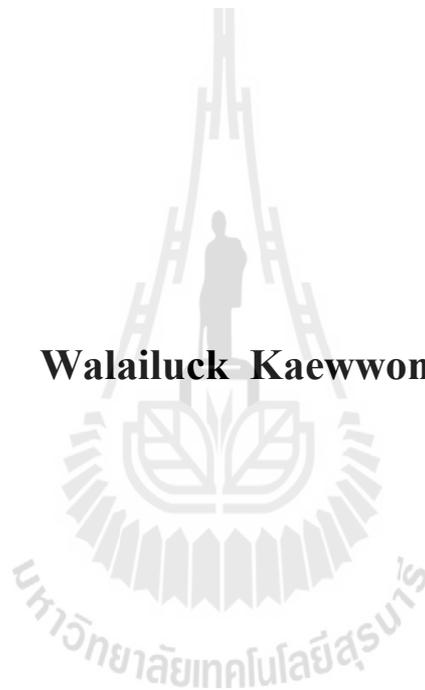


**UTILIZATION OF FERMENTED CASSAVA PULP BY
YEAST (*Saccharomyces cerevisiae*) AS A PROTEIN
SOURCE FOR MEAT GOATS**

Walailuck Kaewwongsa



**A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Animal Production Technology**

Suranaree University of Technology

Academic Year 2011

การใช้กากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ
(*Saccharomyces cerevisiae*) เป็นแหล่งโปรตีนสำหรับแพะเนื้อ

นางสาววลัยลักษณ์ แก้ววงษา



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2554

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***(Saccharomyces cerevisiae)* AS A PROTEIN SOURCE**
FOR MEAT GOATS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

(Assoc. Prof. Dr. Pongchan Na-Lampang)

Chairperson

(Asst. Prof. Dr. Pramote Paengkoum)

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วลัยลักษณ์ แก้ววงษา: การใช้กากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ เป็นแหล่งโปรตีนสำหรับแพะเนื้อ (UTILIZATION OF FERMENTED CASSAVA PULP BY YEAST (*Saccharomyces cerevisiae*) AS A PROTEIN SOURCE FOR MEAT GOATS) อาจารย์ที่ปรึกษา: ผู้ช่วยศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 126 หน้า.

วัตถุประสงค์ของการทดลองนี้คือ การศึกษาผลของการหมักกากมันสำปะหลังด้วยยีสต์แซคคาโรไมเซส เซรีวิซิเอ เพื่อใช้ทดแทนการใช้โปรตีนจากกากถั่วเหลืองในสูตรอาหารแพะเนื้อต่อปริมาณการกินได้ การย่อยได้ กระบวนการหมักในกระเพาะรูเมน ปริมาณไนโตรเจนที่ถูกกักเก็บ จำนวนประชากรจุลินทรีย์ในกระเพาะรูเมน และสมรรถนะการผลิตของแพะเนื้อ

การทดลองที่ 1 การทดสอบการเพิ่มโปรตีนของกากมันสำปะหลังหมักด้วยยีสต์แซคคาโรไมเซส เซรีวิซิเอ ทำวางแผนการทดลองแบบสุ่มสมบูรณ์ที่จัดกลุ่มทดลองแบบแฟคทอเรียล โดยแบ่งปัจจัยที่ต้องการศึกษาดังนี้ ปัจจัยเอและปัจจัยบี คือระดับของการใช้ยีสต์แซคคาโรไมเซส เซรีวิซิเอ 4 ระดับ (0, 0.5, 2.5 และ 5.0 เปอร์เซ็นต์) และระยะเวลาที่ทำการหมัก 4 ช่วงเวลา (0, 1, 3 และ 5 วัน) ตามลำดับ ทำการศึกษาเฉพาะส่วนใช้แพะเนื้อเพศผู้ และที่ผ่านเจาะกระเพาะรูเมน จำนวน 3 ตัว น้ำหนักเฉลี่ย 20 ± 5 กิโลกรัม แพะทดลองได้รับอาหารขึ้น 1.5 เปอร์เซ็นต์ของน้ำหนักตัว และได้รับอาหารฟางข้าวอย่างเต็มที่ เพื่อประมาณค่าความสามารถในการย่อยได้ของโปรตีน ด้วยการใช้เทคนิคถุงในล่อน และการศึกษาในหลอดทดลองโดยใช้เทคนิคทดสอบการย่อยด้วยเอนไซม์เปปซิน-แพนครีเอติน จากการทดลองพบว่าการหมักกากมันสำปะหลังด้วยยีสต์ 5 เปอร์เซ็นต์ เป็นเวลา 5 วัน ทำให้กากมันสำปะหลังหมักมีคุณค่าทางโปรตีนหยาบ และโปรตีนแท้สูงที่สุดคือ 31.6 และ 29.0 เปอร์เซ็นต์ ($P < 0.05$) ความสามารถและประสิทธิภาพของการย่อยได้วัตถุแห้ง และอินทรีย์วัตถุพบว่าไม่มีความแตกต่างกันระหว่างกลุ่มทดลอง อย่างไรก็ตามการย่อยได้รวมของโปรตีนหยาบ (กระเพาะรูเมน-ลำไส้เล็ก) มีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) เมื่อเพิ่มระดับการใช้ยีสต์

การทดลองที่ 2 ใช้แพะเนื้อเพศผู้เจาะกระเพาะรูเมน จำนวน 10 ตัว มีน้ำหนักเฉลี่ย 20 ± 5 กิโลกรัม ทำการสุ่มสัตว์เข้าทดลองตามแผนการทดลองแบบจัดรัสละตินที่ทำซ้ำหลายจัดรัส อาหารที่ใช้ในการทดลอง คือ การทดแทนการใช้โปรตีนจากกากถั่วเหลืองด้วยกากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ ที่ระดับ 0, 25, 50, 75 และ 100 เปอร์เซ็นต์ โปรตีนในสูตรอาหารขึ้น โดยทุกกลุ่มการทดลองได้รับสูตรอาหารที่มีระดับโปรตีนหยาบให้เท่ากับ 14 เปอร์เซ็นต์โปรตีน แพะทดลองจะได้รับอาหารขึ้น 1.5 เปอร์เซ็นต์ของน้ำหนักตัว และได้รับฟางข้าวเป็นแหล่งอาหารหยาบแบบเต็มที่ จากการทดลองพบว่าการเพิ่มขึ้นของระดับการทดแทนการใช้กากถั่วเหลืองด้วยกากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอในอาหารขึ้น ไม่มีผลกระทบต่อค่าความเป็นกรด-ด่าง

ในกระเพาะรูเมน, ค่าแอมโมเนีย-ไนโตรเจน, ค่าการย่อยได้ของโภชนะที่กิน และปริมาณการสังเคราะห์ไนโตรเจนของจุลินทรีย์ แต่อย่างไรก็ตามค่าพลาสมายูเรียไนโตรเจน, กรดไขมันที่ระเหยได้รวมหลังการกินอาหาร, ปริมาณแบคทีเรียกลุ่มที่ย่อยสลายเซลลูโลส และสมมูลไนโตรเจน มีค่าเพิ่มขึ้นอย่างนัยสำคัญทางสถิติเมื่อมีการเพิ่มขึ้นของระดับการทดแทนการใช้กากถั่วเหลืองด้วยกากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ ที่ระดับ 75 เปอร์เซ็นต์ ยิ่งไปกว่านั้นพบว่ามีการเปลี่ยนแปลงของน้ำหนักตัวสูงที่สุด คือ 50 กรัมต่อวัน ในแพะที่ได้รับอาหารชั้นที่มีทดแทนการใช้กากถั่วเหลืองด้วยกากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ ที่ระดับ 75 เปอร์เซ็นต์ จากการศึกษาพบว่าการใช้กากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ ที่ระดับ 75 เปอร์เซ็นต์ เป็นแหล่งโปรตีนที่เหมาะสมที่จะใช้ทดแทนการใช้กากถั่วเหลืองซึ่งส่งผลต่อแพะระยะที่กำลังเจริญเติบโตในส่วนของปริมาณการกินได้ และสมรรถนะการผลิต

การทดลองที่ 3 การใช้กากมันสำปะหลังหมักยีสต์แซคคาโรไมเซส เซรีวิซิเอ เป็นแหล่งโปรตีนทดแทนการใช้กากถั่วเหลืองในสูตรอาหารชั้น ต่อปริมาณการกินได้ กระบวนการหมักในกระเพาะรูเมน สมรรถนะการเจริญเติบโต และคุณภาพซากของแพะ โดยใช้แพะเนื้อเพศผู้จำนวน 24 ตัว มีน้ำหนักเฉลี่ย 18 ± 5 กิโลกรัม วางแผนการทดลองแบบสุ่มในบล็อกสมบูรณ์ แบ่งกลุ่มทดลองเป็น 4 กลุ่ม ตามการเพิ่มขึ้นของระดับของการให้อาหาร คือที่ระดับ 1.0, 1.5, 2.0 และ 2.5 เปอร์เซ็นต์ต่อน้ำหนักตัวตามลำดับ อาหารชั้นประกอบไปด้วยกากมันสำปะหลังหมักยีสต์ เป็นแหล่งโปรตีนจากการทดลองพบว่า อัตราการเจริญเติบโต, ปริมาณไนโตรเจนที่ถูกกักเก็บ, ปริมาณการกินได้ของวัตถุดิบทั้งหมด, ความสามารถในการย่อยได้ของโปรตีนหยาบ, การย่อยได้ของโภชนะที่กิน (อินทรีย์วัตถุ, โปรตีนหยาบ, เยื่อใยที่ไม่ละลายในสารฟอกที่เป็นกลาง และเยื่อใยที่ไม่ละลายในสารฟอกที่เป็นกรด) และค่าพลังงานที่ใช้ประโยชน์ได้จากอาหารที่กิน มีการเพิ่มขึ้นแบบเป็นเส้นตรงอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) เมื่อมีการเพิ่มขึ้นของระดับการใช้กากมันสำปะหลังหมักยีสต์ในอาหารชั้น ยิ่งไปกว่านั้นแพะที่ได้รับอาหารชั้นที่มีกากมันสำปะหลังหมักยีสต์ที่ระดับการให้อาหาร 2.0 และ 2.5 เปอร์เซ็นต์ของน้ำหนักตัว มีอัตราการเจริญเติบโต, สังเคราะห์ไนโตรเจนของจุลินทรีย์ และสมมูลไนโตรเจน มากกว่าแพะที่ได้รับอาหารชั้นที่มีกากมันสำปะหลังหมักยีสต์ที่ระดับการให้อาหาร 1.0 และ 1.5 เปอร์เซ็นต์ของน้ำหนักตัว มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) แต่อย่างไรก็ตามพบว่าไม่มีความแตกต่างกันทางสถิติ ($P > 0.05$) ของความสามารถในการย่อยได้ของโภชนะ, ค่าความเป็นกรด-ด่างในกระเพาะรูเมน, ค่าแอมโมเนีย-ไนโตรเจน, ปริมาณการสังเคราะห์ไนโตรเจนของจุลินทรีย์ และองค์ประกอบของซากแพะที่ได้รับระดับของการให้อาหารที่แตกต่างกัน

จากการทดลองทั้ง 3 การทดลอง พบว่าการหมักกากมันสำปะหลังด้วยยีสต์แซคคาโรไมเซส เซรีวิซิเอ 5 เปอร์เซ็นต์ เป็นเวลา 5 วัน ทำให้เพิ่มคุณภาพทางโปรตีนที่ส่งผลต่อความสามารถในการย่อยได้ ในที่นี้แนะนำให้ใช้กากมันสำปะหลังหมักยีสต์ทดแทนการใช้กากถั่วเหลืองได้ถึง

75 เปอร์เซ็นต์ ในอาหารชั้นของพะเนื่อที่มีการให้ฟางข้าวเป็นแหล่งอาหารหยาบ สามารถเพิ่มระดับของการให้อาหารได้ถึง 2.5 เปอร์เซ็นต์ของน้ำหนักตัว เมื่อมีการใช้กากมันสำปะหลังหมักยีสต์ 75 เปอร์เซ็นต์ เป็นแหล่งโปรตีนทดแทนการใช้กากถั่วเหลืองซึ่งจะให้ผลดีต่อพะเนื่อในส่วนของปริมาณการกินได้ และสมรรถนะการผลิต



สาขาวิชา เทคโนโลยีการผลิตสัตว์
ปีการศึกษา 2554

ลายมือชื่อนักศึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

WALAILUCK KAEEWONGSA : UTILIZATION OF FERMENTED
CASSAVA PULP BY YEAST (*Saccharomyces cerevisiae*) AS A PROTEIN
SOURCE FOR MEAT GOATS. THESIS ADVISOR : ASST. PROF.
PRAMOTE PAENKOU, Ph.D., 126 PP.

