IMPROVEMENT OF CASSAVA PULP NUTRIENTS BY YEAST FERMENTATION WITH CHICKEN MANURE AND ITS EFFICACY ON DIGESTIBILITY, HAEMATOLOGICAL PARAMETERS AND GROWTH PERFORMANCE OF BARROW PIGS

By
Mr. Simon ANTHONY KAYOMBO

A Thesis Submitted in Partial Fulfillment of the Requirements
for Master of Science (BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
Graduate School, Silpakorn University
Academic Year 2021

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โดย
Mr.Simon Anthony Kayombo

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีววิทยาศาสตร์เพื่อเกษตรกรรมที่ยั่งยืน แผน ก แบบ ก 2 (หลักสูตรนานาชาติ)
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2564

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Field of Study	(BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
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Graduate School Silpakorn University in Partial Fulfillment of the Requirements for the Master of Science

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621120009 : Major (BIOSCIENCE FOR SUSTAINABLE AGRICULTURE)
Keyword : Cassava pulp, Chicken manure, Yeast Fermentation, Pigs, Saccharomyces cerevisiae

MR. SIMON ANTHONY KAYOMBO : IMPROVEMENT OF CASSAVA PULP NUTRIENTS BY YEAST FERMENTATION WITH CHICKEN MANURE AND ITS EFFICACY ON DIGESTIBILITY, HAEMATOLOGICAL PARAMETERS AND GROWTH PERFORMANCE OF BARROW PIGS THESIS ADVISOR : ASSISTANT PROFESSOR PATTARAPORN POOMARIN, Ph.D.

ABSTRACT

One of the constraints of animal production is feed availability and cost. The aim of this research was to investigate the use of chicken manure (CM) as a nitrogen source to *Saccharomyces cerevisiae* yeast fermentation of cassava pulp and its efficacy on nutrient digestibility, haematological parameters, and growth performance of barrow pigs. Four fermentation experiments of cassava pulp were conducted in different treatment conditions and fermentation times in order to improve the nutrient status of cassava pulp. The results showed that the yeast fermented cassava pulp with chicken manure (YFCP) had crude protein increase from 1.99% of the plain cassava pulp to 8.54% in YFCP ($p < 0.05$), while the crude fibre of YFCP decreased from 15.63 to 13.85%. The best improvement of YFCP was used in the formulation of diets as a replacement of maize at 4 levels (0%, 5%, 10%, and 15%). Twenty-four castrated male pigs (57.13 ± 3.29 kgs bodyweight) were separated into 4 groups, with 6 pigs for each group. The results revealed that the nutrient digestibility was significantly different ($p < 0.05$) among groups. The control diet (0%YFCP) had the greatest digestibility percentages in all categories included dry matter, crude protein, crude fibre, gross energy, and total phosphorus. Among YFCP replacement diets, the 15%YFCP generally demonstrated 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 normal ranges of haematological pigs' references. In conclusion, the results confirmed that YFCP can be used in replacement up to 15% in maize-soybean pig diets without any harmful effects.

ACKNOWLEDGEMENTS

First, I would like to thank the Almighty God for blessings of healthy life, study opportunities, and successful completion of my master’s degree program. Then my sincere gratitude extends to the following institutions and people to whom this study would be impossible.

To Thailand International Postgraduate Program (TIPP) under Thailand International Cooperation Agency (TICA) for a full funded scholarship of master’s degree of Bioscience for Sustainable Agriculture program. Their financial support is greatly valued.

To my work supervisor, employer (Wanging’ombe district), Ministry of foreign affairs and East Africa Cooperation under the government of United Republic of Tanzania for their study leave permission to develop my career abroad.

To the Dean of Animal science and agriculture Technology (ASAT), Silpakorn University Assoc. Prof. Watcharaporn Ruamthum (Ph.D.), together with chairperson of Bioscience of Sustainable Agriculture program Asst. Prof. Chaowanee Laosutthipong (Ph.D.) and all staff, for admission and their technical facilitation of my study.

To my enthusiastic research advisor Asst. Prof. Pattaraporn Poommarin (Ph.D.), my research co-advisor Asst. Prof. Panida Duangkaew (Ph.D.) and my academic advisor Assoc. Prof. Mana Kanjanamaneesathian. Their instructions, supervision, recommendations, corrections, follow-ups and encouragement empowered me to finish this study on time.

To all my fellow students represented by our senior doctoral candidate Phy. Manaw Sangfuang, my classmates (Felis, Kyaw, Leo, Pheak, Thithi and Zaw) and all other Thai friends who reduced boredom and homesickness to me in and out of class. This companionship was very important and made life easy and joyful, otherwise life would have been very difficult.

To my uncles (Gerald, Ignas and Adriano Gumbo), my siblings (Edward, Anna, Abdon, Maria Masukuzi, Paul and Peter Kayombo) who participated in financing my education from primary to college. They generously supported me, regardless of the poor resources they had.

To my wife Josephine Moi and our daughters Nyotahelen and Nurucesilia

Kayombo for agreeing to stay lonely during my study time. Their prayers and hopeful waiting had encouraged me.

Lastly, I would like to dedicate this work to my beloved mother Helena and her young sister Cecilia together with my grandmother Maria Gumbo who cared for me in early life. Their difficult life gave me motivation and passion to study hard so that one day I can change their life. Unfortunately, they passed away before the success time. Let God place their souls in peace in heaven.

Simon ANTHONY KAYOMBO

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Description
AIA	Acid-insoluble ash
ANOVA	Analysis of variance
AS	Ammonium sulfate
ASAT	Animal Science and Agriculture Technology
ADF	Acid-detergent fiber
BSA	Bioscience for Sustainable Agriculture
Ca	Calcium
CIT	International Center for Tropical Agriculture
CM	Chicken manure
CRD	Completely randomized design
CP	Crude protein
Cr_2O_3	Chromic oxide
CF	Crude fiber
CPu	Cassava pulp
CV	Coefficient of variation
DAP	Diammonium phosphate
DM	Dry matter
DMRT	Duncan’s New Multiple Rang Test
dL	Deciliter
DOAE	Department of Agriculture Extension
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FCP	Fermented cassava pulp
fL	Femtoliters
GE	Gross energy
g	Gram
ha	hectare
HCN	Hydrogen Cyanide
kg	kilogram
KU	Kasetsart University
L	Litter
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MPV	Mean platelet volume

ME	Metabolizable Energy
MEP	Methylerythritol Phosphate
mg	milligram
MJ	Mega Joules
mmt	Million metric ton
No.	Number
NDF	Neutral Detergent Fiber
NRC	National Research Council
P	Phosphorus
p	Probability
ppm	Parts per million
PCV	Packed Cell volume
pg	picogram
pH	Potential of Hydrogen
std	Standard deviation
T	Treatment
THB	Thai Baht
TICA	Thailand International Cooperation Agency
TiO_2	Titanium dioxide
TIPP	Thailand International Postgraduate Program
TM	Treatment with Molasses
TS	Treatment with Sugar
TSH	Tanzanian shillings
RBC	Red Blood Cells
RCBD	Randomized Completely Block Design
Y	Yeast
YFCP	Yeast Fermented Cassava Pulp
WBC	White Blood Cells
WHO	World Health Organization
w/v	weight to volume ratio
ul	microliter
%	Percent
<	Less than
>	Greater than
&	And
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Rationale of the study

Pig production is one of the important economic activities which contribute income and food to the increasing population. Pork plays a major role in food security and nutrition for reducing competition of high religion acceptable meat such as beef, chicken, sheep, and goat meat (Legg, 2017). Its prolificacy and intensively favor higher meat production in a short period (Gourmelen & Moan, 2004). According to FAO (2020), pig meat is the second most utilized meat after poultry and contributes 109.28 million metric ton (32.49%) out of 336.36 million metric ton. One of the constraints of pig production especially in poor resource farmers is the inaccessibility of feed or/and the high price of feed which may be higher up to 70% (Manyelo et al., 2020). Pigs compete for food with humans such as cereals and soybeans. Rice and maize are preferred for human consumption such that they cannot be spared for feeding pigs (Muhanguzi et al., 2012). Therefore, it is important to find diverse sustainable feedstuffs which are cheap, available, and precisely can replace conventional pig feeds.

Since 2000, the production of cassava (*Manihot esculent* Crantz) in the world has increased by 60% (Montagnac et al., 2009). This increase may be accelerated by its agronomic practice because cassava plants require comparatively lower inputs. Moreover, the cassava plant can be a good alternative in the challenge of climatic change because it is drought-resistant, can be grown in the mountainous slope of low fertility and acid soil tolerant, (Food & Organization, 2013; Montagnac et al., 2009). This plant can be a sustainable opportunity for livestock keepers to utilize it as alternative animal feed to reduce production costs.

The utilization of industrial by-products in monogastric animals will reduce food competition and increase profitability in livestock production (Falvey, 2015). Cassava pulp is a solid moist fibrous material remaining after starch extraction from cassava roots. This residue is sold at a low cost and may reach up to 30% of the whole original cassava roots depending on the efficiency of cassava starch processing factories (Ghimire et al., 2015). The nutritional problem of cassava pulp is higher fibre content up to 26.9%, and low crude protein less than 2.2% (Heuzé et al., 2016a). Several efforts have been done by scientists to solve this problem. Nowadays scientists can improve the cassava pulp by fermenting with microorganisms and using it as feed in the livestock with a higher nutritional value than before (Sugiharto, 2019).

