacement for maize in a soybean-maize based diet in this study. The nutrients' digestibility, haematological parameters, and growth performance were determined to evaluate the effect of the improved cassava with chicken manure as a pig feed. The information gained from this study will help the farmers to depend on available cheap raw materials hence sustainability.

Objectives

The general objective of this study was to evaluate the efficacy of the improved cassava pulp by fermentation with chicken manure and yeast on nutrients digestibility, haematological parameters and growth performance of pigs. Specifically, it sought to answer the following:

1. Increase crude protein and decrease crude fibre content of a cassava pulp.
2. Examine the level of digestibility of the improved cassava pulp in barrow pigs.
3. Assess the impact of the improved cassava pulp on haematological parameters in barrow pigs.
4. Assess the growth performance of the barrow pigs fed with the improved cassava pulp.

METHODS

Location, material collection, and preparation

This experiment was conducted at Silpakorn university farm, Phetchaburi IT campus, Thailand from March to June 2021. Chicken manure was collected from layer pens at university farm, removed trashes, and dried in a hot air oven at 60 °C for 3 days, then milled by using an electric flour mill machine. The ingredients for experimental diets were purchased from the EK animal feed store, Tha-yang district, Phetchaburi province, Thailand. Yeast bought at nearby campus store. Pigs were purchased from SC farm, Cha-am District, Phetchaburi province, Thailand.

Experimental design

A total of 24 castrated cross breed (large white ×landrace) × duroc jersey) male pigs average 57.13 ± 3.29 kg body weight was arranged in Randomized Complete Block Designed (RCBD) by using different initial weights as blocks. There were 4 treatment diets with 6 block replications.

Animal care and management

Before the experimental period, all pigs were adjusted by a feeding control diet for 5 days. On day one, each pig was weighted using a digital electronic weighing scale for initial weight and allotment into 4 treatments with 6 replications. Each pig was separated into a 1.00×1.20 metres pen and equipped with a feeder and water nipple. The animals were fed the experimental diets ad libitum, the ration added 2 times, morning 8.00 A.M. and evening 5.00 P.M for 28 days. On day-21st, all pigs were started to feed with 0.20% of chromic oxide (Cr_2O_3) as a digestibility indicator for 7 days. Approximately 200 grams of fresh clean samples of feces were collected from each pig morning and evening for five days and preserved in the refrigerator until analysis. On day-28th, approximately 5 milliliters of blood samples were randomly collected from 3 pigs in each treatment by 18-gauge needle size at jugular vein for haematological parameter analysis. Finally, all 24 pigs were measured for their final body weight.

Fermentation of cassava pulp

Cassava pulp fermentation was prepared by a method of Nukraew et al., (2019) with modification. Eighty-four kilograms of cassava pulp (70%) were thoroughly mixed with 34.8 kg of dried milled chicken manure (29%). Yeast *Saccharomyces cerevisiae* (1.2 kg as 1% of total weight) was thoroughly mixed with 4.8 kg of molasses (4% of total weight) plus 36 litres of water (30% of total weight) using a blender and paused for 30 minutes. Then all ingredients were thoroughly mixed together again by using spade on a polythene sheet. Finally, the feed was anaerobically packed tightly in plastic containers inside lined with polythene bags, then left to ferment for 20 days. After 20 days the containers were opened and the yeast fermented cassava pulp with chicken manure (CPCM) was dried under the sunlight for 2 days. Lastly, the CPCM samples were taken from each container for determination of pH, gross energy, and chemical composition by proximate chemical analysis (AOAC, 1990). The remaining were used for experimental diet formulations.

Experimental diets

The experimental diets were formulated using a maize-soybean based diet with CPCM inclusion levels of 0%, 5%, 10%, and 15% in replacement of maize (Table 1). The diet's formulation was based on National Research Council (1998) recommendation of growing pigs (BW 50-80 kg).