UTILIZATION/MEAT GOATS/*Saccharomyces cerevisiae*/CASSAVA
PULP/FERMENTED/REPLACEMENT/SOYBEAN MEAL

The objective of this study was to investigate the effects of fermented cassava pulp by yeast (*Saccharomyces cerevisiae*) for replacing protein soybean meal in meat goat rations with respect to feed intake, digestibility, rumen fermentation, nitrogen retention, rumen microbes, and productivity of the meat goat.

In experiment I, the protein enrichment of cassava pulp fermentation by *S. cerevisiae* was tested. The experiment was assigned in a 4x4 factorial arrangement in a completely randomized design (CRD). Factor A and B were the level of *S. cerevisiae* (0, 0.5, 2.5, 5.0 %) and the times of fermentation (0, 1, 3, and 5 days), respectively. *In situ* studies were conducted in three male meat goats and rumen cannulated with an average weight of 20±5 kg. The goats were fed with concentrate at 1.5% body weight and rice straw was offered *ad libitum*. An estimation of rumen undegradable protein was tested by the *in situ* nylon bag technique and *in vitro* pepsin-pancreatin digestion technique. The results showed that the highest crude protein and true protein contents were 31.6 and 29.0% dry matter (DM), respectively in the fermented cassava pulp with 5% *S. cerevisiae* after a 5 day period (P<0.05). The potential and effective degradability of DM and organic matter (OM) were not

significant among treatments. However, total crude protein digestibility (rumen-intestinal) increased ($P < 0.05$) with increasing levels of yeast.

In experiment II, ten male rumen cannulated for growing goat with an average body weight of 20 ± 5 kg, were randomly assigned in double 5 x 5 Latin Square Design. The dietary treatments were the replacement of soybean meal (SBM) by fermented cassava pulp with *S. cerevisiae* (FCSC) at 0, 25, 50, 75 and 100% CP in of concentrate. All diets were formulated to contain isonitrogenous feed rations and formulated to meet 14 % CP. The goats were fed with concentrated diet at 1.5% of body weight and rice straw was offered *ad libitum*. The results showed that increasing level of SBM by FCSC in the diet did not affect ruminal pH, ammonia nitrogen ($\text{NH}_3\text{-N}$), digestible nutrient intake and microbial N supply. However, plasma urea nitrogen (PUN), total volatile fatty acids (TVFA) on post feeding, cellulolytic bacteria and nitrogen balance increased ($P < 0.05$) with increasing level of FCSC 75% replacement. Moreover, The highest body weight change were 50 g/d in goats fed with FCSC replacement of SBM 75% concentrate. Based on this results using 75% FCSC as the main source of protein to completely replace SBM was beneficial to growing goats in terms of feed intake and productive performance.

In experiment III, the substitution of FCSC as a protein replacement for SBM in concentrated diets on feed intake, rumen fermentation, growth performance, and carcass quality of goats was implemented. Twenty-four male meat goats with body weight of 18 ± 5.0 kg were used. The experiment was assigned in a randomized complete block design (RCBD). There were four different dietary treatments: with increased feeding levels at 1.0, 1.5, 2.0 and 2.5% BW, respectively. The concentrate contained yeast fermented cassava pulp as the main protein source. The results showed

that average daily gain (ADG), N retention, total DM intake, CP digestibility, digestible nutrient (OM, CP, NDF, ADF) intake and ME intake increased linearly ($P < 0.05$) with increasing feeding levels of FCSC concentrate diets. Moreover, goats fed with FCSC at 2.0 and 2.5% BW were significantly ($P < 0.05$) in ADG, microbial N supply and N balance than goats fed with FCSC at 1.0 and 1.5% BW. However, there were no significant differences ($P > 0.05$) in nutrient digestibility, ruminal pH, $\text{NH}_3\text{-N}$, microbial N synthesis and carcass composition in goats fed at different feeding levels.

It can be concluded from the results of the three experiments conducted in this research, that fermented cassava pulp with 5% *S. cerevisiae* for a 5 day period, increased CP content improved degradability. It is suggested that fermented cassava pulp by yeast could replace up to 75% of SBM in increased diet of meat goats fed rice straw as roughage. Feeding levels were increased to 2.5 % by using 75% FCSC as the main source of protein as the complete replacement of SBM would be beneficial for meat goats in terms of feed intake and productive performance.

School of Animal Production Technology Student's Signature _____

Academic Year 2011

Advisor's Signature _____

Co-advisor's Signature _____

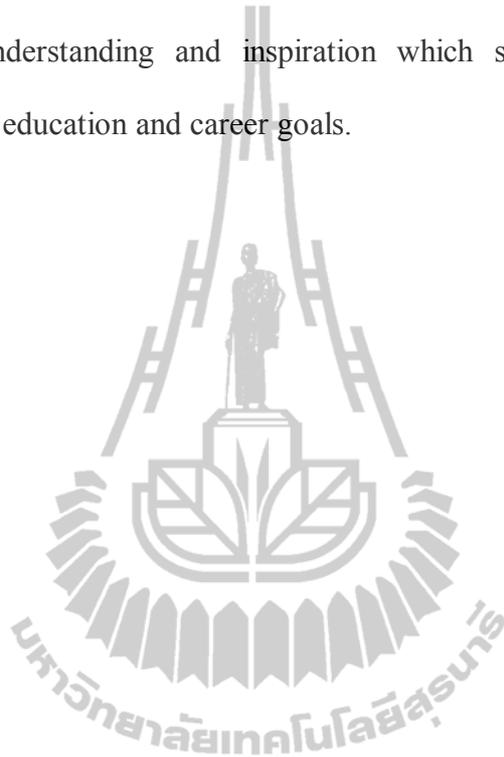
Co-advisor's Signature _____

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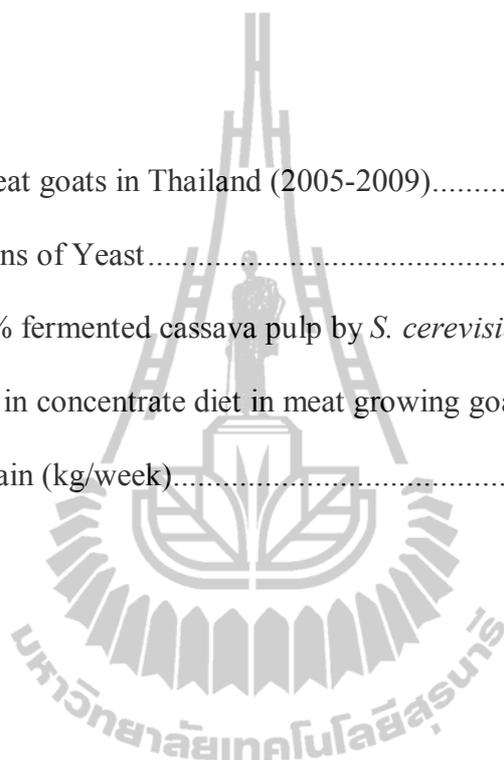
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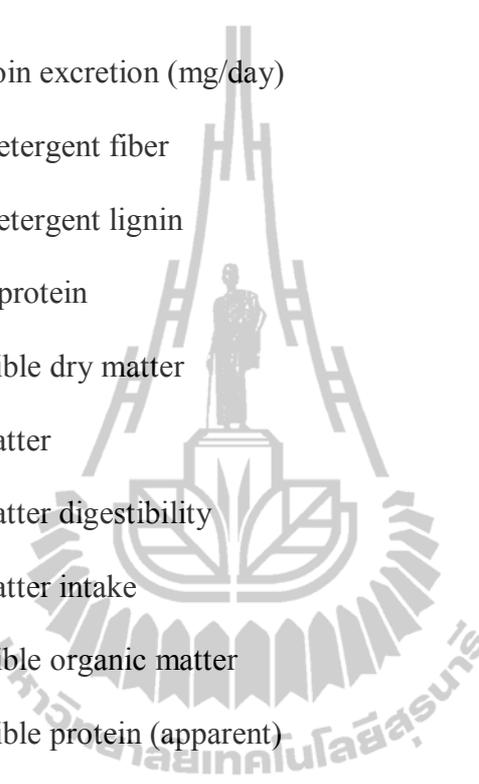
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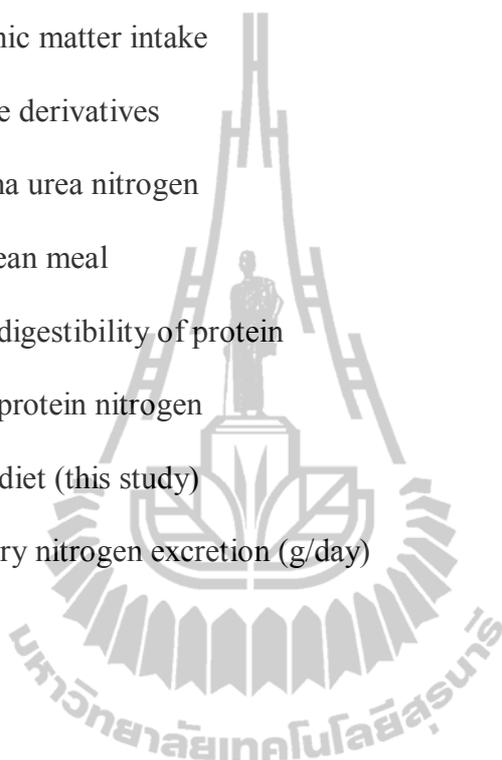
LIST OF ABBREVIATIONS



A	=	Allantoin excretion (mg/day)
ADF	=	Acid detergent fiber
ADL	=	Acid detergent lignin
CP	=	Crude protein
DDM	=	Digestible dry matter
DM	=	Dry matter
DMD	=	Dry matter digestibility
DMI	=	Dry matter intake
DOM	=	Digestible organic matter
DP	=	Digestible protein (apparent)
FCSC	=	Fermented cassava pulp by yeast (<i>Saccharomyces cerevisiae</i>)
GC	=	Gas chromatography
HPLC	=	High-performance liquid chromatography
N	=	Nitrogen
NA	=	Nitrogen absorbed nitrogen
NB	=	Nitrogen balance (g/day)
NDF	=	Neutral detergent fiber
NI	=	Nitrogen intake
NPN	=	Non-protein nitrogen
OM	=	Organic matter

LIST OF ABBREVIATIONS (Continued)

OMD	=	Organic matter digestibility
OMI	=	Organic matter intake
PD	=	Purine derivatives
PUN	=	Plasma urea nitrogen
SBM	=	Soybean meal
TD	=	True digestibility of protein
TPN	=	True protein nitrogen
U	=	Urea diet (this study)
UN	=	Urinary nitrogen excretion (g/day)



CHAPTER I

INTRODUCTION

1.1 Rationable of the Study

In Thailand, goats are predominantly raised by small-holder farmers, particularly in Muslim community. According to the report of Department of Livestock Development in 2010, October with a population 380,277 goats are kept by 36,629 families. Nowadays, Thai farmers are more interested in meat goat business because the meat goats are easy to rise. They can be bred relatively fast and fed by a wide range of roughage. In situations where concentrate feed supplements are expensive, farmers should be capable of formulating their own feeds based on available farm resources and their economical viability (Wanapat, 1999). The quality of the roughage has got a low nutrient and feeding value due to the fact that high temperature leads the grass has got high lignin content. In general, goats raised in small-holder farms are fed with local roughages or some agricultural by-products. Research on feeds and feeding is then directed to the effects of these roughages on performances of goats. So, Feeding is base on concentrated feed in order to meet all requirements on growth of goats. In the concentrated feed, protein is the most expensive component in the ration. The protein feed is mainly from soybean meal and fish meal, the demand has been increasing. As the result, the price is inevitably getting expensive and causing high cost for farmers resulting in lower income. Finding the alternative protein feed is a solution for reducing the feeding cost.