Yeast fermented cassava pulp (YFCP) is one of the improved cassava pulps which can help animal producers to supplement energy and protein source to their animals (Huu & Khammeng, 2014). Also, it is a way of environmental conservation because the cassava pulp as a waste if not treated will produce an unpleasant smell and putrefaction hence environmental pollution (Soewarno et al., 2012). Moreover, the presence of yeast and higher fibre may act as probiotic and prebiotic and bring the nutraceuticals effect to animals (Chirinang & Oonsivilai, 2018; Shareef & Al-Dabbagh, 2009; Sugiharto et al., 2017)

For efficiency increase of crude protein in cassava pulp, inorganic nitrogenous fertilizers are required to the microorganisms as a source of nitrogen during fermentation (Hang et al., 2019). However, these commercial inorganic fertilizers are expensive and sometimes are not available in rural areas. The aim of this research was to investigate the use of chicken manure as a nitrogen source to *Saccharomyces cerevisiae* yeast fermentation of cassava pulp and its effect on nutrients digestibility, haematological and growth performance of barrow pigs. Possibly this is the first research to provide an alternative for the commercial inorganic source of nitrogen by organic source (chicken manure). The work may influence the sustainable reuse of farm by-products.

1.2 Objectives of the study

i. To increase crude protein and decrease crude fiber content of cassava pulp by yeast fermentation with chicken manure.

ii. To evaluate the efficacy of cassava pulp improved by yeast fermentation with chicken manure on nutrients digestibility, hematological parameters and growth performance of barrow pigs.

1.3 Hypothesis of the study

i. If cassava pulp is fermented by yeast with chicken manure, then crude protein and crude fiber content will be improved.
ii. If cassava pulp improved by yeast fermentation with chicken manure, then nutrients digestibility, hematological parameters and growth performance of pigs fed experimental diets will have positive effects or equal to the control diet.

1.4 Scope of the study

i. Improvement of cassava pulp nutrient composition including crude protein and crude fibre by yeast fermentation with chicken manure
ii. Feeding efficacy of the improved cassava pulp on nutrients digestibility, hematological parameters, and growth performance of barrow pigs.

Note; The hydrocyanic acid (HCN) as 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 sure the YFCP had very low HCN, less than 10 mg/kg recommended by FAO and WHO, due to the combined processing methods.

CHAPTER 2

LITERATURE REVIEW

2.1 Cassava production

Cassava plant (*Manihot esculenta* Crantz) is the most multipurpose important tropical root crop. According to FAO estimates, 280 million tonnes of cassava was produced worldwide in 2012. Africa accounted for 56%, Asia for 30%, and Latin America and the Caribbean for 14% of the total world production, with an increase of 60% since 2000 (Howeler, 2013). This increase is caused by the demand for cassava as food in Africa and as animal feed in Asia (Morgan & Choct, 2016). As shown in Table 1, according to the world atlas website on April 25, 2017, the best 20 cassava producing countries in the world, all come from tropical regions, the top three are Nigeria, Thailand, and Indonesia (Nag, April 25, 2017).

Table 1: The best twenty countries cassava producer in the world

Rank	Country	Production (in tons)
1	Nigeria	47,406,770
2	Thailand	30,227,542
3	Indonesia	23,936,920
4	Brazil	21,484,218
5	Angola	16,411,674
6	Ghana	15,989,940
7	Democratic Republic of the Congo	14,611,911
8	Viet Nam	9,757,681
9	Cambodia	7,572,344
10	India	7,236,600
11	Malawi	4,813,699
12	United Republic of Tanzania	4,755,160
13	Cameroon	4,596,383
14	China, mainland	4,585,000
15	Mozambique	4,303,000
16	Benin	3,910,036
17	Sierra Leone	3,810,418
18	Madagascar	3,114,578
19	Uganda	2,979,000
20	Rwanda	2,948,121

Source: Nag (2017)

In Asia, cassava is grown to some extent all over the tropical and subtropical countries in the less fertile sidehills areas (Onwueme, 2002). In 2003, Asia produced 30% of all cassava in the world, and it is used mainly as animal feed, starch extraction, and some for human food (Howeler, 2006).

In Thailand, cassava is the third most important economic crop (Treesilvattanakul, 2016). Even though cassava is not a staple food in Thailand; however, it plays a major role in the economy as food, feed, and fuel (Treesilvattanakul, 2016). Also, Thailand is one of the top producers of cassava starch and cassava roots (Win, 2017). Cassava is mainly grown as a cash crop in the arid and non-irrigated areas in Thailand. In 2014, approximately 1.5 million hectares of cassava were planted by Thai farmers, and cassava productivity was 71.71 tans/ha (Praneetvatakul & Vijitsrikamol, 2017). In 2016, over 31 million tons of cassava were produced in Thailand. The most grown varieties are Rayong 1, Rayong 5, Rayong 60, Rayong 90, and Kasetsart 50. (Keaokliang et al., 2018) (Table 2).

Table 2: Cassava varieties grown in Thailand

S/N	Variety	Planted Area (ha)	Percentage of production
1	Rayong 1	383,973	36.8
2	Rayong 5	136,228	13.1
3	Rayong 60	216,602	20.7
4	Rayong 90	150,377	14.4
5	Kasetsart 50	156,910	15.0
	Total	**1,044,090**	**100**

Source: DOAE (1999)

Cassava processing is required to reduce hydrogen cyanide (HCN), the toxic antinutritional substance, prior to consumption. Hydrogen cyanide or prussic acid is a poisonous gas or liquid that is produced by cyanogenic glycosides as a protective mechanism after damage from many plants. Cyanogenic glycosides may be found in different cyanogenic food plants, for example, taxiphyllin in bamboo shoots, linamarin in cassava (Siritunga, 2003). Cassava has two major cyanogenic glycosides, (i), linamarin 80 – 95%, and (ii) lotaustralin 5 - 20% of total glycosides (Cereda, 1996; Montagnac, 2009). There are several ways of reducing HCN in cassava, including drying and fermentation. Crushing and sun-drying cassava root remove 96 to 99% of total cyanogens, (Montagnac, 2009).

Fresh cassava pulp has HCN of 72.2 mg/kg, (Srisaikham et al., 2018). However, the level of HCN should be reduced to below 10 mg/kg as recommended by FAO and WHO through the process such as cell disruption, drying, drop in pH, and strong heating (Lounglawan, Khungaew, & Suksombat, 2011). Also, about 98% of the free cyanide was lost by ensiling cassava roots with poultry litter for 8 weeks, (Tewe, 1992). A fermentation period of 3 to 5 days is enough to reduce cyanide (Ekwe et al., 2009).

2.2 Cassava pulp

Cassava pulp, also known as pomace, is a solid moist fibrous material remaining after starch extraction from cassava root. This residue may reach up to 30% of the whole original cassava root depending on the efficiency of cassava starch processing (Aro et al., 2010; Ghimire et al., 2015; Djuma'ali et al., 2011). Thailand is the world-leading country for cassava starch exporters. About 93% of starch in the world was exported from Thailand and Vietnam (Khempaka et al., 2013).

Cassava pulp is produced approximately 300 kilograms from 1 ton of cassava root (Ghimire et al., 2015). This waste some time was dumped in the lowland and pollutes the environment. Under anaerobic condition, cassava pulp is fermented and generate methane which contributes to global warming (Ghimire et al., 2015).

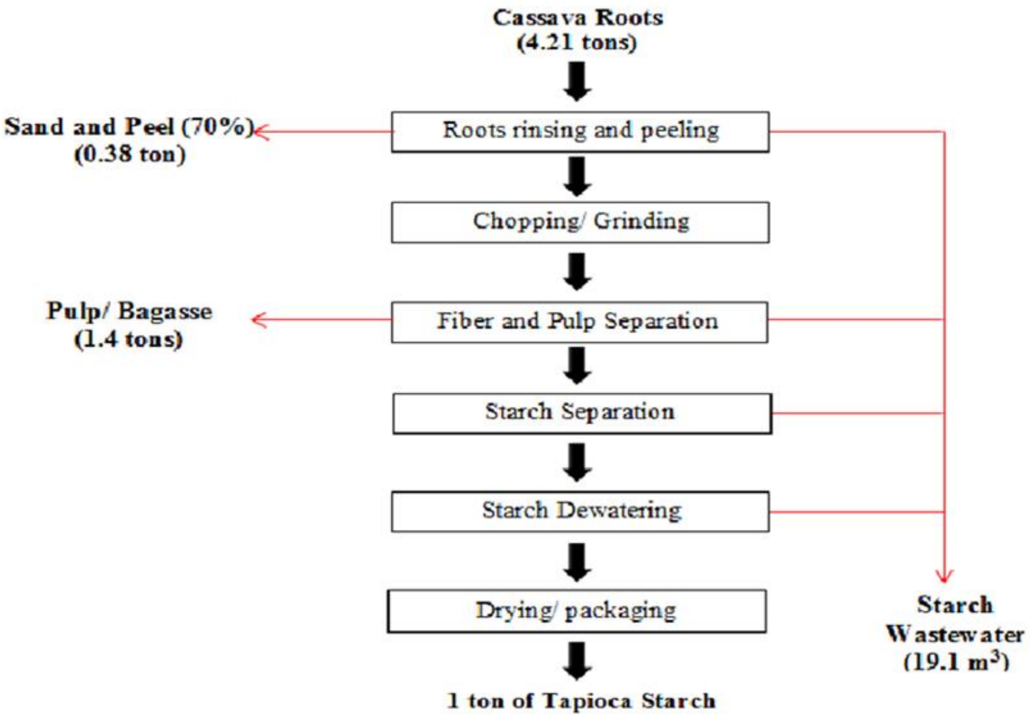


Figure 1 Cassava root processing in starch industry and pulp production

Source: Sulaiman, & Rahim (2014).