Table 1: Ingredients in the four experimental diets

Ingredient	Treatment diets			
	0%CPCM	5%CPCM	10%CPCM	15%CPCM
Maize/corn	66.55	61.08	55.58	50.10
SBM, 44% CP	21.30	21.36	21.44	21.52
CPCM	0.00	5.00	10.00	15.00
Rice bran	10.00	10.00	10.00	10.00
Palm Oil	0.19	0.62	1.05	1.45
L-Lysine, 98%	0.02	0.03	0.03	0.03
MCP, 21% P	0.27	0.31	0.35	0.41
Limestone	1.00	0.93	0.88	0.82
Choline Chloride, 2%	0.02	0.02	0.02	0.02
Vitamin-Mineral Premix	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00
Calculated nutrient composition (% of as fed basis)				
Dry matter, %	88.02	87.73	87.43	87.16
Metabolizable energy, kcal/kg	3,270	3,271	3,271	3,270
Crude protein, %	15.50	15.50	15.51	15.51
Crude fibre, %	4.15	4.52	4.88	5.25
Ether extract, %	2.17	2.50	2.81	3.37
Calcium, %	0.51	0.51	0.51	0.51
Total phosphorus, %	0.45	0.45	0.45	0.45
Available phosphorus, %	0.22	0.23	0.23	0.24
Lysine, %	0.75	0.75	0.75	0.75
Methionine, %	0.25	0.24	0.24	0.23

Statistical analysis

The data were analyzed by using one-way ANOVA. Treatment means were statistically compared by Duncan's New Multiple Range Test (DMRT) using the R program, at $p < 0.05$.

RESULTS

Chemical compositions of cassava pulp and chicken manure were analyzed before use in fermentation. Cassava pulp showed higher crude fiber, and gross energy, but lower in crude protein and pH compared to chicken manure (Table 2). The mixture of cassava pulp, yeast, and chicken manure with molasses was fermented for 20 days (CPCM). The chemical compositions of the CPCM were significantly changed after fermentation. The crude protein was slightly increased by 1.03%, while dry matter, crude fiber, gross energy, and pH were decreased by 1.19%, 0.72%, 34.4 kcal/kg, and 1.06, respectively (Table 3). CPCM was included in the formulation of experimental diets at 4 levels and the chemical composition of each formula was shown in table 4.

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

Parameters	**Cassava pulp**	**Chicken manure**
Dry matter (%)	87.03 ± 0.25	95.45 ± 0.08
Crude protein (%)	1.99 ± 0.19	17.90 ± 021.
Crude fibre (%)	15.63 ± 0.73	13.78 ± 0.77
Gross energy (kcal/kg)	3,456.667 ± 50.38	2,913.10 ± 14.06
pH value	4.67 ± 0.11	7.62 ± 0.06

Note :*Data are expressed as mean ± standard deviation (n = 3).*

Table 3. The chemical composition of cassava pulp mixed with chicken manure and yeast (CPCM) before and after fermentation.

Nutrients	Fermentation days		% CV	*p* value
	0	20		
Dry matter (%)	67.31 ± 0.54 [a]	66.12 ± 0.36 [b]	0.69	< 0.001
Crude protein (%)	7.51 ± 0.26[b]	8.54 ± 0.22 [a]	3.36	< 0.05
Crude fibre (%)	14.57 ± 0.24[a]	13.85 ± 0.11[a]	4.20	< 0.001
Gross energy (kcal/kg)	3154.93 ± 17.53[a]	3120.53 ± 15.37[b]	0.54	< 0.001
Total ash (%)	14.80 ± 0.37[a]	13.99 ± 0.38[b]	3.30	< 0.001
pH value	7.50 ± 0.03[a]	6.44 ± 0.08[b]	0.88	< 0.001

Note :*Results are in dry matter basis and are expressed as mean ± standard deviation (n =3) .The different superscript letters within rows are statistically different by DMRT Test (p ≥ 0.05)*

Table 4. Chemical composition of analysed experimental diets (as fed basis)

Item	Treatment diet			
	0%CPCM	5%CPCM	10%CPCM	15%CPCM
Dry matter, %	88.70	89.20	89.67	90.00
Gross energy, kcal/kg	3821.37	3830.03	3763.33	3752.07
Crude protein, %	14.81	14.44	14.00	15.54
Crude fibre, %	2.54	3.17	3.60	4.13
Ether extract, %	1.98	2.05	2.45	3.13
Total Ash, %	4.08	4.49	4.93	5.86
Total Calcium	0.92	1.14	1.35	1.64
Total Phosphorus, %	0.55	0.70	0.61	0.70
Chromic oxide, %	0.11	0.13	0.14	0.11