Among microorganisms considered as possible food sources, yeast has attracted perhaps the greatest interest. It represents, in fact, the only sort of microbial protein that has in the past been used as a food to an extent worth mentioning. As a feed, yeast (*Sacchromyces cerevisiae*) has a well established place for many decades (Chumkhunthod, 2000; Kockva-kratochivilova, 1990; Berry, 1989 and DeMot, 1990). The main reason why yeast is generally used in animal production due to the fact that yeast production technology is a well known procedure in the intrusdial sector. The nutritive values and compositions of yeast has been classified to be an excellent single cell protein source. Moreover, yeast is used as a pre-digester for many feedstuffs such as wine production. Supplemental yeast as prebiotic for ruminants have been shown to improve nutrient digestion and rumen fermentation (Polviset, Wachirapakorn, Alhaidary, Mohamed, Beynen, and Yuangklang, 2010; Sommart, Wanapat, Wachirapakorn, Chanthai, and Toburan, 1991).

Cassava pulp is a by-product from cassava-producing ethanol industry. Cassava pulp is consisted medium starch, low nitrogen and high fiber fractions. However, cassava pulp is conceivable for use as ruminant feed ingredient due to the chemical composition of itself. Before cassava pulp can be used in ruminant ration, it would be reasonable to improve the quality. As mention above, yeast has been known to improve the protein quality of many feedstuffs. Thus, yeast must be also improve the protein quality of cassava pulp. In the trial of Oboh (2006) who studied the nutrient enrichment of cassava peels using a mixed culture of *S. cerevisiae* and *Lactobacillus spp* by the solid media fermentation techniques. The result of the analysis of the fermented cassava peels revealed that there was an increase in the protein content of the cassava peels fermented with wastewater from fermented cassava pulp when

compared to unfermented peels (8.2%). This increase was highest in the cassava peel fermented with wastewater from the inoculated cassava pulp (21.1%) (Ubalua, 2007). Furthermore, Shrasen, Abbot, and Battey (1970) described a process in which the yeast *Candida utilis* was enzymatically hydrolyzed cassava in a submerged culture to produce a product containing 35% crude protein on a dry weight basis. Iyayi and Losel (2001) reported protein enrichment of cassava through less expensive means is therefore desirable. Fungal or yeast fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in solid state. The attractive characteristics in the use of microorganism for single cell protein include (1) their fast growth rate even in semi solid and solid media: (2) their high level of protein: (3) their comparable good nutritional values and (4) their easy genetically modification to growth under specific conditions on particular substrates.

This study was interested in the utilization of cassava pulp fermentation with yeast *S. cerevisiae* in growing goat diets. The first part of *in vitro* study was to investigate the fermentation cassava pulp with yeast *S. cerevisiae* on the nutritional quality of the product. The second part of *in vivo* study was contributed to the knowledge of different protein sources from cassava pulp fermentation by *S. cerevisiae* in concentrate diets of goats fed low quality roughages (rice straw).

1.2 Research Hypothesis

1.2.1 Fermentation of cassava pulp with yeast *S. cerevisiae* would be enhanced the nutritional quality of product.

1.2.2 Utilization of fermented cassava pulp with yeast *S. cerevisiae* for meat goats production would be improved the rate of degradation of rumen undegradable protein.

1.2.3 Fermented cassava pulp with yeast *S. cerevisiae* would be alternative protein source to replace the expensive ingredient in meat goats without any adversely effect on productive performance.

1.3 Research Objectives

The main objective of this research was to utilize of fermented cassava pulp with yeast *S. cerevisiae* in dietary of meat goats. The specific objectives were:

1.3.1 To determine the optimal level of yeast *S. cerevisiae* and incubation times as protein source to future enhance utilization in goat's rations.

1.3.2 The purpose of this study was to determine fermented cassava pulp with yeast *S. cerevisiae* replacement protein source from soybean meal in Thai native x Anglo-Nubian crossbred meat goat diets.

1.3.3 To investigate the optimal feeding levels of concentrated feed in terms of efficiency of rumen fermentation, nutrient digestibilities and productive Thai native x Anglo-Nubian crossbred meat goats.

1.4 Scope of the Study

These researches intended to study fermented cassava pulp with yeast *S. cerevisiae* to enhance the nutritional quality of cassava pulp and the effects of fermented cassava pulp with *S. cerevisiae* replacement soybean meal in growing

meat goat's diets on rumen fermentation of Thai native x Anglo-Nubian crossbred meat goats from Nakhon Ratchasima province.

1.5 Expected Results

1.5.1 Fermentation of cassava pulp with yeast *S. cerevisiae* would be enhanced the nutritional quality of product.

1.5.2 Fermented cassava pulp with yeast *S. cerevisiae* can be used to improve the rate of degradation of rumen undegradable protein.

1.5.3 Fermented cassava pulp with yeast *S. cerevisiae* can be used as a protein source to replace soybean meal in goat diet.

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CHAPTER II

REVIEW OF LITERATURE

2.1 Goats production in Thailand

The world goat population is 550 million, 94% of this total is found in the Mediterranean, Asia and Africa (Lan et al., 2007). Furthermore, in a few European countries such as Italy and Hungary, the making of goat cheese is of major interest and has motivated the setting-up of a genetic improvement program. Goat's meat is also consumed in many regions of the world. In the USA, the demand for goat's meat exceeds availability (Glimp, 2005). Similarly in Thailand, goat production has increased steadily during the past 5 years because of the increased demand for goat's meat by ethnic groups who prefer goat's meat in their diet. The number of goats is highest in southern of Thailand, especially Yala province while the number of goats is lowest in northeast Thailand (Division of Planning, Department of Livestock Development, 2007). The production of meat goats in Thailand has increased in recent years (Supakorn, 2009). In Thailand, about 80% of goats were found mostly concentrated in center and southern part of Thailand. The demand for goat's meat tends to exceed the meat available (Division of Planning, Department of Livestock Development, 2007) and showed that Table 2.1.

According to the department of livestock division reported, currently the demand in goat meat and products involved with goat was rising both in the domestic market and the foreign one such as Malaysia and Kuwait. However the supply of goat

produced in Thailand was not enough to the demand, it could fulfill only 60% of the total demand. Currently, there were 400,000 goats raised in the country, mostly in the south, a Muslim-dominated region, where most of the Muslim families raised no more than five goats each (Bumroongpuk, 2009) Economic traits in goats can be divided into 4 main types; growth, reproduction, meat and milk traits.

Table 2.1 Number of meat goats in Thailand (2005-2009).

Locations	Number of meat goats (head/year)				
	2005	2006	2007	2008	2009
Northern	55,310	56,149	86,373	49,315	61,368
Northeastern	13,974	15,014	21,423	17,522	20,363
Middle	109,681	111,742	162,926	139,011	160,278
Southern	159,390	141,245	174,052	138,668	141,787
Total	338,355	324,150	444,774	344,516	383,796

Source: Department of Livestock Development (2010).

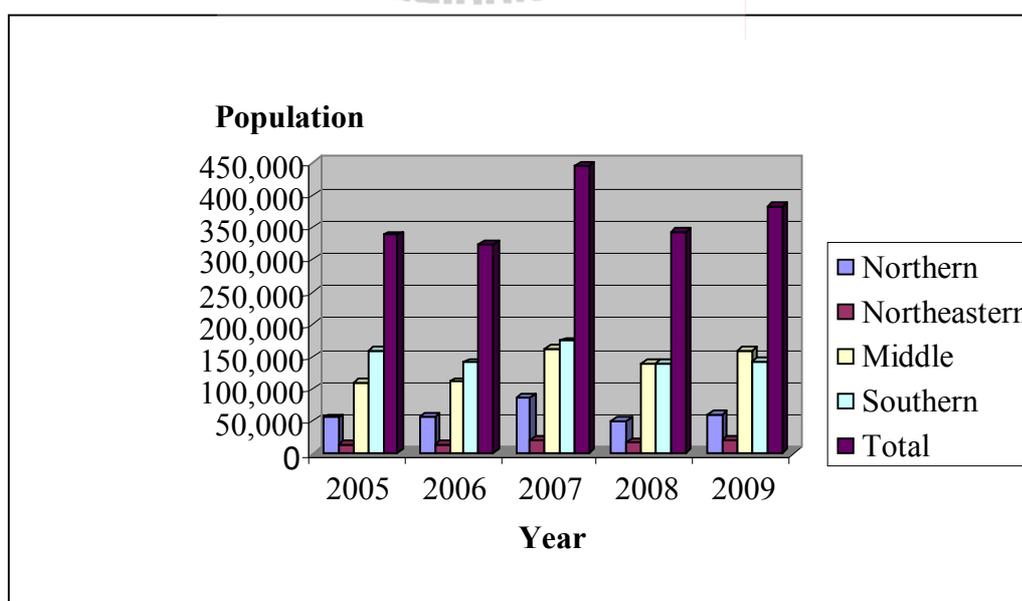


Figure 2.1 Number of meat goats in Thailand (2005-2009).

In addition, disease resistance is an important trait in small ruminant herds. These traits are controlled by polygenes and are affected by environmental factors. The breeding improvement for these economic traits of goats in the past had been achieved by selection based on only phenotypic expression. In addition, disease resistance is an important trait in small ruminant herds. (Supakorn, 2009). In general, the main breeds of Thai goats are Thai native goat or the cross-bred of Thai native with Anglo-nubien breed or Saanen (Pralomgarn, 1987; 1995). The increasing number of goats certainly aggravates the grazing, and takes a bad turn of the degradation of grassland. The grazing system also contributes to the destroyed environment for the higher nitrate contamination of surface and groundwater, pathogens contamination, and also methane emissions (Siegford et al., 2008).

2.2 Nutritional characteristics of ruminants and goats

Ruminant animals have evolved the ability to utilize vegetative plant material as their sole source of nutrition (Hofman, 1989). This utilization occurs via a symbiotic relationship with rumen microorganisms that ferment polysaccharides in vegetative plant material that cannot be easily digested and utilized by mammalian enzymes (Hungate, 1966). There are several types of ruminants: those that consume grasses are grazers, those that consume brush material are browsers, and those that consume both are intermediate feeders. Goats consume both grasses and brush material, and are therefore considered intermediate feeders, more specifically intermediate browsers (Pande et al., 2002; Lyons et al., 1998). Concentrate ingredients are commonly used in ruminant feeding systems to supply both protein and energy to the animal. By-product feeds are increasingly important in ruminant feeding systems (Moore-Colyer, Hyslop,

Longland, and Cuddeford, 2000). Although concentrates, such as grain, are fed extensively to ruminant livestock, because of the digestive capacity of goats to utilize brushy materials, forages represent the most important and valuable feed resource for this ruminant (Jung and Allen, 1995). In goat meat, growth rate is very important because farmers earn money from the meat production. To maximize the growth rate, feeding plays a crucial role. A goat needs to get sufficient energy for their growth. Therefore, roughage together with concentrate feed need to meet all requirements of the goats.