2.2.1 Nutrient composition in cassava pulp

Nutrients are molecules in the feed that all organisms require to make energy, grow, develop, and reproduce. The organisms break down food into small parts and absorb it into the body for nourishment. The nutritional composition of cassava root is the nutrients present in cassava in terms of quality and quantity. These nutrients are crude protein, lipids, ether extract, and micronutrients (vitamins and minerals). The nutrition composition in the field of animal nutrition is determined by proximate analysis. The nutrient composition of cassava pulp is shown in Table 3. It contains comparatively high fibre and low protein composition for use as monogastric animal feed (Heuzé, 2016).

Table 3 Nutrient's composition of cassava pulp

Main analysis	Unit	Average	Minimum	Maximum
Dry matter	as % feed	89.2	83.5	94.8
	% DM	18.4*		
Crude protein	% DM	2.2	1.1	3.4
Crude fiber	% DM	16.7	12.1	26.9
NDF	% DM	36.7	7.3	46.7
ADF	% DM	19.3	3.3	35.2
Lignin	% DM	3.6		
Ether extract	% DM	0.6	0.2	2.0
Ash	% DM	4.3	1.5	6.5
Gross energy	MJ/kg DM	16.2	14.7	17.5

Source: Heuzé et al. (2016) * from Keaokliang et al., 2018

2.2.2 Nutritional improvement of cassava pulp by fermentation

Fermentation is a metabolic process that involves the chemical breakdown of organic materials through the action of enzymes from microorganisms such as yeasts, bacteria, or other microorganisms. Fermentation of cassava pulp is one of the less expensive ways of increasing the quality of the cassava pulp. The important factors in fermentation are microorganisms, substrate, moisture content, and temperature, (Okrathok et al., 2018).

Yeast (*Saccharomyces cerevisiae*) fermentation of cassava pulp increases crude protein, true protein, and lysine, but also lowers cyanide content and crude fiber (Khampa et al., 2011). *S. cerevisiae* is the most valuable species because of its lifestyle of 'make-accumulate-consume'(Hagman et al., 2011). It produces ethanol which is toxic for most microbial species. After accumulation of the ethanol, *S. cerevisiae* consumes it hence promoting its own growth (Parapouli et al., 2020; Thomson et al., 2005).

In order to accelerate fermentation and increase proteins, nitrogen is usually supplemented in the fermentation for protein synthesis by microorganisms. These are urea, ammonium sulfate, diammonium sulfate, and ammonium nitrate (Norrapoke et al., 2018; Sugiharto, 2019). Urea is used mostly in ruminant animals at the recommended level. In monogastric animals, it is not commonly used since it causes damage to gastrointestinal mucosa thus reducing nutrient absorption and decreasing the growth performance (Patra & Aschenbach, 2018). Most previous research reported that yeast requires 120 to 140 milligrams of nitrogen per litre, but later research reported yeast requires 267 and others reported 400 milligrams of nitrogen per litre in 200 grams of glucose (Jiranek et al., 1995; Mendes-Ferreira et al., 2004). Table 4 showed some research works related to the improvement of cassava pulp with different microorganisms at different fermentation time.

Table 4 Examples of cassava pulp improvement by fermentation

Ingredient's formula	**Improvement**		**Fermentation Time**	**Source**
	Before	**After**		
Cassava pulp (% 92.9 DM) Urea (4.05%) DAP (1%) Yeast (*Saccharomyces cerevisiae)* (2.02%)	CP 2% DM 29.4%	CP 18% DM 19.2%	9 days	Sengxayalth & Preston, (2017)
Cassava pulp Urea *A. charticola*	CP 2.14% CF 25.6%	CP 11.3% CF 22.7%	4 days	Sugiharto, (2019)
Cassava pulp Urea (2%) DAP (1.5%) *Aspergillus niger* *Bacillus subtilis*	CP 1.2% CF 26.2%	CP 14.9% CF 21.8%	21 days	Hang et al. (2019)

2.2.3 Comparative cost of cassava pulp to conventional energy feedstuffs

The common feedstuffs used for pigs are maize, rice, and soybean. Maize and rice are the main staple food in tropical regions (Ceballos et al., 2004). Under good management, cassava has a lower production cost compared to maize, rice, and soybean. Cassava also is an alternative to the challenge of climate change because it can tolerate drought and low nutrient soil (Save, 2013). The following data (Table 5) is a comparative price of feed ingredients from EK animal feed store Tha yang – District, Phetchaburi province in Thailand.

Table 5 Comparative cost of different ingredients compared to cassava

Ingredient	Cost/kg (THB)
Cassava pulp	4.5
Corn/Maize	9.6
Fresh Rice Bran	10.3
Soybean meal, 46% CP	18

Note; The price given above according to EK animal feed store Tha yang – District, Phetchaburi province, Thailand.

2.3 Chicken manure as animal feed ingredient

Chicken manure or poultry litter has been used directly as pig feed by some livestock keepers in different places. For example, Nigerians use dried poultry manure to feed pigs. In southeast Asia, farmers construct layer cages 1.5 meters above the pig pens, thus the excreta fall in pens and consumed direct leading pigs to get 6.3 – 14.6% of layer manure (DM) in their ration (Flachowsky, 1997). Chicken manure should be dried at 40 to 60ºC to remove harmful microorganisms prior to use as animal feed.

Dried chicken manure has a nitrogen of 3.5% and high protein at 422 g/kg which exceeds all forages (range from 110 to 200g/kg) (Trevino, Ornelas, & Barragan, 2002). The crude protein of chicken manure ranges from 15.4% to 31% (Lanyasunya et al., 2006; Trevino et al., 2002). The use of this waste at the recommended level does not show any negative effect, such that finishing pigs can replace up to 66% of maize fraction diet (Adesehinwa et al., 2010).

2.4 Nutrient digestibility in pigs

Nutrient digestibility is a percentage of a feedstuff that is digested in the digestive tract and absorbed into the body for various metabolism activities. There are three main methods for the determination of nutrient digestibility: 1) total collection/convention method, 2) Marker/Index method, and 3) Substitution/ difference method (Zhang & Adeola, 2017).

The collection method is a technique whereby total feed intake and total feces are collected using indicators such as chromic oxide (Cr_2O_3) or ferric oxide. The adjustment period is 5-7 days and the collection period is 4-6 days. It is recommended that 1 g of ferric oxide added to 100 g of feed is sufficient for pigs up to 50 kg body weight and 2 g of ferric oxide added to 100 g of feed will be adequate for pigs above 50 kg body weight (Liu et al., 2000).

The marker method is used when feces cannot be all collected. The indigestible marker is added to a diet and the concentration of marker in feces is higher than in feed, (Wang et al., 2016). The marker should be easy to analyze, non-toxic, indigestible, not interfere in digestibility, not interfere with the ride of passage, not decrease palatability (Moughan et al., 1991). The common indigestible compound used as markers is acid-insoluble ash (AIA), titanium dioxide (TiO_2), chromic oxide (Cr_2O_3), which is added to the level of 0.1 to 0.5% (Olukosi et al., 2012). Percentage of nutrient digestibility can be calculated using the formula shown below;

$$\%\,\boldsymbol{Nutrient\ Digestibility = 100 - 100}\left[\frac{\%\,marker\ in\ feed}{\%marker\ \ in\ faeces}\right]\boldsymbol{x}\left[\frac{\%\,nutrients\ in\ faceses}{\%\,nutrients\ \ in\ feed}\right]$$

The average crude protein digestibility coefficiency for pig ranges 64 to 95% depend on dietary fibre and mineral contents (Noblet, 1993). The increase of dietary fibre causes a decrease in the apparent faecal digestibility of crude protein and fat (Noblet & Perez, 1993).

2.4.1 Effect of cassava on nutrient digestibility of pigs

Apparent crude protein digestibility of integral cassava root silage with wastewater and yogurt was reported as 60.67 to 66.43% (Araújo et al., 2016). The inclusion level of dried whole cassava plant in the diets of barrow pigs up to 60% did not affect the performance and serum metabolites (Akinfala & Tewe, 2001). There is no negative effect on nutrient digestibility and nitrogen retention of pigs' digestibility of ether extract. Also, the crude fibre was improved when fermented cassava pulp was included in the diet. The fermented cassava pulp can be incorporated in diets of barrow pigs up to 12% (Huu & Khammeng, 2014). Table 6 showed crude protein digestibility of fermented cassava pulp from different research works.

Table 6 Crude protein digestibility of cassava or fermented cassava pulp diet in pigs

Animals	%CP Digestibility	Recommended level (%)	Source
15 weaned male pigs	84.9	12	Huu & Khammeng, (2014)
18 castrated male pigs	60.67	25	Araújo et al. (2016)
32 pigs	85		Taysayavong (2018)

2.5 Influence of diets on hematological parameters

The hematological parameters are all constituents that are related to blood or blood-forming organs (Waugh & Grant, 2001). These are the hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), lymphocyte, neutrophil, platelet (PLTs), mean platelet volume (MPV), hematocrit (PCV), etc. (Coronado, 2014). Complete blood count (CBC) is one of the hematological tests which evaluate the parameters that circulate in the blood. It is a reliable test for evaluating the health status of an animal (Doyle, 2006). Diets fed to animals affect positive or negative to the blood parameters of pigs (Etim, Offiong, Williams, & Asuquo, 2014). Therefore, the quality of feed or feed toxicity can be assessed through hematological parameters (Aro & Akinmoegun, 2012). The following is the range of hematological values for swine.