Barrow pigs were fed with experimental diets with different levels of CPCM for 28 days. The nutrient's digestibility was evaluated using the chromic oxide marker method at the last week of treatment. The percentage of nutrient digestibility showed a significant difference among the treatment group ($p < 0.05$) (Table 5). The control diet without CPCM had the highest nutrient digestibility. The inclusion of CPCM reduced nutrient digestibility of dry matter, crude protein, crude fiber, and gross energy, except the total phosphorus which showed significantly lowest digestibility in 10%CPCM. In general, the 15%CPCM was the second-best in nutrient digestibility followed by 5% CPCM diets and the 10%CPCM was the least.

Table 5. Nutrient's digestibility of barrow pigs fed with 0%, 5%, 10%, and 15% of CPCM diets

Nutrient	Treatment diet				% CV	*p value*
	0% CPCM	5% CPCM	10% CPCM	15% CPCM		
Dry matter, %	83.74 ± 0.16[a]	79.79 ± 0.10[c]	74.12 ± 0.09[d]	80.62 ± 0.06[b]	0.13	< 0.001
Crude protein, %	83.17 ± 0.65[a]	79.02 ± 1.29[b]	72.51 ± 0.97[c]	78.13 ± 0.89[b]	1.25	< 0.001
Crude fibre, %	29.99 ± 8.72[a]	21.69 ± 6.76[bc]	20.44±0.34[c]	28.56 ± 2.65[ab]	22.25	0.02
Gross energy, %	84.16 ± 0.11[a]	80.66 ± 0.23[c]	74.86 ± 0.34[d]	81.72 ± 0.15[b]	0.28	< 0.001
Total Phosphorus, %	73.01 ± 1.52[a]	72.98 ± 5.99[a]	60.47 ± 2.33[b]	75.34 ± 1.89[a]	4.88	< 0.001

Note: *Results are expressed as mean ± standard deviation (n = 6). The different superscript letters within rows of mean are statistically different by DMRT Test (p ≤ 0.05)*

The haematological test of complete blood count (CBC) of barrow pigs was determined at the terminal date to investigate the influence of experimental diets on the pig health status. The results showed that all parameters in all four experimental diets were not significantly different ($p > 0.05$) (Table 6). However, there was a slightly higher haematocrit (PCV) in animals fed diet with 10%CPCM and lymphocytes in animals fed 5%CPCM. In addition, an increasing trend of monocytes can be seen in 10%CPCM and 15%CPCM.

Table 6. Haematological test results of pigs fed with 0%, 5%, 10%, and 15% of CPCM diets

Parameter	Treatment				% CV	*p value*
	0% CPCM	5% CPCM	10% CPCM	15% CPCM		
Hemoglobin(g/dl)	12.97 ± 0.31	13.23 ± 0.57	13.25 ± 0.78	12.90 ± 0.1	3.49	0.74
Hematocrit (%)	42.30 ± 0.89	44.27 ± 1.28	51.30 ± 8.49	43.27± 0.23	7.41	0.21
RBC (M/uL)	7.20 ± 0.35	7.08 ± 0.42	7.07 ± 0.12	7.12 ± 0.25	4.53	0.96
MCV (fL)	58.87 ± 3.96	62.63 ±2.15	61.10 ± 5.37	61.10± 5.37	5.42	0.60
MCH (pg)	18.03 ± 1.00	18.70 ± 0.70	18.80 ±1.42	18.13 ±0.56	4.86	0.69
MCHC (g/dL)	30.70 ± 0.95	29.87 ± 1.16	30.75 ± 0.35	29.80± 0.10	2.7	0.42
Platelet (K/uL)	228.33 ± 52.05	156.67 ± 117.20	248.00 ± 46.67	237.67 ± 61.44	36.31	0.54
WBC (K/uL)	17.83 ± 0.61	14.93 ± 3.024	17.05 ± 0.21	17.20 ± 1.40	10.84	0.31
Neutrophil (%)	45.00 ± 3.00	33.00 ± 6.08	39.00 ± 4.24	30.67 ±17.6	27.82	0.39
Lymphocyte (%)	49.67 ± 3.06	64.00 ± 7.00	55.00 ± 8.49	53.33 ± 7.09	11.57	0.13
Monocyte (%)	1.67 ± 0.58	1.00 ± 0.00	4.00 ± 4.24	13.33 ± 19.63	208.59	0.50
Eosinophil (%)	3.67 ± 0.58	1.67 ± 0.58	2.00 ± 0.00	2.67 ± 2.89	63.00	0.50