2.3 Goat nutrition and performance

Sanon (2007) note that goats are highly prolific, producing twins or triplets (most tropical breeds) allowing owners to build flocks quickly. The goat is often used as a first step towards livestock ownership, as with a large flock part can be sold and replaced by sheep or cattle. Many other attributes of goats are reported, including small size and low price, which is ideal for slaughter at any celebration, and to sell when cash is needed. They are easy to manage by children and can survive in harsh conditions (low availability of vegetation in the arid areas, feed rich in fibre and low in nitrogen, lack of water and heat stress). All these attributes lead to the connotation that the goat is “a poor man’s cow” (Mahatma Gandhi, great Indian Leader) quotation taken up by Peacock (1996) who qualified it as “a poor person’s bank”. The feeding strategies of goats, reported by Luginbuhl and Poore (1998), consist of selecting grasses when the protein content and the digestibility are high, but switching to browse when their nutritive value may be higher. However, where browse is not available, goats can feed on grasses and crop residues such as cereal straws, but tend to prefer

less coarse grasses (Devendra and McLeroy, 1982). Luginbuhl and Poore (1998) noted that goats are not able to digest cell walls as well as cattle because the feed stays in their rumen for a shorter period of time. On the other hand, Morand-Fehr (2005) reported similar retention time of feed particles in the whole digestive tract of sheep and goats eating the same quantity of good quality forage, but the retention time of goats receiving poor quality forage was longer. Hence sheep and goats have similar patterns of digestion of moderate to high quality forages, but goats are better in digesting forages rich in cell walls and poor in nitrogen. This seems to be related to their ability to recycle urea nitrogen (Silanikove, 2000). In addition, goats are efficient in the use of water and have a low rate of water turnover per unit of body weight (Devendra and McLeroy, 1982). The adaptation of goats to water shortage in hot environments in the tropics has been explained by low water turnover and the ability to resist desiccation (they do not sweat, and lose less water in feces and urine). The DM intake of goats, indicating the capacity to utilise feed voluntarily, depends on the breed (meat or milk) and the environment. Thus in the tropics, intake of 4 to 5% of live weight has been reported for dairy goats and 3% for meat goats (Devendra and McLeroy, 1982). The growth rate and mature weight of goats vary widely in different parts of the world, due to differences in breeds and level of nutritional management. However, Luginbuhl and Poore (1998) noted that the goat has lower rate of weight gain and do not fatten like cattle and sheep; thus to achieve maximum potential, goats need high quality feed and require optimum balance of many different nutrients.

2.4 Feeding requirements for goat

The goat is not able to digest the cell walls of plants as well as the cow because feed stays in its rumen for a shorter time period. A distinction as to what is meant by "poor quality roughage" is necessary in order to make decisions concerning which animal can best utilize a particular forage. Trees and shrubs, which often represent poor quality roughage sources for cattle, because of their high tannin content, highly lignified stems and bitter taste, may be adequate to high in quality for goats. This is because goats can manipulate their lips as they browse, and thereby avoid eating the stems, they don't mind the taste, they have the ability to detoxify tannins, and as a result they benefit from the relatively high levels of protein and cell soluble found in the leaves and buds of these plants. On the other hand, low quality hay with high cell wall and low protein, can be used by cattle but will not provide even maintenance needs for goats because goats don't utilize the cell wall as efficiently as cattle. In addition, goats must consume a higher quality diet than cattle because their digestive tract size is smaller with regard to their maintenance energy needs. Relative to their body weight, the amount of feed needed by meat goats is approximately twice that of cattle. When the density of high quality forage is low and the stocking rate is low, goats will still perform well because their grazing/browsing behavior allow them to select only the highest quality forage from that on offer. Thus, they are able to perform well in these situations, even though their nutrient requirements exceed those of most domesticated ruminant species.

2.5 Nutrient requirements for goats

Meat goats require nutrients for body maintenance, growth, reproduction, pregnancy, and production of products such as meat, milk and hair. The groups of nutrients that are essential in goat nutrition are water, energy, protein, minerals and vitamins. The nutrient requirements of bucks, young goats and does with a high production potential and at various stages of development and production. Goats should be grouped according to their nutritional needs to more effectively match feed quality and supply to animal need. Weanlings goats, does during the last month of gestation, lactating does and yearlings should be grouped and fed separately from dry does and mature bucks which have lower nutritional needs.

2.5.1 Protein requirements

Protein is usually the most expensive component of the goat diet. As for energy, lush leafy forage and browse, and tree leaves contain sufficient protein to cover the nutrient requirements of every goat on the farm. Concentrates that are high enough in protein to serve as supplements are whole cottonseed, soybean meal, wheat middlings and corn gluten feed. Protein is required both as a source of nitrogen for the ruminal bacteria and to supply amino acids for protein synthesis in the animal's body. When the level of protein is low in the diet, digestion of carbohydrates in the rumen will slow and intake of feed will decrease. Inadequate levels of protein in the diet can negatively affect growth rate, milk production, reproduction and disease resistance because insufficient amino acids are getting to the intestines to be absorbed by the body. Unlike energy, excess of protein is not stored in the body of the goat; it is excreted in the urine as urea. Therefore, it is important for animals to have access to enough protein to cover their nutritional requirements. Protein requirements vary with

developmental and physiological stages and level of production (Palomkarn, 1995; Pande, Kemp, and Hodgson, 2002; Poore and Luginbuhl, 2002; NRC, 2006).

2.5.2 Energy requirements

Energy comes primarily from carbohydrates (sugars, starch, and fiber) and fats in the diet. Lush leafy forage and browse, and tree leaves contain sufficient energy to cover the nutrient requirements of every goat on the farm. Feeds that are high in energy are corn and other feed grains, whole cottonseed, wheat middlings, soybean hulls, and corn gluten feed. Bacteria that are present in the rumen of goats ferment sugars, starches, fats and fibrous carbohydrates into volatile fatty acids. These acids are absorbed and used for energy. Fat is efficiently used for energy, but the amount that can be included in the diet is limited. Usually added fat should not represent more than 5% of a diet because it depresses ruminal fermentation. For example, if whole cottonseed (perhaps with as much as 25% fat) is used as a supplement, it should not be more than 20% of the diet. Whole cottonseed also contains a good level of protein and phosphorous, and fed at 0.5 to 1.0 lb per day makes an excellent supplement to low quality forage. If the diet consumed by goats contains an excess of energy, that extra energy will be stored in the body as fat, mainly around the internal organs (Pande, Kemp, and Hodgson, 2002; Poore, and Luginbuhl, 2002; NRC, 2006).

2.6 Production of cassava in Thailand

Cassava (*Manihot esculenta* Crantz) is grown in tropical countries in Africa, Asia, and Latin America, with 70% of the world's cassava production coming from Nigeria, Brazil, Thailand, Indonesia, and the Democratic Republic of the Congo.

Cassava root can be used to produce cassava chips, cassava pellets, and cassava starch, which are in high demand throughout the world (FAO, 2008; Khempaka, Molee, and Guillaume, 2009). In Thailand, cassava starch is a large and growing industry with about 10 million tons of fresh cassava roots used for the production of starch, generating at least 1 million tons of pulp annually (Sriroth, 1994). Cassava is widely cultivated in tropical areas and used as food and animal fodder (Kosugi et al., 2009). The main concentration of the crop is now found in the northeast of Thailand, especially in Nakhon Ratchasima province. Cassava has excellent drought tolerance properties and can be planted in almost all types of soil. Therefore, the planted area has rapidly increased. Cassava is grown by a large number of farmers, who own small plots of land (about 0.5-2 ha). The main application for the large quantities of waste material produced each year, after drying, is as a low value animal feed or fertilizer. Due, in part, to the processing practice in Thailand, a large amount of starch remains in the pulp (up to 50±60%, dry basis) (Grace, 1977; Balagopalan, Ray, Sheriff, and Rajalekshmy, 1994); in addition the pulp is also high in moisture (60±70%). These factors combine to create a difficult drying process that is both inefficient and expensive. Poorly dried or fresh pulp spoils rapidly in the humid warm tropical environment as microorganisms quickly multiply on this substrate high in nutrients (Sriroth, Chollakup, Chotineeranat, Piyachomkwan, and Oates, 2000). Cassava pulp, the by-product from the procedure produces cassava flour, which very the inventory is left abandon that might cause way pollution problem from bin rotten of its waste when, can not eradicate get in a correct way and is appropriate, and have using, cassava waste dries out to go to mix is the feed for animals. In fact, cassava pulp is cheap starchy raw material with low protein content. Animal feed is another high value added from

cassava pulp. Recently, many studies have been conducted on cassava pulp utilization, for instance, the production of citric acid and reducing sugar from cassava pulp (Lowongwatana, 1985; Chotineeranat, 1996). Cassava pulp can be used to produce ethanol. Ethanol or ethyl alcohol, an alternative biofuel which is used to blend with gasoline to obtain a product called gasohol, can be produced from various carbohydrate-containing materials by yeast fermentation. In Thailand, there are many economic crops that can be used as the raw material for ethanol production including sugar cane, cassava, rice, sweet sorghum and corn. Use of cassava pulp as raw material in ethanol production not only reduces waste material created from the cassava starch industry, but also lowers the cost of ethanol production (Srinorakutara, Kaewvimol, and Saengow, 2006). Ethanol is increasingly used as an alternative fuel in the transportation sector. In general, fuel ethanol is produced mainly from sugar cane, corn, and cassava. However, a dramatic increase in ethanol production using the crops mentioned earlier may not be practical, because these same crops are important sources of food and feed, and expansion of fuel ethanol production using these crops could lead to shortages and price increase in food and feed. (Kosugi et al., 2009).

2.7 Processing cassava for animal feeds

Animal feed has always been a major limiting factor in the growth of the livestock industry in developing countries. Most of the feed ingredients are imported and a large proportion of foreign exchange is spent for this purpose. Availability of animal feed is one of the greatest constraints to the expansion of the livestock industry in developing countries. Apart from the high and fluctuating costs, some of the ingredients used in mixed feeds, notably cereal grains, are in high demand for human

consumption. In view of the dwindling supply of the conventional feed resources and the shortage of foreign exchange for importation, alternative sources produced locally within these countries are being investigated. The compositions of cassava waste approximately contains carbohydrate 68.4%, fiber 13.45%, protein 2.15%, fat 0.16% and ash 4.59% (Srinophakun, Srinophakun, Krusong, and Limpai boon, 1999). Thailand cassava pulp is always sold as a cheap animal feed material in substitution of rice bran and broken rice. Therefore, through the solid-state fermentation, protein content in the cassava pulp can be increased. Fermented cassava pulp then can be used as the protein material in feed which is not only reduce the environmental problem of cassava flour production factory but also lower the cost of animal feed (Wattanachaisaereekul, 2000).

2.8 Need for protein enrichment of cassava using microbial

The economic feasibility of using cassava based rations for animals depends mainly on the price of cassava in relation to alternate energy sources and the price of the supplementary protein sources to be added to balance the protein requirements of animals to be fed. Because of the very low protein content of the cassava tubers, any substitution of cassava for cereals in compounded feeds necessitates the inclusion of a considerable amount of supplementary protein. Experimental studies conducted by (Gomez, Cawacho, and Maner, 1976) showed that a swine feeding programme based on cassava meal required approximately 60 to 65% more protein supplement than a similar feeding programme using maize as an energy sources. Therefore, in developing countries the potential for cassava use as animal feed depends mainly on the availability of cheap protein sources. An alternate approach is to enrich cassava flour

with microbial protein. The microbial enrichment process is relatively cheap and the enriched product can increase the potential of cassava as a feed (Balagopalan, Padmaja and George, 1990).