Table 7 Range of hematological values for swine

Parameters	Unit	Range of values	Range values for Over 6 weeks pigs
Hemoglobin	g/dl	10.0 – 16.0	
Hematocrit (PCV)	%	32.0 – 50.0	
RBC	M/uL	5.0 – 8.0	
MCV	fL	50.0 – 68.0	47.5 – 59.2
MCH	pg	17.0 – 21.0	16.3 – 20.6
MCHC	g/dL	30.0 – 34.0	
Platelet	K/uL	325 - 715	118.9 – 522.9
WBC	K/uL	11.0 – 22.0	
Neutrophil	(%)	28.0 – 51.0	
Lymphocyte	%	39.0 62.0	
Monocyte	%	2.0 – 10.0	0.0 – 3.7
Eosinophils	%	0.0 – 10.0	

Source: Coronad, (2014); Low state university (2011).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experiment I: Improvement of cassava pulp nutrients by yeast fermentation with chicken manure

3.1.1 Location, materials collection, and preparation

This experiment was conducted at the laboratory of the 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 a 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 ground into fine using pestle and mortar, mixer grinder, and grinding mill machine for *in vivo* big batch.

3.1.2 Experimental design and methods

3.1.2.1. Fermentation I

This experiment was performed in 9(A) × 4(B) factorial arrangement in complete randomized design (CRD) with three replications to investigate the dry matter, crude protein, crude fibre, gross energy, and pH. Factor A was arranged into 9 treatments and factor B was set as 4 different fermentation times (0, 10, 20, and 30 days). The details of treatments were shown in Table 8. All treatments with nitrogen source supplementation were hypothetically calculated approximately as 5% crude protein.

Cassava pulp fermentation was prepared by a method of Huu & Khammeng (2014) with modification. Cassava pulp and chicken manure were weighted according to each treatment composition (Table 9). Yeast solution (10% w/v) was prepared in 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 obtain 1% yeast inoculation. Ammonium sulfate solution was added to obtain the 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. Figure 2 showed the preparation of cassava fermentation.

Table 8 Treatment group with their hypothetical percentage of nitrogen

Treatment compositions					**Nitrogen (N)**					
					CPu 0.32	**Y 6.11**	**AS 21.00**	**CM 1.6**	**Total N**	**% CP**
T	**% CPu**	**% Y**	**% AS**	**% CM**	**Contribution of N**					
1	100				0.32	0.00	0.00	0.00	0.32	2.00
2	99	1			0.32	0.06	0.00	0.00	0.38	2.36
3	98		2		0.31	0.00	0.42	0.00	0.73	4.59
4	71			29	0.27	0.00	0.00	0.46	0.82	5.13
5	97	1	2		0.31	0.06	0.42	0.00	0.79	4.95
6	90	1	1.5	7.5	0.29	0.06	0.32	0.13	0.82	5.10
7	83.5	1	1	14.5	0.27	0.06	0.21	0.24	0.82	5.14
8	76.5	1	0.5	22	0.24	0.06	0.11	0.35	0.83	5.17
9	70	1		29	0.22	0.06	0.00	0.46	0.83	5.21

Note: AS = ammonium sulfate, CM = chicken manure, CP = Crude protein, CPu = cassava pulp, N = nitrogen, T = treatment, Y = yeast.

Table 9 Description of preparing fermentation in each treatment

Treatment	**Cassava pulp (g)**	**10% Yeast solution (ml)**	**20% AS solution (ml)**	**Chicken manure (g)**	**Sugar (g)**	**Water (ml)**	**Total (g)**
T1	100.00	0.00	0.00	0.00	0.00	20.00	100
T2	99.00	10.00	0.00	0.00	1.00	10.00	100
T3	98.00	0.00	10.00	0.00	0.00	10.00	100
T4	71.00	0.00	0.00	29.00	0.00	20.00	100
T5	97.00	10.00	10.00	0.00	1.00	0.00	100
T6	90.00	10.00	7.50	7.50	1.00	2.50	100
T7	83.50	10.00	5.00	14.50	1.00	5.00	100
T8	76.50	10.00	2.50	22.00	1.00	7.50	100
T9	70.00	10.00	0.00	29.00	1.00	10.00	100

1. Grinded cassava pulp

2. Grinded cassava chicken

3. Ammonium sulfate

4. *Saccharomyces cerevisiae* yeast

5. Table sugar

6. Mixing cassava pulp and manure

7: Yeast activation with table sugar

8: Put up the yeast inoculant in cassava pulp

9: Fermenting anaemically

10: Storage in freezer after reached specific fermentation

Figure 2 Preparation of cassava pulp fermentation.

3.1.2.3 Fermentation II

The fermentation II was designed as 5(A) × 3(B) factorial arrangement in complete randomized design (CRD) with three replications to evaluate crude protein. Factor A was arranged into 5 treatments and factor B was set as 3 different fermentation times (0, 10, and 20 days). Five treatments included T1, T2, T5, T9, and T10 of fermentation I were selected to evaluate the effect of temperature and water on fermentation. The details of treatments were shown in Table 8. Treatment T1, T2, T5, and T9 were incubated in the incubator at 37 °C and T10 was placed at room temperature. When reaching the specific fermentation time, the samples were stored in the freezer until analysis. All treatments with nitrogen source supplementation were hypothetically calculated approximately as 5% crude protein.

Cassava pulp fermentation was prepared by the same procedures of fermentation I. The difference was the water added. In fermentation II, water was adjusted to 40% and stored in the incubator at 37 °C.

3.1.2.3 Fermentation III

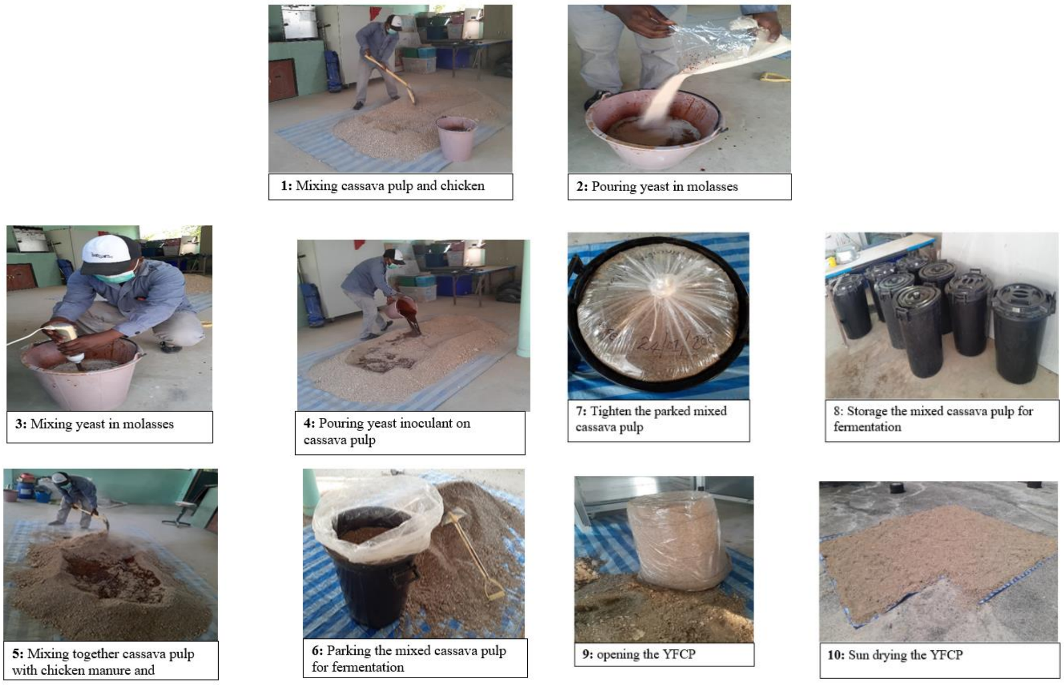
The fermentation was designed as 2(A) × 3(B) factorial arrangement in complete randomized design (CRD) with three replications to evaluate the crude protein before and after fermentation. Factor A was arranged into 2 treatments and factor B was set as 3 different fermentation times (0, 10, and 20 days). The details of treatments were shown in Table 10 below**.**Preparation of cassava pulp fermentation was the same as in fermentation I except, yeast activated with 20% solution of molasses, and moisture adjusted to 30% instead of 40% and stored at room temperature then compared with sugar activation.

Table 10 Ingredient composition of fermented cassava pulp in fermentation III

Treatments	Composition (%)
T9S	Cassava pulp (70%), chicken manure (29%), yeast (1%) + table sugar
T9M	Cassava pulp (70%), chicken manure (29%), yeast (1%) + molasses

3.1.2.4 Fermentation IV

Cassava pulp fermentation for in vivo experiment was done by a combination method of Huu & Khammeng (2014) and Nukreaw 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 of *Saccharomyces cerevisiae* yeast (1.2 kg for 1% of total weight) was mixed with molasses (4.8 kg for 4% of total weight), in 36 liters of water (30% of total weight) using a hand blender 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 average of 20 kgs each container, then left for 20 days at room temperature. After 20 days, the fermented cassava pulp was sun-dried for three days. The samples were taken from each container for laboratory analysis and the remained were used for experimental diets formulation.



1: Mixing cassava pulp and chicken

2: Pouring yeast in molasses

3: Mixing yeast in molasses

4: Pouring yeast inoculant on cassava pulp

7: Tighten the parked mixed cassava pulp

8: Storage the mixed cassava pulp for fermentation

5: Mixing together cassava pulp with chicken manure and

6: Parking the mixed cassava pulp for fermentation

9: opening the YFCP

10: Sun drying the YFCP

Figure 3 Preparation of yeast fermented cassava pulp for in vivo experimental diets formulation.