Note: *Results are expressed as mean ± standard deviation (n = 3).*

After 28 days of treatment, the growth performance parameters were evaluated. As shown in table 7, the results revealed that there were no statistically significant differences among groups of diets ($p \leq 0.05$). However, the animals fed with the control diet (0%CPCM) had a slightly higher mean body weight gain of 25.8 kg. The control diet and 15%CPCM had a better feed conversion ratio of 2.84 and 2.86, respectively. Also, a diet containing 15% CPCM had a low feed intake compared to other diets.

Table 7. Growth performance of pigs fed with 0%, 5%, 10%, and 15% of CPCM diets

Item	Treatment diet				% CV	*p value*
	0% CPCM	5% CPCM	10% CPCM	15% CPCM		
Feed intake per animal (kg)	73.34 ± 9.06	73.50 ± 8.19	73.59 ± 9.67	67.56 ± 13.49	10.01	0.61
Body weight gained (kg)	25.80 ± 2.73	22.96 ± 1.94	22.48 ± 4.66	23.61 ± 4.97	15.88	0.32
Average daily weight gain (ADG), gram/day	921.43±0.10	820 ± 0.07	802.86 ± 0.17	843.21 ± 0.18	15.81	0.33
Feed conversion ratio (FCR)	2.84 ± 0.48	3.20 ± 0.53	3.27 ± 0.76	2.86 ± 0.97	22.62	0.52
Feed cost per 1 kg (BHT)	11.89	11.78	11.67	11.55		
Meat cost /kg gained (THB)	33.77	37.70	38.16	33.03		

Note: *Results are expressed as mean ± standard deviation (n = 6), The different superscript letters within rows of mean are statistically different by DMRT Test ($p \leq 0.05$). The feed conversion ratio was calculated as feed intake divided by body weight gain. Body weight gain was calculated as final body weight minus initial body weight. Feed cost per 1 kg meat gained calculated as FCR times feed cost of 1 kg. Feed intake obtained by summation of feed fed to each experimental animals in 28 days. Meat cost per 1kg gained calculated by multiplying FCR and feed cost per 1 kg.*

DISCUSSION

Cassava pulp can be one alternative choice to lower the feed costs, but the low protein and high fibre content limit its uses. The improvement of cassava pulp nutritive value could promote its uses especially in monogastric animals. Nitrogen inorganic fertilizers have been verified to improve crude protein content of cassava pulp when fermented with beneficial microorganisms, (Sugiharto, 2019). Alternatively, in order to reduce the use of inorganic fertilizers and promote the use of organic by-products, the present study attempted to investigate the use of chicken manure as an organic nitrogen source in the fermentation of cassava pulp.

Analysis of chemical compositions of cassava pulp used in this study showed that crude protein and crude fiber levels were comparable to those reported by Heuzé et al., (2016). The crude protein, crude fiber, and pH of chicken manure are also related to previous reports (Flachowsky, 1997; Lanyasunya et al., 2006). The crude protein of cassava pulp increased from 1.99 to 7.51% after being mixed with chicken manure and yeast, then to 8.54% after fermentation. The final crude protein in this study related to 7.91%, as reported by (Iyayi & Losel, 2001), who fermented cassava pulp with one percent of *S. cerevisiae* and peptone for 21 days. The ability of yeast to utilize the supplemented organic and inorganic nitrogen was reported previously (Jiranek et al., 1995; Kayombo et al., 2021; Magasanik & Kaiser, 2002). However, the improved crude protein level at 1.03% in this work was low compared to 8.9%, as reported in (Sengxayalth & Preston, 2017), which use 4.05% urea, 1% diammonium phosphate, and 2.02% yeast. The low increment of crude protein may be due to the low initial nitrogen supplementation and yeast inoculation. Also, the low increase of crude protein content of this work may be caused by higher pH from chicken manure which interferes with optimal conditions of pH 5 for *S. cerevisiae* yeast (AL-Sa'ady, 2014). Dry matter, crude fiber, and gross energy decreased after 20 days of fermentation, which may be caused by the conversion of cassava pulp carbohydrate to protein (Kasprowicz-Potocka et al., 2016). However, the nutritional status of CPCM revealed in this work is lower compared to maize which has an average dry matter of 86.30%, crude protein 9.4%, crude fibre 2.5%, and gross energy of 4,469.41 kcal/kg (Heuze et al., 2017).