Oboh (2006) studied the nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus* spp solid media fermentation techniques. Three treatments (mixing of 150 ml of wastewater from fermented cassava pulp with 200 g of washed, dried and ground cassava peels and mixing of 150 ml of waste water from the fermented inoculated pulp with 200 g of washed, dried and ground cassava peels) were observed for fermentation. The unfermented cassava peels served as control. The result of the analysis of the fermented cassava peels revealed that there was an increase in the protein content of the cassava peels fermented with wastewater from fermented cassava pulp when compared to unfermented peels (8.2%). This increase was highest in the cassava peel fermented with wastewater from the inoculated cassava pulp (21.1%) (Ubalua, 2007). Shrasen, Abbot, and Battey (1970) described a process in which the yeast *Candida utilis* fermented enzymatically hydrolyzed cassava in a submerged culture to produce a product containing 35% crude protein on a dry weight basis. Gregory (1977) using *Aspergillus fumigatus* 1-21 A fermented whole cassava in a non aseptic continuous fermentation system to produce single cell protein containing 37% crude and 27% true proteins. The fungi was a non revertible sporogonous mutant of *A. fumigatus* 1-21. This product was fed to rats and produced good growth responses. *Rhodospseudomonas gelatinosa*, a photosynthetic bacterium, was cultivated on cassava starch medium under aerobic dark and anaerobic light conditions. During fermentation of fufu, lactic acid bacteria, yeast

and other bacteria contribute significantly to starch breakdown, acidification, detoxification and flavour development (Sobowale, Olurin, and Oyewole, 1991).

2.9 Kinetic of yeast fermented cassava

For year ruminant nutritionists have attempted to manipulate the microbial ecosystem of the ruminant with hope of increasing production. Currently, the most common means for enhancing ruminant production is through the use of chemical feed additive (Woodward, 1995). *S. cerevisiae* is the most popular yeast used in alcoholic fermentation step of wine production. The role of yeast in wine production is to converse sugars in grape juice into ethanol. Nowadays, many commercial strains of yeast exit. Approximately 60% of *S. cerevisiae* used in France are killer yeast (Sakesit, 2000).

2.10 History of microbial supplement diets in the world

Krehbiel, Rust, Zhang, and Gilliland (2003) refer to historical information pertaining to the use of bacterial direct fed microbial has been reviewed (Stern and Storrs, 1975; Newman and Jacques, 1995; Yoon and Stern, 1995). In his book, *The Prolongation of Life*, Metchnikoff (1908) first proposed that consuming lactobacilli capable of living in the intestinal tract was desirable (Yoon and Stern, 1995). He suggested that longevity of the Bulgarians was partly due to their consumption of a fermented milk product and that lactobacilli present in the fermented product prevented disease caused by enteropathogens. Metchnikoff's (1908) postulation led to several studies on the efficacy of the *Lactobacillus* species during the 1920's (Stern and Storrs, 1975). Stern and Storrs (1975) reported that the early

popularity of *Lactobacillus acidophilus* therapy in the United States reached its peak by about the mid-1930s, and then faded. Following World War II, antibiotics came into use and were often so efficient that they destroyed all the intestinal bacteria (Mannheim, 1951). The net effect was an increase in the incidence of “antibiotic diarrhea” and related side effects, and interest in acidophilus therapy for restoration of normal intestinal microorganisms began to be renewed. Since then (mid-1950s), there has been a slow but steady increase in the study of bacterial bacterial direct fed microbial for humans and animals. However, production responses of growing and lactating ruminants and interest in the corresponding mode of action of bacterial DFM have not occurred until more recently (Yoon and Stern, 1995).

2.11 Mode of action of yeast in the rumen

Yeasts as probiotics are gaining popularity among fattening systems, because yeasts are sturdy, with high viability under a range of environmental conditions and can be cultured easily (Tripathi, Karim, Chaturvedi and Verma, 2008). Aqueous extracts prepared from *S. cerevisiae* stimulated the growth of certain rumen microorganisms. Recently, Girard (1996) reported the presence of both heat-labile (probably lipidic) and heat-stable (short chain peptides) stimulation factors in different yeast cells fractions. Yeast has been shown to provide vitamins (especially thiamin) to support the growth of rumen fungi (Chaucheyras, Fonty, Bertin, and Gouet, 1995). High dicarboxylic acids, particularly malic acid, content of the yeast has also been shown to be the possible cause of stimulation (Nisbet and Martin, 1990; 1991) *in vitro*, but it does not appear to cause the most important effects of yeast *in vivo* (Newbold, Wallace, and McIntosh, 1996). Removal of oxygen, which would inhibit the growth of

the strictly anaerobic bacteria of the rumen, was also suggested. Rumen contents are essentially anaerobic, but low concentration of dissolved O₂ can be detected during the daily feeding cycle. O₂ enters the rumen while the animal is eating, both with the feed and the saliva. The increase in redox potential observed after the meal in sheep observed by Mathieu et al. (1996) is mainly due to the supply of oxygen in the rumen during feed intake, mastication and water intake (Figure 2.2). The ability of different strains of *S. cerevisiae* to stimulate the viable count of bacteria in the rumen appears to be related to their ability to remove oxygen from rumen fluid, since respiration-deficient mutants of *S. cerevisiae* failed to stimulate bacterial numbers (Newbold, Wallace, and McIntosh, 1996).

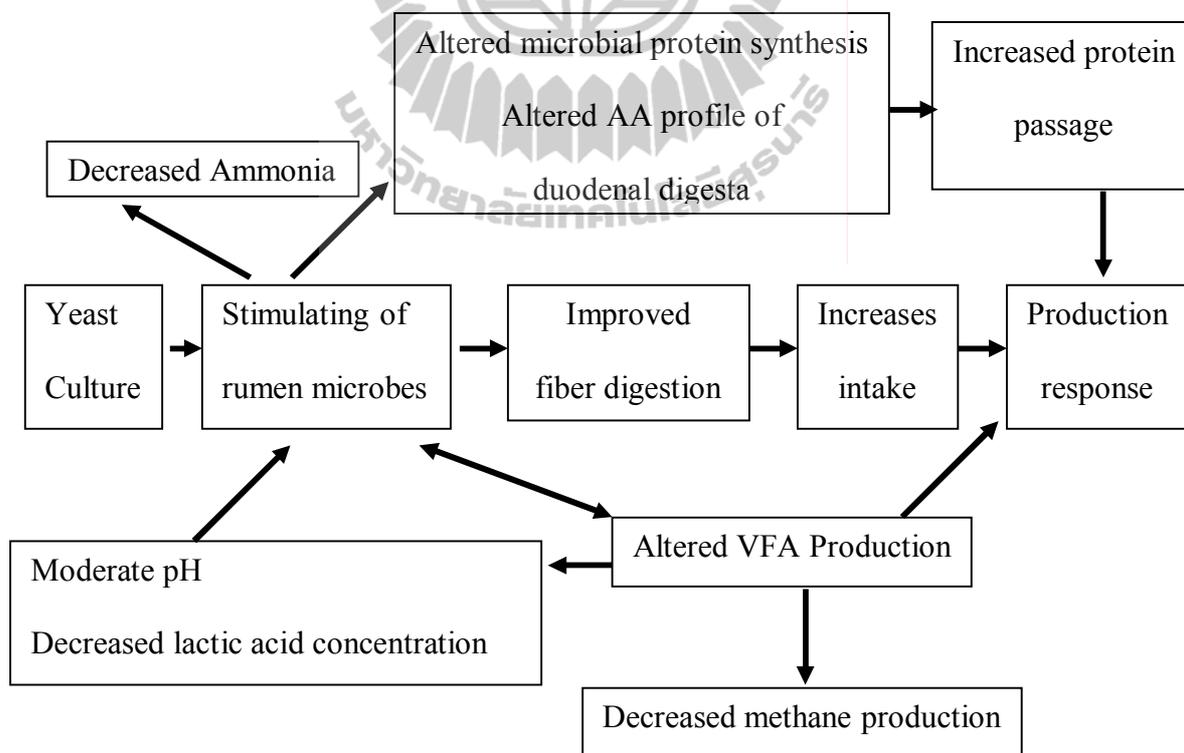


Figure 2.2 Mode of actions of Yeast.

McDonald, Edwards, Greenhalgh, and Morgan, 2002.

2.12 Type of microbials for animal productions

A live microbial feed supplement that beneficially affects the host animal. Claims include reduced early mortality, increased growth rate, improved feed conversion, egg quality and animal health (e.g., prevent disease and/or aid recovery from illness). Lactic acid producing strains (e.g., *Lactobacillus* and *Streptococcus*) are common components of bacterial direct fed microbial (DFM). The original concept of feeding bacterial DFM to livestock was based primarily post-ruminal effects. Thus, previous reports in the literature indicate that supplying DFM that contribute to the ability of the rumen ecosystem to manage lactic acid production and utilization can be beneficial, even for animals that do not have clinical acidosis (Martin and Nisbet, 1992; Raeth-Knight, 2007; Yang, Beauchemin, Vedres, Ghorbani, Colombatto, and Morgavi, 2004; Kung, 2001).

2.12.1 Fungal direct-fed microbials

Fungal DFM have been popular additions to ruminant diets for many years. Fungal based-DFM are beneficial via interactions in the rumen. Several reasons for improvements in ruminal fermentation from feeding fungal DFM have been suggested. First, DFM have caused beneficial changes in activity and numbers of rumen microbes. For example, the numbers of total ruminal anaerobes (Dawson, Neuman, and Boling, 1990).

In general, three types of additives are available. First, some products contain and guarantee “live” yeast. Most of these products contain various strains of *S. cerevisiae*. Second, other additives contain *S. cerevisiae* and culture extracts, but make no guarantee for live organisms. Third, there are fungal additives based on *Aspergillus oryzae* fermentation end products that also make no claim for supplying live microbes

(Kung, 2001). Reports on performance responses of ruminants, including lambs, fed on yeast and yeast cultures have been variable. Growth rate and efficiency of gain were similar or reduced (Agarwal et al., 2002; Erasmus, 2005; Mahender, Prasad, Reddy, 2005; Kim et al., 2006; Kawas et al., 2007), while others suggested improved weight gain, feed consumption and feed efficiency of gain on yeast supplementation (Williams and Newbold, 1990; Wallace and Newbold, 1993; Lesmeister, Henrich, and Gabler, 2004; Stella et al., 2007).

2.12.2 Bacterial direct-fed microbials

There are many bacterial-based direct fed microbial that are sold for use in ruminant diets with more specific applications. These products often contain lactobacilli with *Lactobacillus acidophilus* being one of the most common microorganisms used. Other commonly used bacteria include various species of *Bifidobacterium*, *Enterococcus*, *Bacillus* and *Lactobacillus*. Most bacterial-based DFM are probably beneficial because they have effects in the lower gut and not in the rumen. For example, *Lactobacillus acidophilus* produces lactic acid, which may lower the pH in small intestines to levels that inhibit the growth of pathogenic microbes (Kung, 2001). *Megasphaera elsdenii* is major lactate utilizing organism in cattle fed high grain diets. Feedlot producer have used direct fed microbial to adapt cattle to high energy diets reducing lactic acidosis. High producing cows in early lactation would be the best candidates for such products because these cows are in negative energy balance and have diets that contain highly fermentable carbohydrates that sometimes leads to acidosis (Kung, 2001). Applications in high producing cows are being explores in the field. *Propionibacteria* have the ability to convert lactic acid and glucose to acetic and propionic acid improving the energy status of early lactation

cows (Hutjens, 2007). Lactic acid producing bacteria convert complex carbohydrates to lactic acid and, for example, acetic acid. In this way, the indigestible complex carbohydrates are transformed into easy-to-digest free fatty acids. Lactic acid itself acts as an inhibitor of pathogens. It stimulates the gut function and aids the re-establishment of the gut pH after stress or antibiotic treatment (Feed specialties, 2007). The mode action and dosage are listed in Table 2.2.

Table 2.2 Bacteria with potential use as direct fed microbial (Hutjens, 2007).