3.1.3 Data collection

Cassava pulp, chicken manure, and samples from fermentation I and IV were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), gross energy (GE), and pH analysis. For fermentation II and III, only DM and CP were evaluated. The crude protein and crude fibre were analyzed using proximate analysis, following the AOAC (2016). Gross energy was determined using a Bomb calorimeter (6200 Isoperibol, Parr Instrument Company, USA). The pH values were determined by dissolving the sample in distilled water (1: 10 ratio) and pH was measured by pH meter (Adwa AD12, Hungary).

3.1.4 Statistical analysis

The mean ± standard deviation of DM, CP, CF, GE, and pH were statistically analyzed using two-way analysis of variance (ANOVA) and compared by Duncan's New Multiple Range Test (DMRT) in R program. Significance was defined by $P < 0.05$.

3.2. Experiment 2: Effects of improved cassava pulp on nutrient digestibility, haematological parameters, and growth performance of barrow pigs.

3.2.1 Location, material collection and preparation

This experiment was conducted at Silpakorn university farm, five kilometers from the faculty of Animal Science and Agricultural Technology, Silpakorn University, Phetchaburi, Thailand from 23rd February to 26th March 2021. The ingredients for experimental diets were purchased from EK animal feed store - Tha yang, Phetchaburi province in Thailand. Yeast fermented cassava pulp (YFCP) from fermentation IV were mixed to formulate pig diets at the university farm warehouse. Animals were bought from SC farm, Rai Mai Pattana, cha-am district, Phetchaburi province, Thailand.

3.2.2 Animals and experimental design

A total of 24 castrated crossbreed ((large white ×landrace) × duroc jersey) male pigs, an average of 57.13 ± 3.29 kg body weight, were arranged in Randomized Complete Blocked Designed (RCBD) by using different initial weight as a block. There were 4 treatment diets with 6 block replications.

3.2.3 Experimental diets

The experimental diets formulated by corn-soybean-based diet with inclusion levels of 0, 5, 10, and 15 % of YFCP in replacement of cornmeal. The diet formulation was based on NRC (1998) recommendation of growing pig (BW 50-80 kg). T1 is a control diet with 0 % YFCP, T2 is a diet with 5% YFCP, T3 is a diet with 10 % of YFCP and T4 is a diet with 15 % of YFCP as described in Table 11.

Table 11 Ingredients in the four experimental diets

Ingredient	**Treatment diet**			
	T1	**T2**	**T3**	**T4**
Maize	66.55	61.08	55.58	50.10
SBM, 44% CP	21.3	21.36	21.44	21.52
YFCP	**0.00**	**5.00**	**10.00**	**15.00**
Rice bran	10.00	10.00	10.00	10.00
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	3270	3271	3271	3270
Crude protein, %	15.5	15.5	15.51	15.51
Crude fibre, %	4.15	4.52	4.88	5.25
Ether extract, %	2.17	2.5	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

3.2.4 Care and management of the experimental animals

(1) Before feeding with the experimental diets, all pigs were adjusted by feeding control diet for 5 days.

(2) On day one, each pig was weighted for initial weight and allotment into 4 treatments with 6 replications.

(3) Each pig was separated into 1.00×1.20 metres pen and equipped with feeder and water nipple.

(4) The animals fed on ad lbitum and ration added 2 times, morning 8.00 A.M. and evening 5.00 P.M for 28 days.

(5) On day 21st, all pigs were started to feed experimental diet adding 0.20% of chromic oxide (Cr_2O_3) as digestibility indicator. Feeding chromic oxide diet for 7 days.

(6) Approximately 200 grams of fresh clean sample of feces were collected from each pig morning and evening for five days and preserved in refrigerator for analysis.

(7) On day-28, approximation of 5 milliliters of blood samples were collected from each pig by 18-gauge needle size at jugular vein for cell blood count analysis (3 pigs from each treatment). Finally, all 24 pigs were determined for their body weight using digital electronic weighing scale.

Figure 4 Experimented pigs in the pens.

3.2.5 Data Collection

The data collected were body weight before and after feeding trial, feed intake, hematological parameters, chemical analysis (DM, CP, CF, GE, calcium, and phosphorus) of feed and fecal samples. Nutrient digestibility was examined using the maker method. Chromic oxide and phosphorus were calculated by absorbance reading of ash soluble of the samples using the Biochrome spectrophotometer (Libra S22) at 880 nm and 370 nm, respectively. Calcium amount was analyzed by absorbance reading from atomic absorption spectrophotometer.

Nutrient’s digestibility was calculated by using the following formular;

$$\%\,\boldsymbol{Nutrient\ Digestibility} = \mathbf{100} - \mathbf{100}\left[\frac{\%\,marker\ in\ feed}{\%marker\ \ in\ faeces}\right]\boldsymbol{x}\left[\frac{\%\,nutrients\ in\ faceses}{\%\,nutrients\ \ in\ feed}\right]$$

3.2.5. Statistical analysis

All data in experiment 2 were expressed as mean ± standard deviation and analyzed by using ANOVA. Treatment means were statistically compared by DMRT using R program at $p \leq 0.05$ for significance level.

CHAPTER 4

RESULTS

4.1. Experiment 1: Improvement of Cassava Pulp Nutrients by Yeast Fermentation with Chicken Manure

4.1.1. Nutritional analysis of YFCP from fermentation I

Before fermentation, the chemical composition (DM, CP, CF, and GE) of cassava pulp and chicken manure used in this study was evaluated (Table 12). Cassava pulp had higher DM, CF, and GE compared to chicken manure which had higher CP. The low protein of 1.99% in cassava pulp reduces its nutritive value as an ingredient of animal feed. Therefore, the improvement of cassava pulp nutritive values has been interesting nowadays.

Table 12 Proximate analysis of cassava pulp and chicken (as fed basis)

Parameters	**Cassava pulp**	**Chicken manure**	
		Batch A	Batch B
Dry matter (%)	87.03 ± 0.25	25.39 ± 0.68*	95.45 ± 0.08
Nitrogen (%)	0.32 ± 0.03	2.23 ± 0.05	2.86 ± 0.06
Crude protein (%)	1.99 ± 0.19	13.94 ± 0.29	17.90 ± 0.24
Crude fibre (%)	15.63 ± 0.73	13.32 ± 1.48	13.78 ± 0.77
Gross energy (kcal/kg)	3456.667 ± 50.38	2386.467 ± 24.31	2913.10 ± 14.06
pH value	4.67 ± 0.11	7.89 ± 0.05	7.62 ± 0.06

Note: Data are expressed as mean ± standard deviation (n = 3), * Dry matter of chicken manure batch A 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 dry matter, crude protein, crude fibre, gross energy, and pH of fermented cassava pulp in each treatment at different fermentation times were determined. A significant interaction between treatment composition and fermentation time was observed in all parameters.

4.1.1.1 Dry matter

The dry matter of YFCP significantly differed by the influence of the treatments and times (Table 13). Treatment with cassava pulp, yeast, and ammonium sulfate (T5) had the highest dry matter content of 76.59 compared to other treatments. The mean of treatments showed that treatment with chicken manure (T9) had the highest dry matter content of 74.55 and the mean of fermentation day showed the highest dry matter of 72.11 at 30 days of fermentation.

4.1.1.2 Crude protein

For crude protein analysis, the changes in the level of crude protein were influenced by both ingredients and fermentation time. The highest level of crude protein at 6.28% was observed in the treatment of cassava pulp fermented with yeast and chicken manure (T9) at 30 days (Table 14). The use of nitrogen sources either ammonium sulfate or chicken manure increased the level of crude protein. Cassava pulp and cassava pulp fermented with yeast without nitrogen source supplementation (T1 and T2) showed low crude protein approximately 1.59 – 2.11% while cassava pulp fermented with yeast and chicken manure (T9) showed the best improvement with 4.84% crude protein. In addition, the results showed that fermentation time had a positive effect on crude protein but was not different among 10, 20, and 30 days (Table 14).

4.1.1.3 Crude fibre

The level of crude fibre significantly varied within treatments and fermentation times. Low fibre is preferred for use as a 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. However, when considering the treatment effect, cassava pulp fermented with yeast and ammonium sulfate (T5) showed the lowest crude fibre (Table 15). It can be noted that fermentation time significantly lowers the crude fibre when increasing the time of fermentation.