The CPCM was formulated in the maize-soybean based diet in replacement to maize from 0% to 15% and was fed the barrow pig for 28 days. Determination of nutrient's digestibility showed that there was significant different between the control diet and the CPCM diets. The lower crude fiber in control diet compare to CPCM diets may influence to the digestibility percentage (Banerjee, 2018). The results of digestibility of 78.13% crude protein, 80.62% dry matter, 81.72% gross energy, and 28.56% crude fibre in present work, is lower compared to 84.9 % crude protein, 86.1% dry matter, 86.3% gross energy and 67.9 % crude fibre as stated in (Huu & Khammeng, 2014) who included 12% of the fermented cassava pulp diet on 15 weaned male pigs. However, the crude protein digestibility reported herein was greater when compared to 60.67 % reported by (Araújo et al., 2016) who used 25% of fermented cassava pulp on18 castrated pigs. The difference may be caused by the level of fermented cassava pulp in the diets and pigs used.

The haematological test was conducted to verify the influence of the CPCM diets that included chicken manure on the pig health. The results of all haematological parameters from the pigs fed with CPCM diets were not different ($p > 0.05$) compared to control diet treatment. Also, all parameters fall within ranges of haematological studies of pigs as reported by (Etim et al., 2014) and within the range of pig haematological references by Weiss & Wardrop (2011). This meant that the diets did not show any opposing effect during experimental periods. However, there were slightly above normal range of haematocrit (PCV). The increase of haematocrit together with red blood cell may be an indicator of efficient in blood making (erythropoiesis) in the experimented animals (Togun et al., 2007). Also, the lymphocytes count in animals fed with 5% CPCM (64 %) was slightly above normal range of 39 - 62%. The increase in neutrophil: lymphocytes ratio may be indicator of stress (Minka & Ayo, 2007); however, the higher lymphocytes lower the ratio thus may indicate less stress in the pigs. Growth performance of CPCM treated pigs were not significant different from those with control diet ($p > 0.05$). These results demonstrated that CPCM can replace the maize up to 15% without any adverse effect on growth performance. This result was supported by previous reports which suggested that different inclusion levels of fermented cassava pulp between 12 to 28% has no harmful effect on growth performance (Hu et al., 2008; Huu & Khammeng, 2014; Sengxayalth & Preston, 2017).

CONCLUSION AND RECOMMNEDATION

Based on this research cassava pulp crude protein can be increased by fermentation with chicken manure and yeast for twenty days. The improved cassava pulp with chicken manure and yeast can be used as alternative protein improved energy ingredient up to15% without any harmful effect on nutrient digestibility, haematological parameters and growth performance of barrow pigs. We recommend that the higher percentage replacement of this improved feed (cassava pulp fermented with chicken manure and yeast) should be investigated to reveal the ideal replacement. Also, the nutraceutical effect should be studied due to the presence of yeast and fibre which can act as probiotic and prebiotic respectively.

LIMITATIONS OF THE STUDY

The hydrocyanic acid (HCN) as an anti-nutritional factor in cassava was not analysed in this research, because the process of crushing the cassava into pulp in starch industries, fermentation, and drying, all are methods of reducing HCN. Thus, we are sure the CPCM had very low HCN, less than 10 mg/kg as recommended by FAO and WHO, due to the combined processing methods.

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