Source	Strain	Dose	Effect
<i>Megasharera elsdonii</i>	B159 407A	8.7×10^6	Prevent lactic acidosis when diets change to higher fermented carbohydrate.
<i>Lactobacillus acidophilus</i>	-	1×10^9	Increase milk yield when feed intake depressed and under stress.
<i>Propionibacteria sp.</i> (P-63) and <i>L. acidophilus</i> (5345)	-	1×10^9 1×10^8	Improve feed efficiency during adaption to higher carbohydrate diets.
<i>Propionibacterium freudenreichii</i> and <i>L. acidophilus</i> (B2FFO4)	-	1×10^9 1×10^8	Improve feed efficiency.
<i>P. acidipropionici</i>	DH42	1×10^9	Increase propionic acid.
<i>P. freudenreichii</i> plus <i>Lactobacillus</i>	-	-	Improve weight gain in calves.

In general, most yeast, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* are destroyed by heat during pelleting. In contrast, bacilli form stable endospores when conditions for growth are unfavorable and are very resistant to heat, pH, moisture and disinfectants. Thus, bacilli are currently used in many applications that require pelleting. Future improvements in strain development may allow use of

heat-sensitive organisms in pelleted feeds. Bacterial products may or may not be compatible with use of traditional antibiotics and thus care should be taken when formulations contain both types of additives. For example, some species of bacilli are sensitive to virginiamycin, and lactobacilli are sensitive to chlortetracycline and penicillin. Information on direct fed microbial and antibiotic compatibility should be available from the manufacturer (Krehbiel, Rust, Zhang, and Gilliland, 2003; Kung, 2001).

2.13 Used of *S. cerevisiae* in ruminants

S. cerevisiae is the most common type of yeast used both in culture and feeding applications. More than 1000 strains of *S. cerevisiae* are listed in the American Type Culture Collection catalogue (ATCC, 1990) and it is still unknown how widespread probiotic activity is among these strains of yeast. Among the different strains applied in practice, little is known concerning the effects of *S. cerevisiae* yeast in domestic animals (Keyser, 2006; Oeztuerk, Schroeder, Beyerback, and Breves, 2005) also stated that based on comparisons of autoclaved and live yeast, yeasts act through prebiotic rather than probiotic effects that other researchers have suggested. The term probiotic has been defined as a live microbial feed supplement, which affects the host animal by improving its intestinal microbial balance. Prebiotics can be defined as a killed (autoclaved) microbial feed supplement, which stimulates microbial metabolism rather than improving microbial balance as observed with probiotics (Krehbiel, Rust, Zhang, and Gilliland, 2003; Oeztuerk, Schroeder, Beyerback, and Breves 2005). Because of the limited data currently available, further research is needed with yeast, particularly with respect to its mode of action, possible performance effects, and overall effects on animal health (Keyser, 2006). The effects of live yeast culture on ruminal microbes are

important in relation to rate and extent of digestion, as well as the overall health of an animal. Callaway and Martin (1997) conducted an experiment to determine the effects of yeast (*S. cerevisiae*) on lactate utilization and cellulose digestion by ruminal bacteria.

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CHAPTER III

PROTEIN ENRICHMENT OF CASSAVA PULP

FERMENTATION BY *Saccharomyces cerevisiae*

3.1 Abstract

The purpose of this study was to determine intestinal digestibility of residual components of cassava pulp solid state fermentation by *Saccharomyces cerevisiae* for animal feed. Three ruminally cannulated animal were used to measure *in situ* rumen dry matter (DM) and crude protein (CP) degradability characteristics of cassava pulp solid state fermentation by *S. cerevisiae*. Nylon bags containing 3 g (as fed basis) of each feed was immersed in duplicate at each time point in the ventral rumen of each goat for 2, 4, 8, 12, 24, 48, and 72 hours. Rumen feed residues from bags of 16 h incubation were used for estimation of lower gut digestibility by the technique of *in vitro* pepsin-pancreatin digestion. The results of the chemical analysis indicated that fermentation was slightly improved ruminal undegradable protein (RUP) of cassava pulp. The highest value of RUP was significantly differ ($P<0.05$) after 5 days of fermentation period. Ruminal undegradable protein content increased ($P<0.05$) with the addition of *S. cerevisiae* in cassava pulp. The present results indicate that fermented cassava pulp can improve protein content and ruminal undegradable protein content.

3.2 Introduction

One of the most important problems in animal husbandry is that the animals could not be fed adequately (Saricicek and Kilic, 2004). Cassava or tapioca (*Manihot esculenta*, Crantz), a root crop is one of the major crop grown, especially in northeast of Thailand. In Thailand, cassava pulp is always sold as a cheap animal feed material in substitution of urea, yeast and other. Therefore, through the solid state fermentation, protein content in the cassava pulp can be increased that can lower the cost of animal feed. Cassava pulp is fermented with yeast (*S. cerevisiae*) for protein enrichment before it is used as the high quality animal feed material (Srinorakutara, Kaewvimol, and Saengow, 2006; Oboh and Akindahunsi, 2005; Ubalua, 2007). Estimations of intestinal digestibility of rumen undegraded protein of feeds are critical in the application of protein evaluation systems for ruminants (Faría-Mármol, González, Rodríguez, and Alvir, 2002). The rate and extent of protein degradation in the rumen is very crucial, as it determines the availability of nitrogen to microorganisms and amino acids in the small intestine to the host animals. The protein consumed by the ruminant should be partly degradable in the rumen, into peptides, amino acids and $\text{NH}_3\text{-N}$ derived from proteolysis to be used in microbial protein synthesis and to improved rumen ecology. It is, therefore, very important to determine the degradability and digestion of different feed ingredients which are grown and used in different locations. Incubation of feeds in nylon bags in the rumen of cannulated ruminants have been used to determine the extent of rumen degradation of the feed protein (Ørskov and McDonald, 1979; Rao and Prasad, 1989; Islam, Ishida, and Nishida, 2002). The feed N which escapes rumen degradation and digestibility can be further measured by a three-step *in vitro* procedure (Calsamiglia and Stern, 1995; Kamalak, Canbolat, Gurbuz,

Ozkan, and Kizilsimsek, 2005). Therefore, the objective of this study was to investigate the effect of the inoculants, *S. cerevisiae* separately and in combined application on changes in fermentation quality and nutrient composition with duration of storage of dry cassava pulp.

3.3 Objective

3.3.1 To determine the optimal level of yeast *S. cerevisiae* and incubation times as protein source to future enhance utilization in goat's rations.

3.3.2 To investigate *in situ* nylon bag technique and digestibility and *in vitro* pepsin-pancreatin digestion technique in evaluated nutritive values of fermented cassava pulp by yeast *S. cerevisiae*.

3.4 Materials and Methods

3.4.1 Experimental design and treatments

The experiment was taken according to a 4x4 factorial arrangements in complete randomized design (CRD). Factor A was the level of *S. cerevisiae* (0, 0.5, 2.5, and 5 g) and factor B was the times of fermentation (0, 1, 3, and 5 days).

The dietary treatments were as follows:

T1 = *S. cerevisiae* at 0% fermented cassava pulp at time 0 day

T2 = *S. cerevisiae* at 0% fermented cassava pulp at time 1 day

T3 = *S. cerevisiae* at 0% fermented cassava pulp at time 3 day

T4 = *S. cerevisiae* at 0% fermented cassava pulp at time 5 day

T5 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 0 day

T6 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 1 day

T7 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 3 day

T8 = *S. cerevisiae* at 0.5% fermented cassava pulp at time 5 day

T9 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 0 day

T10 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 1 day

T11 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 3 day

T12 = *S. cerevisiae* at 2.5% fermented cassava pulp at time 5 day

T13 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 0 day

T14 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 1 day

T15 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 3 day

T16 = *S. cerevisiae* at 5.0% fermented cassava pulp at time 5 day

Layout:

Factor A Yeast supplemented (g)	Factor B Date to fermentation (day)			
	D(0)	D(1)	D(3)	D(5)
Y(0)	Y(0)D(0)_T1	Y(0)D(1)_T2	Y(0)D(3)_T3	Y(0)D(5)_T4
Y(0.5)	Y(0.5)D(0)_T5	Y(0.5)D(0)_T6	Y(0.5)D(3)_T7	Y(0.5)D(5)_T8
Y(2.5)	Y(2.5)D(0)_T9	Y(2.5)D(0)_T10	Y(2.5)D(3)_T11	Y(2.5)D(5)_T12
Y(5)	Y(5)D(0)_T13	Y(5)D(0)_T14	Y(5)D(3)_T15	Y(5)D(5)_T16

3.4.2 Sample preparation

Cassava pulp was collected from the factory of cassava starch production in Nakhon Ratchasima province, Thailand. It was dried in hot air oven at 60°C for 48 hour or until it was dried completely before performing the experiment. The yeast culture used in this experiment contains as the effective agent living non-pathogenic yeast of the *S. cerevisiae* in the minimum amount of 1×10^{13} CFU/1 g.

About 1 kg of cassava pulp (98 %DM) is used for fermentation. The moisture content of cassava pulp was adjusted to 50% by adding 10% urea, 1.25% molasses. Three samples were prepared by mixing cassava pulp with 0, 0.5, 2.5, and 5.0% of *S. cerevisiae*. A control sample contained no *S. cerevisiae*. The above samples were incubated for 0, 1, 3, and 5 days, dried, ground through 2 mm sieve stored pending chemical analysis and mobile bags studies.

3.4.3 Animals and ruminal degradability

Three, ruminally cannulated growing goats with an average weight of 15 ± 2.5 kg and 8-10 month of ages were used to determine ruminal degradability and intestinal digestibility of fermented cassava pulp. Each animals fitted permanent rumen cannulae, were kept individual pens (0.9×1.4 m²). The animals were fed a maintenance concentration diet and roughage (rice straw). The daily feed was offered in two equal portions, one at 08.30 am and the other at 04.30 pm. Drinking water was freely available.

Dry matter (DM), organic matter (OM), and crude protein (CP) degradation rate in the rumen were determined in the three meat goats. Nylon bags (4×7 cm²) made from polyester cloth with average pore sizes of 45 μ m. Ørskov and McDonald (1979) were filled with approximately 5 g of a test sample. All samples were prepared in triplicates and incubated in the rumen of each animal for 2, 4, 8, 12, 24, 48, and 72 hours. After removal from the rumen, bags were doused with clean water to halt fermentation and were rinsed until wash water ran relatively clear. Bags were stored at $<0^{\circ}\text{C}$ to a wait further processing. Once removed from storage, bags were thawed to room temperature and washed in a domestic washing machine until the wash water ran completely clear. Samples were air-dried in a 60°C convection oven to a

constant mass, then air-equilibrated and weighed to determine residue mass. Residues were then removed and composited by duplicates within goat. Bag residue and original forage samples were ground to pass through a 1-mm screen and subsequently analyzed for DM, OM, CP according to the procedure of AOAC (1990), Analyzed NDF, and ADF (Goering and Van Soest, 1970). The bags were weighed and measured according to Ørskov and McDonald (1979).

3.4.4 *In vitro* pepsin-pancreatin digestion procedure

Samples of the feed residue from nylon bags at 16 hours incubation time, the bags were removed from the rumen and were immediately washed with cold tap water until clear, and dried in a forced air oven at 60°C for 48 hours, after determining N content, were put into a 50 ml centrifugation tube in quantities equivalent to 15 mg of N. 10 ml of a 0.1 N HCl solution (pH 1.9), containing 1 g/L of pepsin (sigma P-7012, Sigma) were added and the samples incubated for 1 hour in a 38°C shaker water bath. After incubation, 0.5 ml of a 1 N NaOH solution and 13.5 ml of a pancreatin solution (0.5 M KH₂PO₄ buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin [Sigma P-7545, Sigma]) were added. The samples were incubated at 38°C for 24 hours in a shaker water bath, and mixed (magnetic stirrer) every 8 hours. After incubation, 3 ml of a 100% (w/v) solution of TCA were added to the tubes to stop enzymatic action and to precipitate undigested proteins. All tubes were mixed and allowed to stand for 15 min. The samples were centrifuged at 10,000 x g for 15 min and the supernatant was analyzed for soluble N by the Kjeldahl method (AOAC, 1990). Pepsin-pancreatin digestion of protein was calculated as TCA-soluble N divided by amount of sample N (nylon bag residue) used in the assay (Calsamiglia and Stern, 1995).