Table 13 Dry matter of fermented cassava pulp in treatments at different fermentation times

Treatment (A)	**Fermentation Day (B)**				**Mean (A)**
	0	**10**	**20**	**30**	
T1	69.74 ± 1.75 ^{jklmn}	61.73 ± 1.49 ^{s}	64.08 ± 1.18 ^{r}	66.85 ± 0.74 ^{opq}	65.60 ± 3.34 ^{F}
T2	72.14 ± 0.99 ^{defghij}	72.59 ± 0.96 ^{cdefghi}	71.00 ± 2.80 ^{bc}	70.93 ± 1.61 ^{ijkl}	71.67 ± 1.67 ^{BC}
T3	67.57 ± 0.58 ^{nopq}	65.36 ± 0.65 ^{qr}	73.54 ± 0.27 ^{cdefg}	73.39 ± 0.62 ^{cdefgh}	69.97 ± 3.77 ^{DE}
T4	68.80 ± 0.96 ^{lmno}	71.09 ± 1.01^{hijkl}	68.38 ± 0.95 ^{mno}	69.30 ± 0.76 ^{klmn}	69.39 ± 1.33 ^{E}
T5	70.41 ± 0.28 ^{ijklm}	69.00 ± 0.83 ^{klmno}	71.05 ± 0.73 ^{hijkl}	76.59 ± 0.25 ^{a}	71.76 ± 3.06 ^{BC}
T6	70.83 ± 0.21 ^{ijkl}	68.03 ± 0.62 ^{mnop}	69.98 ± 0.39 ^{jklm}	71.38 ± 0.45^{ghijk}	70.05 ± 1.38 ^{DE}
T7	71.97 ± 0.49 ^{efghij}	66.07 ± 0.88 ^{pqr}	74.31 ± 0.19 ^{abcde}	71.04 ± 0.41 ^{hijkl}	70.85 ± 3.18 ^{CD}
T8	71.80 ± 1.52^{fghij}	68.42 ± 1.82 ^{mno}	74.40 ± 0.32 ^{abcd}	74.97 ± 0.06 ^{abc}	72.40 ± 2.89 ^{B}
T9	73.87 ± 0.14 ^{bcdef}	75.95 ± 0.35 ^{ab}	73.80 ± 0.15 ^{bcdef}	74.59 ± 0.29 ^{abc}	74.55 ± 0.93 ^{A}
Mean (B)	70.79 ± 2.00 ^{w}	68.69 ± 4.12 ^{x}	71.17 ± 3.39 ^{y}	72.11 ± 3.00 ^{z}	
% CV	1.36				
p - value					
A	< 0.001				
B	< 0.001				
A x B	< 0.001				

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 ($p \leq 0.05$)

Table 14 Crude protein of fermented cassava pulp in treatment at different fermentation times

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^{Y}	3.74 ± 1.15^{X}	3.59 ± 1.08^{X}	3.86 ± 1.33^{X}	
% CV	10.67				
***p* -value**					
A	< 0.001				
B	< 0.001				
A x B	< 0.001				

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 ($p \leq 0.05$)

Table 15 Crude fibre of fermented cassava pulp in each treatment at different fermentation times

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^{X}	13.23 ± 0.79^{Y}	12.97 ± 0.81^{Y}	12.40 ± 0.89^{Z}	
% CV	4.86				
p -value					
A	< 0.001				
B	< 0.001				
A x B	< 0.001				

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 ($p \leq 0.05$).

4.1.1.4 Gross energy

Gross energy was affected by treatments and fermentation times (Table 16). Treatment of cassava pulp with yeast (T2) at 0 days had the highest energy of 3484.89 kcal/kg. In addition, considering the treatment factor, yeast fermented cassava pulp (T2) and yeast fermented cassava pulp with ammonium sulfate (T5) showed the highest mean energy among treatments. It could be noted that treatment using chicken manure caused a reduction in gross energy. Longer fermentation decreased the gross energy but there was no significant difference among 10, 20, and 30 days.

4.1.1.5 pH values

The pH condition is an important parameter for microbial activities. Yeast cells metabolism is efficient in a slightly acidic environment. Therefore, pH values in cassava fermentation were determined in this study. The results demonstrated that pH values 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 days 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 increase of fermentation time lowered the pH value from 6.21 to 5.63 at 30 days (Table 17).

Table 16 Gross energy of fermented cassava pulp in each treatment at different fermentation times

Treatment	**Fermentation Day (B)**				**Mean (A)**
(A)	**0**	**10**	**20**	**30**	
T1	3373.18 ± 118.32^{abc}	3329.66 ± 74.37^{abcd}	$3291.40 \pm 37.26^{abcde}$	$3191.94 \pm 97.17^{bcdef}$	3296.55 ± 102.04^{AB}
T2	3484.89 ± 47.92^{a}	$3278.14 \pm 37.91^{abcde}$	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}	$3249.82 \pm 23.98^{abcde}$	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}	$3342.34 \pm 102.09^{abcd}$	3330.48 ± 63.59^{abcd}	3337.46 ± 98.49^{A}
T6	$3325.32 \pm 224.55^{abcd}$	$3267.56 \pm 42.89^{abcde}$	3310.98 ± 86.16^{abcd}	3200.57 ± 51.37^{bcde}	3276.11 ± 117.90^{AB}
T7	$3276.44 \pm 160.04^{abcde}$	$3232.31 \pm 101.46^{abcde}$	$3110.94 \pm 11.95^{cdefg}$	$3116.50 \pm 38.29^{cdefg}$	3184.05 ± 120.91^{BC}
T8	3205.85 ± 32.95^{bcde}	$3144.66 \pm 83.77^{cdefg}$	$3122.88 \pm 39.20^{cdefg}$	$3113.10 \pm 76.35^{cdefg}$	3146.62 ± 65.04^{C}
T9	$3254.61 \pm 329.99^{abcde}$	3086.48 ± 33.91^{defg}	$3133.08 \pm 135.82^{cdefg}$	3025.96 ± 55.78^{efg}	3125.03 ± 18.78^{C}
Mean (B)	3302.23 ± 191.94^{Y}	3182.03 ± 149.35^{X}	3216.22 ± 168.66^{Y}	3180.79 ± 160.37^{Y}	
% CV	3.28				
p -value					
A	< 0.001				
B	< 0.001				
A x B	< 0.001				

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 ($p \leq 0.05$).

Table 17 The pH of fermented cassava pulp in each treatment at different fermentation times

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 ^{X}	6.27 ± 1.38 ^{X}	5.75 ± 1.18 ^{Y}	5.63 ± 0.92 ^{Z}	
%CV	2.19				
p -value					
A	< 0.001				
B	< 0.001				
A x B	< 0.001				

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 ($p \leq 0.05$).

4.1.2. Crude protein analysis of YFCP from fermentation II

The results showed that crude protein levels were influenced by treatments and fermentation times but not influenced by the interaction between treatments and times ($p \leq 0.05$). Treatment with a source of nitrogen (T5, T9, and T10) had higher crude protein compared to those with no source of nitrogen (T1 and T2). Also, there is a significant increase of crude protein at different times of fermentation. The highest increase of crude protein (6.04%) was observed in treatment with chicken manure stored in an incubator (T9). Fermentation days for 20 days showed the highest increase of crude protein (4.63%).

Table 18 Crude protein of yeast fermented cassava pulp in fermentation II

Treatment	**Fermentation times (days)**			**Mean (A)**
	0	**10**	**20**	
T1	2.08 ± 0.18	2.10 ± 0.16	2.20 ± 0.36	2.13 ± 0.22^{E}
T2	2.56 ± 0.04	2.40 ± 0.10	2.54 ± 0.10	2.50 ± 0.10^{D}
T5	5.10 ± 0.20	5.10 ± 0.20	5.61 ± 0.11	5.28 ± 0.31^{C}
T9	5.80 ± 0.10	5.91 ± 0.07	6.41 ± 0.26	6.04 ± 0.31^{A}
T10	5.10 ± 0.10	6.29 ± 0.08	6.38 ± 0.28	5.92 ± 0.20^{B}
Mean (B)	4.13 ± 1.74^{X}	4.36 ± 1.83^{X}	4.63 ± 1.94^{Y}	
% CV	3.9			
p value				
A	< 0.001			
B	< 0.001			
A x B	0.1			

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 ($p \leq 0.05$)

4.1.3. Crude protein analysis of YFCP from fermentation III

The treatment T9 in fermentation II was selected due to the highest percentage of mean crude protein together with the room temperature condition which is ideal for the field environment. Treatment T9 was modified in fermentation III by the addition of energy activations by table sugar (T9S) and molasses (T9M). The results showed that crude protein was significantly influenced by the energy activation source and fermentation time but not their interaction effect ($p \leq 0.05$).

Table sugar activation of YFCP provided a higher crude protein level compared to molasses (Table 19). Fermentation for 20 days resulted in the increase of crude protein from 6.84% to 8.64%. However, even though table sugar had the best result, the YFCP activated with molasses was selected due to it is a sugar by-product with a low price which is more suitable for bulk fermentation in a feeding trial.

Table 19 Crude protein of YFCP with table sugar (TS) or molasses (TM) activation

Treatment	**Fermentation times (days)**			**Mean**
	0	**10**	**20**	**(A)**
T9S	7.50 ±0.90 ^{b}	8.65 ±0.54 ^{a}	8.96 ±0.72 ^{a}	8.70 ± 0.66 ^{A}
T9M	6.17± 0.08 ^{b}	6.37± 0.35 ^{b}	8.32± 0.37 ^{a}	6.95 ± 1.06 ^{B}
Mean (B)	6.84± 1.4 ^{Y}	7.51± 1.31 ^{Y}	8.64± 0.62 ^{X}	
p value				
A	< 0.001			
B	< 0.01			
A: B	< 0.05			
% CV	7.15			

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 ($p < 0.05$)

4.1.4. Nutrient analysis of YFCP from fermentation IV

Nutritional analysis of YFCP of fermentation IV was presented in Table 20. There were significant changes in dry matter, crude protein, gross energy, ash, and pH of YFCP in unfermented (day 0) and fermented YFCP on day 20. The dry matter, gross energy, ash, and pH were found slight decrease while crude protein was increased from 7.51% to 8.54% (Table 20). This YFCP was used as an ingredient in the diet formulation in experiment 2 in replacement of cornmeal.

Table 20 The chemical composition of YFCP in fermentation IV

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^{b}	4.20	< 0.001
Gross energy (kcal/kg)	3154.93 ± 17.53 ^{a}	3120.53 ± 15.37 ^{b}	0.539	< 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 Duncan’s New Multiple Range Test ($p \leq 0.05$)

4.2 Experiment 2: Effects of improved cassava pulp on nutrient digestibility, haematological parameters, and growth performance of barrow pigs.

4.2.1 Nutrients digestibility of YFCP diets in barrow pigs

The percentage of nutrient digestibility showed in Table 23. They were calculated by data of chemical analyses of the diets (Table 21) and the faeces of experimental pigs (Table 22). It showed that the nutrients digestibility is significant different ($p < 0.05$). The trend of the highest to the lowest digested was GE, DM, CP, TP and CF. The control diet (0% YFCP) had higher ($p < 0.05$) nutrient percentage digestibility except, total phosphorus which was higher in a diet containing 15% YFCP. The 15% YFCP diet was the second for higher nutrient digestibility followed by 5% YFCP diets and the 10% YFCP was the least.

Table 21 Chemical composition of YFCP diets (as fed basis)

Item	Treatment diet			
	0% YFCP	**5% YFCP**	**10% YFCP**	**15% YFCP**
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

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

Table 22 Chemical composition of faecal samples

Item	Treatment diet's faeces			
	0% YFCP	**5% YFCP**	**10% YFCP**	**15% YFCP**
Dry matter, %	95.21 ± 0.89	95.28 ± 0.46	95.29 ± 0.18	96.01 ± 0.30
Gross energy, kcal/kg	3996.02 ± 27.76	3916.63 ± 46.86	3884.08 ± 55.13	3774.07 ± 32.83
Crude protein, %	16.46 ± 1.15	16.02 ± 1.17	15.80 ± 1.78	18.71 ± 1.46
Crude fibre, %	11.64 ± 1.11	13.11 ± 1.00	11.76 ± 1.71	16.24 ± 0.49
Ash, %	17.30 ± 0.66	19.89 ± 1.03	21.21 ± 1.52	20.40 ± 0.91
Total phosphorus, %	0.98 ± 0.03	1.00 ± 0.07	0.99 ± 0.05	0.95 ± 0.04
Chromic oxide, %	0.69 ± 0.31	0.70 ± 0.63	0.56 ± 0.29	0.59 ± 0.54

Note: Results are in dry matter basis and are expressed as mean ± standard deviation (n = 6).

Table 23 Nutrient's digestibility of barrow pigs fed with 0, 5, 10, 15% of YFCP diets

Nutrient digestibility	Treatment diet				% CV	*p* *value*
	0% YFCP	**5% YFCP**	**10% YFCP**	**15% YFCP**		
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

The results are expressed as mean ± standard deviation (n = 6). The different superscript letters within rows are statistically different by Duncan's New Multiple Range Test ($p < 0.05$)

4.2.2 Hematological parameters of pigs fed with YFCP diets

The complete blood count of experimental pigs was determined to investigate the influence of YFCP on the health of YFCP feeding pigs. The results (Table 24) showed that all parameters in all four experimental diets were not statistically significant ($p \leq 0.05$). However, there was a slightly higher hematocrit in animals fed with 10% of YFCP diet. The increase in lymphocytes was found in animals fed with 5% of YFCP. In addition, an increasing trend of monocytes shell be noted.

4.2.3 Growth performance of pigs fed experimental diets

The growth performance of 24 pigs fed four different experimental diets was determined (Table 25). The results showed that they were no statistically significant differences among groups of diets on final body weight, body weight gained, and feed conversion ratio ($p \leq 0.05$). However, the animals fed with control diet (T1) had slightly higher mean body weight gained of 25.8 kg and a better feed conversion ratio of 2.84 kg followed by animals fed diet containing 15% of YFCP (T4) which had a mean gain of 23.61kg with 2.86 feed conversion ratio.

Table 24 Complete blood count of pigs fed 0, 5, 10, 15 % of YFCP diets

Parameter	**Treatment**				**% CV**	***P value***
	0% YFCP	**5% YFCP**	**10% YFCP**	**15% YFCP**		
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 ± 3.02	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).

Table 25 Growth performance of pigs fed with 0, 5, 10, 15% of YFCP diets.

Item	**Treatment diet**				**% CV**	***p value***
	0% YFCP	**5% YFCP**	**10% YFCP**	**15% YFCP**		
Number of pigs	6	6	6	6		
Period (days)	28	28	28	28		
Initial body weight (kg)	56.95 ± 2.57	57.19 ± 3.21	57.12 ± 2.48	57.25 ± 4.9	6.00	0.99
Final body weight (kg)	82.75 ± 3.76	80.15 ± 4.73	79.60 ± 4.69	80.86 ± 5.13	5.70	0.67
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, gram/day	921.43±0.10	820 ± 0.07	802.86 ± 0.17	843.21 ± 0.18	15.81	0.33
Feed intake per animal (kg)	73.34± 9.06	73.50 ± 8.19	75.06 ± 9.67	67.56 ± 13.49	10.01	0.61
Average daily feed intake per animal (kg)	2.62	2.63	2.63	2.41		
Feed conversion ratio (FCR)	2.84 ± 0.48	3.20 ± 0.53	3.34 ± 0.76	2.86 ± 0.97	22.62	0.52
Feed cost per 1 kg diet (THB)	11.8928	11.78228	11.67008	11.5506		
Meat cost per 1 kg gained (THB)	33.77	37.70	38.99	33.03		

Note: Results are expressed as mean ± standard deviation (n = 6), FCR is calculated by feed intake divide by body weight gain. Feed cost per 1 kg gained was calculated by multiplying feed cost of 1 kg diet with FCR.

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1. Experiment 1: Improvement of Cassava Pulp Nutrients by Yeast Fermentation with Chicken Manure

Feed costs especially protein ingredients, contribute to higher production costs in the animal industry. Cassava pulp can be one alternative to lower feed costs, but the low protein and higher fibre content limit its uses. The improvement of cassava pulp helps to increase nutrition value that will influence its uses. Nitrogen inorganic fertilizers have been verified to improve cassava pulp nutrients when fermented with beneficial microorganisms, as reported by Sugiharto (2019). In order to solve 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 of yeast in the fermentation process of cassava pulp. Three different fermentations were performed before selection for feeding trial in the *in vivo* experiment in barrow pigs.

5.1.1. Ingredients used

Ingredient's analysis showed that cassava pulp used in these fermentations had a crude protein of 1.99% (Table 12). This result related to Keaokliang et al. (2018) whereby samples from four different starch factories in the northeast of Thailand, the mean crude protein 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 those reported by Heuzé et al. (2016) which had an average DM of 89.2% and GE of 3868.56 kcal/kg. The crude fibre of the cassava ingredient in this study was 15.63% which was lower than in Aro et al. (2010) which had 19.3%. Crude fibre content is influenced by the presence of cellulose in cassava roots (Apiwatanapiwat et al., 2011). However, low crude fibre ingredients are preferred to monogastric animal feed.

The chicken manure had a higher crude protein of 13.93% compared to cassava pulp 1.99%. But this was still lower when compared to 15.4% crude protein of chicken manure from smallholder farmers in Kenya reported by Lanyasunya et al, (2006). The crude fibre is a result of indigestible materials, due to the concentration of cell walls and ash, (Flachowsky, 1997). The gross energy of chicken manure of 2386.47 kcal/kg in this work was low compared to an average of 2664 kcal/kg of chicken manure from 9 farms in Spain (Quiroga et al., 2010). The chicken manure has comparable low gross energy due to low content of carbohydrate (Lanyasunya, et al., 2006).

5.1.2. Decrease of dry matter

The dry matter of YFCP is significantly influenced by the treatments and times. It seemed that chicken manure increases the dry matter of improved cassava pulp. In the fourth fermentation, the dry matter decreased from 67.31% at day 0 to 66.12% on day 20 of fermentation. This decrease of dry matter may be caused by the conversion of cassava pulp carbohydrate to yeast protein as reported by Du Thanh Hang et al., (2019). However, the higher content of dry matter is preferred because it represents the higher amount of nutrients that are available in feed samples to the animals.

5.1.3. Increase of crude protein

Fermentation of cassava pulp in this study revealed that crude protein in plain cassava pulp significantly increased when fermented by *Saccharomyces cerevisiae* with chicken manure as a nitrogen source in all four fermentations. The last fermentation experiment showed the highest crude protein at 8.54%. The nitrogen source increased crude protein first before fermentation. The fermentation increased crude protein due to the ability of yeast *S. cerevisiae* to utilize carbon from carbohydrates of cassava pulp and nitrogen from chicken manure (Parapouli et al., 2020). The *S. cerevisiae* cells may utilize organic and inorganic nitrogen in chicken manure because of the presence of three permeases enzymes (MEP 1, MEP 2, and

MEP 3) which facilitate the entrance of ammonia into the cell and link it with glutamate dehydrogenase then combine with glutamine by glutamine synthetase (Jiranek et al., 1995; Magasanik & Kaiser, 2002).

The percentage increase from first to fourth fermentation were 1.98, 0.61, 2.15, and 1.03 % respectively. The trend showed that fermentation with low moisture at 20% in first fermentation and 30% in third fermentation had a higher increase percentage compared to 40% and 30% plus 4% molasses in fermentation 2 and 3. This may be caused by reduced porosity of substrate which limits the oxygen transfer within a substrate hence low production of enzyme and initial growth as reported in (AL-Sa'ady, 2014; Camacho-Ruiz et al., 2003). Also, the lowest increase of crude protein in the second fermentation could be due to a high temperature of 37 °C which is higher than the optimum growth temperature of *S. cerevisiae* at 30°C (AL-Sa’ady, 2014).