3.4.5 Sample analysis

The nutritional composition of *S. cerevisiae* fermented cassava pulp product was evaluated using the protein content of crude protein (CP), non-protein nitrogen (NPN), true protein and feed residues from bags after the 16 hours incubation using Kjeldahl method. The supernatant was analyzed for soluble N by Kjeldahl method (AOAC, 1990). All samples were analyzed for DM, Ash, and CP according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by using the method of Goering and Van Soest (1970).

3.4.6 Data analysis

Data for ruminal and intestinal degradation of DM and CP were fitted to the exponential equation following procedure described by Ørskov and McDonald (1979).

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

Where:

p = disappearance rate at time t

a = as intercept representing the portion of DM or CP solulized at initiation of incubation (time 0)

b = the fraction of DM or CP potentially degradable in the rumen

c = a rate constant of disappearance of fraction b

t = time of incubation

The nonlinear parameters a, b, and c are estimated by an iterative least squares procedure. The effective degradability of DM (EDDM) or of CP (EDCP) were calculated using the following equation.

$$\text{EDDM or EDCP} = a + (bc/(c+k))$$

Where:

k was the estimate rate of out flow from the rumen (0.05/hour)

Calculations as described by Subuh, Rowan, and Lawrence (1996) were used for ruminal, post-ruminal and total tract protein disappearance using the nylon bag technique. Data of three step procedure were calculated as described by Calsamiglia and Stern (1995). Crude protein degradability was calculated as a percent of total CP.

$$\% \text{ CP degradability} = [(\text{initial CP} - \text{post incubate CP})/\text{initial CP}] \times 100.$$

3.4.7 Statistical analysis

Data of *in vitro* three-step procedure was calculated as described by McNiven, Prestlokken, Mydland, and Mitchell (2002). Data were analyzed by SAS (1985). The statistical analysis of all data *in vitro* procedure was made according to the following model:

$$Y_{ij} = \mu + \delta_{ij} + \varepsilon_{ij}$$

Where:

Y_{ij} = the criteria under study

μ = overall mean

δ_{ij} = feed source effect (or treatment of roughage diet)

ε_{ij} = residual.

3.5 Results and Discussion

3.5.1 Chemical composition of feed sources

The chemical compositions of fermented cassava pulp with different level of yeast (*S. cerevisiae*) are presented in Table 3.1. Generally, wide variations existed in the chemical composition of investigated feedstuffs. The protein content gradually augmented with time because the yeast converts nitrogen source to protein. The highest of crude protein and true protein were 31.6 and 29.0% DM, respectively, in the fermented cassava pulp with 5% *S. cerevisiae* at 5 day period.

Table 3.1 Chemical composition of fermented cassava pulp with different level of yeast (*S. cerevisiae*) (% dry matter basis).

Treatments		Chemical compositions (% DM basis)							
Yeast	Day to fermentation	DM	CP	Ash	NDF	ADF	ADL	TP	NPN
Yeast (0 g)	0	74.2	8.7	3.9	31.6	19.0	5.0	5.6	3.1
	1	69.2	9.2	3.6	28.2	18.7	3.1	5.9	3.3
	3	73.7	12.8	3.4	27.6	18.9	3.3	9.5	3.2
	5	72.5	16.2	3.2	27.3	19.2	5.2	13.5	2.7
Yeast (0.5 g)	0	72.0	15.5	3.6	27.8	19.1	4.4	13.0	2.5
	1	69.5	19.1	3.7	31.9	18.9	3.1	16.7	2.4
	3	73.3	20.2	2.8	26.9	18.5	3.1	18.2	1.9
	5	82.0	20.0	3.9	25.1	19.1	3.5	17.8	2.2
Yeast (2.5 g)	0	77.5	14.5	3.4	24.6	18.2	3.0	11.8	2.7
	1	80.0	19.9	3.8	29.0	17.8	3.8	17.4	2.3
	3	78.6	19.9	3.1	25.9	17.1	3.0	17.6	2.0
	5	80.6	22.6	3.6	26.0	19.1	3.3	20.0	1.9
Yeast (5.0 g)	0	75.9	17.7	3.4	26.7	19.1	3.7	15.5	1.7
	1	77.4	22.6	3.3	26.9	19.0	4.3	20.0	1.9
	3	75.8	23.5	4.4	28.0	18.6	3.7	21.0	1.8
	5	73.2	31.6	3.7	25.5	19.4	4.8	29.0	2.0
SEM		0.66	0.98	0.06	0.06	0.36	0.16	0.12	0.08
Effects	Yeast	**	**	**	**	**	**	**	**
	Day	**	**	**	**	**	**	**	**
	Yeast x Day	**	**	**	**	**	**	**	**

DM = Dry matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, TP = True protein, NPN = Non protein nitrogen. True protein = Crude protein (CP) - NPN).

The chemical composition of the fermented cassava pulp by yeast studied is presented (Table 3.1). The crude protein content of the fermented cassava pulp by yeast products showed the concentrations of true protein and NPN were numerically lower than in soybean meal (Borucki Castro, Phillip, Lapierre, Jardon, and Berthiaume, 2007). Cassava waste from starch industry utilized as animal feed fermentation with *Rhizopus and Rhizopus sp.* 26R. The protein was enriched to 24% after the fermentation (Putipipatkajon and Srinophakun, 1999).

3.5.2 Rumen disappearances

The rumen undegradable protein (RUP) was determined by the *in vitro* nylon bag technique. Table 3.2-3.4 showed the *in vitro* intestinal digestion of feeds. RUP were increased with the increased the incubation time for all treatments. In this experiment, cassava pulp was a very fine dusty particle, which would be lost easily from the bag in the rumen. The result was in agreement with Cone, Kamman, Van Gelder, and Hindle (2002) who found that source of starch had effect on degradability in rumen.

Table 3.2 Percentage of DM, disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats.

Items	g yeast/kg fermented cassava pulp																P-value			
	Y0g				Y0.5g				Y2.5g				Y5g				SEM	Y	D	YxD
	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5				
DM disappearance, (%)																				
a	4.8	9.7	12.2	12.6	10.3	15.5	17.1	14.7	13.4	13.2	17.3	17.9	7.4	10.3	17.8	11.4	0.7	0.1	0.96	0.65
b	74.2	90.3	87.8	82.5	89.7	84.5	82.9	77.7	80.1	68.4	82.7	81.4	92.6	79.2	82.2	74.4	1.1	0.1	0.31	0.89
c	0.04	0.02	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.05	0.03	0.02	0.02	0.04	0.02	0.03	0.001	0.25	0.92	0.97
Effective degradability, (ED, %)																				
0.05	39.2	35.4	42.7	42.0	38.9	38.2	46.1	44.3	41.3	46.4	45.4	44.8	35.9	47.7	43.4	36.3	0.7	0.4	1.00	0.79
0.08	30.8	27.7	34.1	33.8	30.6	31.3	37.9	36.3	33.5	38.6	37.5	37.1	27.5	38.7	35.9	29.1	0.7	0.3	0.98	0.70

a = as intercept representing the portion of DM or CP solulized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, DM = Dry matter.

Table 3.3 Percentage of OM disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats.

Items	g yeast/kg fermented cassava pulp																P-value			
	Y0g				Y0.5g				Y2.5g				Y5g				SEM	Y	D	YxD
	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5				
OM disappearance, (%)																				
a	14.9	19.6	23.4	18.0	19.7	23.3	22.7	18.8	22.0	26.8	27.3	26.8	22.1	23.8	28.0	21.5	0.60	0.50	1.00	0.50
b	67.2	80.4	71.4	82.0	80.3	63.4	62.2	72.5	78.0	73.2	72.7	73.2	73.4	69.0	71.8	78.5	1.00	0.40	0.80	0.20
c	0.04	0.02	0.03	0.02	0.02	0.03	0.05	0.05	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.01	0.37	0.84	0.97
Effective degradability, (ED, %)																				
0.05	45.8	45.5	48.7	43.4	42.9	45.2	52.3	53.3	49.1	52.9	52.3	49.8	48.9	50.9	51.7	43.0	0.60	0.60	0.90	0.90
0.08	38.2	38.1	41.6	36.0	36.0	39.1	45.2	45.1	41.5	45.6	45.2	43.1	41.6	43.7	44.9	36.5	0.60	0.60	0.90	0.80

a = as intercept representing the portion of DM or CP solubilized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, OM= Organic matter.

Table 3.4 Percentage of CP disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) incubated in the rumen growing goats.

Items	g yeast / kg fermented cassava pulp																Effects			
	Y0g				Y0.5g				Y2.5g				Y5g				SEM	Y	D	Yx
	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5	D0	D1	D3	D5				
CP disappearance, (%)																				
a	3.8	19.8	15.4	16.2	18.4	22.5	22.2	20.0	25.9	27.9	30.6	27.8	22.9	25.2	25.9	20.0	1.13	ns	ns	ns
b	60.2	74.7	70.8	83.8	66.4	77.5	66.6	70.2	57.8	72.1	69.4	72.2	68.8	74.8	74.1	80.0	1.16	*	**	ns
c	0.07	0.03	0.03	0.02	0.02	0.02	0.04	0.04	0.04	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	*	ns	ns
Effective degradability, (ED, %)																				
0.05	38.9	47.8	41.9	40.2	37.3	44.7	51.8	51.2	51.6	48.5	50.4	48.5	48.6	46.6	47.1	42.9	0.81	ns	ns	ns
0.08	31.9	40.2	34.7	33.0	31.6	38.0	44.4	43.4	45.1	42.3	44.5	42.3	41.6	40.2	40.7	36.0	0.80	ns	ns	ns

a = as intercept representing the portion of DM or CP solubilized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, CP = Crude protein.

Table 3.5 CP disappearances of fermented cassava pulp with different level of yeast (*S. cerevisiae*) in rumen and intestine of meat goats at 16 h incubated time.

Treatments		CP disappearance (%)		
Yeast	Day to fermentation	Rumen	Intestine	Total
Yeast (0 g)	0	47.7	1.58	49.3
	1	57.2	1.89	59.1
	3	82.4	0.70	83.1
	5	77.1	1.15	78.3
Yeast (0.5 g)	0	73.7	3.00	76.7
	1	80.2	2.58	82.8
	3	87.8	1.61	89.5
	5	85.4	1.11	86.5
Yeast (2.5 g)	0	70.2	2.78	72.9
	1	81.2	3.12	84.3
	3	89.7	0.87	90.6
	5	89.7	0.77	90.5
Yeast (5.0 g)	0	74.9	5.22	80.2
	1	92.6	0.58	93.2
	3	91.1	1.12	92.3
	5	91.6	2.87	94.5
SEM		2.21	0.22	2.17
P value	Yeast	**	**	**
	Day	ns	ns	ns
	Yeast x Day	ns	ns	ns

CP = Crude protein.

The values of CP intestinal digestibility of fermented cassava pulp with *S. cerevisiae* were somewhat higher, however the observed differences between treatments were not significant ($P < 0.05$).

Incubation of bags in the rumen before insertion into the duodenum increased intestinal undegradable CP (Stern, Bach, and Calsamiglia, 1997; de Boer,

Murphy, and Kennelly, 1987). In the *in vitro* enzymatic technique or three step technique, there were also numerous factors influencing the value, e.g. the time of incubation (Cone, Kamman, Van Gelder, and Hindle, 2002), group of feedstuffs (Tomankova, and Kopecny, 1995), variety of enzyme (Roe, Chase, and Sniffen, 1991), pH of buffer and enzyme concentration (Licitra, Van Soest, Schadt, Carpino, and Sniffen, 1999).

3.6 Conclusions

The results obtained from this experiment could have a great impact on animal feed especially using local resources-based diets. The present results indicate that fermentation of cassava pulp by yeast can improve CP content and rumen undegradable protein. This method could be more useful for routine feed evaluation without the need for a rumen cannulated animal.