The molasses activator treatment had a higher percentage increase of 2.15% compared to 1.46% of table sugar. This may be influenced by other nutrients such as protein, fermentable sugar, compared to only sucrose in table sugar (Zohri et al., 2018).

Times of fermentation and nitrogen supplementation also affected the level of crude protein. As fermentation time increased also the crude protein level increased. These increases in crude protein are higher than those reported by Animashahun et al. (2013) and (Sugiharto et al., 2015) due to the absence of nitrogen source, different times of fermentation, and microorganisms used. The efficiency of crude protein improvement was more when there is a nitrogen sources for protein synthesis, (Bañuelos et al., 2016) but low if no nitrogen source as seen in treatment T1 which had only a 0.23% increase.

Chicken manure showed the possibility to be an alternative source of nitrogen to replace the inorganic fertilizer in cassava pulp fermentation. The 1.98% and 2.15% increase of crude protein in first and third fermentation are comparably related to (Du Thanh Hang et al., 2019) which reported 2.05% increment of crude protein in their study using a combination of 2% urea and 1.5% diammonium phosphate as a nitrogen source. However, the 1.98% increase in crude protein in this work was lower than 4.31% increase reported by (Iyayi & Losel, 2001), which used an easy availability of nitrogen source from 2% of peptone, and 8.9% increased crude protein (Sengxayalth & Preston, 2017), which utilized 4.05% urea together with 1% of diammonium phosphate as nitrogen supplementation to 2.02% yeast. Moreover, the wild microorganisms in chicken manure also significantly increased crude protein in T4 of the first fermentation (3.88 to 4.68 in 30 days of fermentation) but this increase was not observed in T1 which had only cassava pulp (Table 14). The 0.8% increase in T4 could be implied that wild microorganisms in chicken manure should be further elucidated and might be candidates for use as fermenters in future studies.

5.1.4. Decrease of crude fibre

The lowest level of crude fibre of improved cassava pulp in this work was 11.4%, observed in cassava pulp with ammonium sulfate (T3) at 20 days. This was a 2.79% decrease from the beginning (Table 15). As the time of fermentation increases also decreases the fibre content but was statistically not different between day 10 and 20. The 2.79% decrease is lower than the 4.9% decrease (Du Thanh Hang et al., 2019). The lower decrease in the present study may be caused by less amount of ammonium sulfate (2%) used compared to the combination of 2% urea and 1.5% diammonium phosphate. The low crude fibre is important in feed because it has higher digestibility compared to higher crude fibre which normally has low digestibility.

5.1.5. Decrease of Gross energy

It could be noted that treatments without chicken manure had higher gross energy (Table 16). This was due to the high percentage of cassava pulp which contains carbohydrates (Apiwatanapiwat et al., 2011), and the low gross energy of chicken manure ingredients. Also, the gross energy decreased with the increase in fermentation time. This may be due to the utilization of carbohydrates in cassava pulp to form other cellular components including protein in the yeast cells (Du Thanh Hang et al., 2019)

5.1.6. Decrease of pH

The pH condition is very important for yeast fermentation to provide a proton gradient for uptake of nutrients and other metabolic processes (Magasanik & Kaiser, 2002). *S. cerevisiae* yeast cells are efficient at a pH of 4 to 5 (AL-Sa'ady, 2014; Lin et al., 2012). The lowest pH value in the present work was 3.84 and 3.91, observed in treatment containing cassava pulp with ammonium sulfate (T3) and cassava pulp with yeast and ammonium sulfate (T5) at 20 days (Table 17). Longer periods of fermentation decreased pH value. However, the trend showed that all treatments containing chicken manure had higher pH. Nevertheless, despite the increase of pH, the best fermentation treatments in terms of higher crude protein observed in cassava pulp fermented with chicken manure. This was possible because *S. cerevisiae* can adapt up to 8.0 alkaline pH by the availability of special genes that utilizes iron and copper in chicken manure (Flachowsky, 1997) for alkaline adaptation (Serrano, Bernal, Simón, & Ariño, 2004).

5.2 Experiment 2: Effects of improved cassava pulp on nutrient digestibility, hematological parameters, and growth performance of barrow pigs.

5.2.1. Nutrient's digestibility of YFCP on experimental pigs

Nutrient digestibility is a percentage of a feedstuff taken into the digestive tract that is absorbed into the body for various metabolic activities. Knowing the digestibility of feedstuff is important because it assures the number of absorbed nutrients in the animal body, hence it's a measure of feed nutritional value and quality (Lawrence et al., 2007). The nutrient digestibility of the present work showed that there is significant different ($p < 0.05$) between the nutrients of the diets. Th control diet had higher percentage digestibility of all nutrients except total phosphorus followed by diet containing 15% YFCP. The highest digestibility in control diets may be caused by the low crude fiber of 2.54 %, higher crude protein of 14.81%**,** and 15.54%, in the 15%YFCP diet compared to other diets (Table 21). Because the lower crude fibre and higher protein influence digestibility of feeds as previously reported

(Banerjee, 2018). The results of crude protein digestibility in this work were lower compared to 84.9 % reported by Huu & Khammeng (2014) who included 12% of the fermented cassava pulp in replacement of maize and soybean diet on 15 weaned male pigs. Also, the digestibility reported in the present work is greater than 60.67 % reported by Araújo et al, (2016) who used 25% of fermented cassava pulp in replacement of maize soybean diet on 18 castrated pigs. The difference may be caused by the level of fermented cassava pulp in the diets, environment, breed, and number of pigs used.

5.2.2. Haematological parameters of pigs fed with YFCP diets

Diets fed to animals have positive (nourishment, immunity) or negative (toxic, pathogens) on health, and their quality can be assessed in blood parameters (Etim et al., 2014). Complete blood count (CBC) is a reliable test for evaluating the health status of an animal (Doyle, 2006). The haematological test conducted in the present work was to verify the safety and quality of the diets included YFCP with chicken manure. The results of all haematological parameters (Table 24) were not statistically different ($p \leq 0.05$) compared to control. The haematological parameters range within several pieces of research of haematological studies of pigs as reported in a review of the influence of nutrition on blood parameters of pigs (Etim et al, 2014). However, there is slightly above the normal range of haematocrit (PCV) 51.3% instead of 32 – 50%. The increase of haematocrits coupled with red blood cells may be an indicator of efficiency in blood making (erythropoiesis) in the experimented animals (Togun et al, 2007). Also, the lymphocytes count in animals fed with 5% of YFCP was higher (64 %) above the normal range (39-62%). An increase in neutrophil: lymphocytes ratio may be an indicator of stress (Minka and Ayo, 2007). Monocytes seem to increase with the increasing level of YFCP but are statistically not significant. Therefore, in general, the haematological parameters from pigs fed with the experimented diets fall within the normal range of pig haematological parameters (Weiss & Wardrop, 2011). This meant that the experimental diets did not show any opposing effect during experimental periods (Togun et al, 2007).

5.2.3. Growth performance of pigs fed experimental diets

Body weight gain, nutrient digestibility, and feed convention ratio are good indicators of feed efficiency (Patience et al., 2015). The results of feed intake, final body weight gained, average body weight gained per day, and feed conversion ratio of the animals fed experimental diets in the present work (Table 25) were not statistically significant **(**$p \geq 0.05$**).** This implies that all three experimental diets included 5, 10, and 15% of YFCP can replace the control diets without any adverse effects on growth performance. This result was supported by Hu et al., (2008), Huu & Khammeng (2014), and Sengxayalth & Preston (2017) which suggested that the inclusion of fermented cassava pulp levels between 12 to 28% in replacement of ingredient in the diet has no effect on growth performance. Though the animals fed control and 15% YFCP diets (T1 and T4) had slightly higher mean body weight gains of 25.8 kg and 23.6 kg, respectively, compared to animals fed with 5% and 10% of YFCP (T2, and T3). The insignificantly different observed may be caused by higher digestibility in these treatments compared to the least. The diet contained 15% YFCP had slightly low feed intake of 67 kg compared to 73 kg of other diets. This was due to observed increased large particles of un-milled cassava pulp which left by pigs. Also, the higher CP of 15.54% in this treatment compared to 14% of other treatments which consumed more may be a reason because, Whittington et al (2007) described that; pigs fed on low protein diets respond by consuming more feed in an order to meet requirements for the limiting nutrients.

5.2.4. Cost analysis

This work also revealed that the inclusion of 5 and 10% of YFCP in diets was not economically different compared to control diets used in this experiment (Table 27). Unless 15% YFCP is used or higher inclusion should be investigated. However, the cost of the ingredients depends on demand and availability of feedstuffs in a specific area (Woyengo et al., 2014). Also, the labor work of preparing YFCP was not included which may increase the cost of this ingredient.

5.3 Conclusion

Based on this study, it can be concluded that chicken manure can be used as a nitrogen source in yeast fermentation of cassava pulp to improve crude protein content and decrease crude fibre of cassava pulp. However, it could lessen the gross energy and interfere with the pH value of an acidic condition during the fermentation process. The best fermentation time is between 10 and 30 days with 20% to 30% moisture and incubation at room temperature. The higher initial nitrogen source in fermentation contributes higher final crude protein composition. Both table sugar and molasses can be used as initial yeast energy sources activators.

The yeast fermented cassava pulp with chicken manure can be used as an alternative improved energy ingredient up to15% in pig diets without any harmful effect on nutrient digestibility, hematological parameters, and growth performance. The inclusion of YFCP below 15% is not economically different. Further study should be done on the higher inclusion level and nutraceutical effect of YFCP to reveal the maximum potential of this feed. Also, plant nitrogenous sources can be investigated as chicken manure alternatives to help farmers who are not happy to work on manure.

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