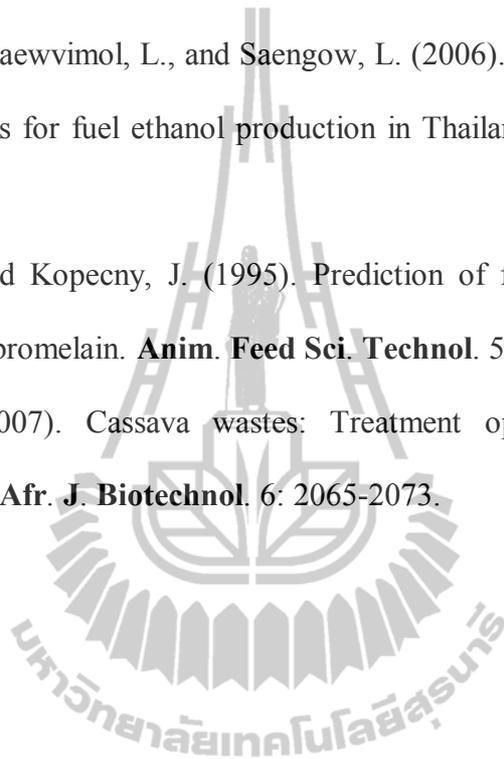
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CHAPTER IV

REPLACING OF SOYBEAN MEAL WITH FERMENTED CASSAVA PULP BY *Saccharomyces cerevisiae* IN THE DIET ON RUMEN FERMENTATION IN MEAT GOAT DIETS

4.1 Abstract

The aim of this study was to determine the effect of replacing soybean meal (SBM) with fermented cassava pulp by *Saccharomyces cerevisiae* (FCSC) in the diet of meat goats. Growing goats were randomly assigned to five dietary treatments according to replicated 5x5 Latin square design. Dietary treatments were five levels of replacement SBM to FCSC at 0, 25, 50, 75, and 100 % of crude protein (CP) in concentrates. The results showed that total dry matter intake (DMI, g/day) was increased ($P < 0.05$) with increasing FCSC. The highest for body weight change was found in 75% replacement of SBM by FCSC. The results suggested that FCSC could replace up to 75% of SBM in concentrate of growing goats fed rice straw as roughage. Based on this result, using 75% FCSC as the main source of protein to completely replace soybean meal was beneficial to growing goats in terms of feed intake and productive performance.

4.2 Introduction

Northeast of Thailand is classified as a hot and dry region of the country. Many researchers have recommended an introduction of goats to the farmers in the northeast region based on the high productivity per year and high ability to utilize low quality roughage which is available in this region. Cassava pulp is always sold as a cheap animal feed material in substitution of urea, yeast and other (Srinorakutara, Kaewvimol, and Saengow, 2006). There has been studied on the improvement of cassava pulp by the solid state fermentation, it found that protein content in the cassava pulp can be increased. However, there are increases in the price of the conventional feed ingredients available due to the competition between the livestock industry and man. The innovative feedstuffs are needed to reduce the productive cost. There has been reported that fermentation of cassava pulp by yeast (*S. cerevisiae*) can be increased protein for animals (Srinorakutara, Kaewvimol, and Saengow, 2006; Oboh and Akindahunsi, 2005; Ubalua, 2007). Therefore, the objective of this study was investigated the effected of different protein sources from the fermented cassava pulp with *S. cerevisiae* on rumen fermentation in meat goats fed low quality roughages since most of small ruminant production in the tropic was based on low quality roughages.

4.3 Objective

This study was to determine the replacing of soybean meal with fermented cassava pulp by yeast *S. cerevisiae* in Thai native x Anglo-Nubian crossbred meat goat diets.

4.4 Materials and Methods

4.4.1 Sample preparation

The cassava pulp, about 1 kg, is used for fermentation process. The moisture content of cassava pulp was adjusted to 50% by mixtures (10% urea, 1.25% molasses and mixed with 5% *S. cerevisiae*). Then, the mixtures were incubated in plastic bag at room temperature (25°C) for 5 days. The treatments are shown in Table 4.1.

4.4.2 Animals and management

In vivo study: Ten male crossbred (Thai native x Anglo-Nubian) goats aged approximately 8 months with an average bodyweight of 20±5 kg (mean±SD), were housed in individual pens. A period was consisted of 21 days of feed intake and body weight gain measurements followed by a week. Animals were randomly divided into 5 treatments according to replicate 5x5 Latin square experiment. The goats were housed in individual pens and allowed 3 weeks to accustom to the experimental conditions. The dietary treatments were the level of replacing of SBM by FCSC at 0, 25, 50, 75, and 100% in concentrate diets. The experimental diets were isonitrogenous and formulated to meet the nutrient requirement of meat goats according to NRC (2006) as presented in Table 4.1. Diets were offered *ad libitum* twice daily at 08.30 and 16.30 hour. Rice straw was offered *ad libitum* and clean water is freely access.

4.4.3 Voluntary feed intake and body weight change

Roughages and concentrate diets were equally fed on morning and afternoon. Orts were weighed daily prior to the morning feeding to determine daily dry matter intake (DMI). Body weight of each animal was measured weekly immediately before the morning feeding.

4.4.4 Experimental procedure

The experiment was consisted of five periods; of 25 days each, viz two weeks of feeding trial followed by 11 days of measurements. The latter consisted of 2 days of adaptation in the metabolic crate, 7 days of digestibility and N balance studies, 2 days of rumen fluid and blood sampling. During the digestibility trail, samples of feed refusal, feces and urine were collected before morning feeding to determine DM and nutrient digestibility and N balance according to Schneider and Flatt (1975).

4.4.5 Sampling methods and chemical analysis

Rice straw and concentrate diets were sampled weekly and dried at 60~65°C in hot air oven for dry matter determination and ground through a 1 mm sieve and then kept in tightly covered plastic containers to make a pool respectively for further approximate analysis. Feeds, orts and feces samples were analyzed for DM, ash, nitrogen content (AOAC, 1990), NDF and ADF (Goering and Van Soest, 1970). During the total collection, feces and urine samples were quantitatively collected and urine sample was acidified with 10% H₂SO₄. Subsequently, 15% of the total amounts was sub-sampled from each animal, and then sample was kept at -20°C for nitrogen utilization (Schneider and Flatt, 1975) and purine derivative analysis according to Chen, Chen, Franklin, Ørskov, and Shand (1992).

Rumen fluid samples from all goats were taken at 0, 3, and 6 hour post feeding by stomach tube connecting with pump. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 ml of the samples were measured and truly with a pipette into the tubes containing 9 ml 10% formalin (v:v, 9:1) as a preserving reagent and then were closed tightly with screw caps for checking the counts of

ruminal protozoa and bacteria counts using the hemacytometer according to Hungate (1966). Another rumen portion was collected for cellulolytic bacteria, proteolytic bacteria, and amylolytic bacteria by using Roll tube technique (Hungate, 1966). And 20 ml of the samples were measured and then put into small plastic bottles containing 5 ml 6 N HCl as a preserving reagent, and then the bottles were closed tightly with screw caps, centrifuged at 3,000 rpm for 10 minutes. Subsequently the ruminal fluid was collected, and then it was stored at -20°C for subsequent analyses of ruminal ammonia N (Bremner and Keeney, 1965) and volatile fatty acids (VFA) by using gas chromatography (GC) analysis (Erwin, Marco, and Emery, 1961). With that, all samples were kept at -20°C until further analysis. The supernatant fluid was analyzed for ammonia N by Kjeldahl method and VFAs (acetate, propionate and butyrate) concentrations by gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m x 0.25 mm x 0.25 μm film (DB-FFAP).

The blood samples from all goats were taken at preliminary feeding 3 and 6 hours post feeding during the digestibility trial of each period were collected from jugular veins into EDTA containing vacuum tubes and were centrifuged at 3,000 rpm for 10 minutes. Subsequently the plasma was collected, and then it was stored at -20°C for subsequent analyses of plasma urea nitrogen determined using a Spectronic R Genesys 5 spectrophotometer according to Mackay and Mackay (1972) (Adapted from Preston, Schnakenberg Andw, and Pfander, 1964).

4.4.6 Statistical analysis

Data were subjected to analysis using the general linear models (Proc GLM) procedure of Statistical Analysis System Institute SAS (1985). Duncan's New

Multiple Range Test and Orthogonal contrast analysis (Steel and Torrie, 1980) were used to compare treatment means.

4.4.7 Experimental location

The experiment was conducted at Suranaree University of Technology's goats farm, The Center for Scientific and Technological Equipment Building 1 and 3, Suranaree University of Technology.

4.5 Results

4.5.1 Chemical compositions

Experimental I: The protein content was gradually augmented by time because the yeast convert nitrogen source to protein. Cassava pulp fermentation with 5 g of *S. cerevisiae* for 5 day was gave the highest in crude protein and true protein at 31.64 and 29.0% DM, respectively. Ingredients and chemical composition of the feed were showed in Table 4.1. The DM, Ash, CP, and NDF contents were similar in all treatments. The analyzed protein contents were slightly higher than calculation. However, the feed cost was dramatically reduced as increased FCSC level.

Table 4.1 Chemical composition of the experimental diets (% DM basis).

Ingredient	Bath/kg ¹	FCSC replacement of SBM (%)					RS
		0	25	50	75	100	
Cassava pulp	4.45	47.5	46.4	45.0	43.6	42.1	
Rice bran	6.36	18.0	18.0	18.0	18.0	18.0	
Plam kernel cake	6.1	10.0	10.0	10.0	10.0	10.0	
Soybean meal	25.0	15.0	11.3	7.5	3.8	0.0	
FCSC	5.0	0	5.3	10.5	15.8	21.0	
Molasses	4.5	8.5	8.1	8.1	7.9	7.8	
Urea	32.2	0.45	0.46	0.48	0.49	0.51	
Dicalcium phosphate	5.5	0.1	0.1	0.1	0.1	0.1	
Premix	30	0.3	0.3	0.3	0.3	0.3	
Total		100	100	100	100	100	
Analyzed chemical composition (% DM basis)							
DM		87.9	86.6	86.5	79.7	76.5	90.3
Ash		8.5	8.9	8.2	8.4	6.6	13.6
CP		13.2	13.2	13.2	13.1	13.1	3.4
NDF		33.0	38.3	38.9	36.8	36.0	74.7
ADF		28.6	30.7	30.6	29.8	28.9	55.5
ADL		8.0	10.9	10.3	10.9	10.7	17.6
Bath/1 kg of feed		8.28	8.90	6.81	6.07	5.34	2.0

FCSC=fermented cassava pulp with yeast (*S. cerevisiae*), RS=Rice straw, DM=dry matter, OM=organic matter, CP=crude protein, NDF=neutral detergent fiber, ADF=acid detergent fiber, ADL= acid detergent lignin, ¹the price of feedstuff recorded on 21 September 2008.

4.5.2 Voluntary feed intake

The concentration, roughage, and total intake of the meat goats were showed that Table 4.2. Concentrate, roughage and total dry matter intakes expressed as g/day were decreased as increasing the level of replacing soybean meal (SBM) with

fermented cassava pulp with yeast (FCSC) ($P < 0.05$). Body weight change, whereas those fed fermented the cassava pulp replacement of soybean meal 75% were higher than other diets, but were not significantly different with increasing proportion of FCSC replacement of SBM. Body weight change, whereas those fed fermented the cassava pulp replacement of soybean meal 75 % were higher than other diets, but were not significantly different with increasing proportion of FCSC replacement of SBM. As one example of the potential benefits of yeast, addition of *S. cerevisiae* to ruminant diets has been observed to improve digestibility of DM; increased ruminal bacteria numbers (Dawson et al., 1990; Oeztuerk et al., 2005); decreased ruminal lactate concentrations (Williams et al., 1991; Callaway and Martin, 1997)

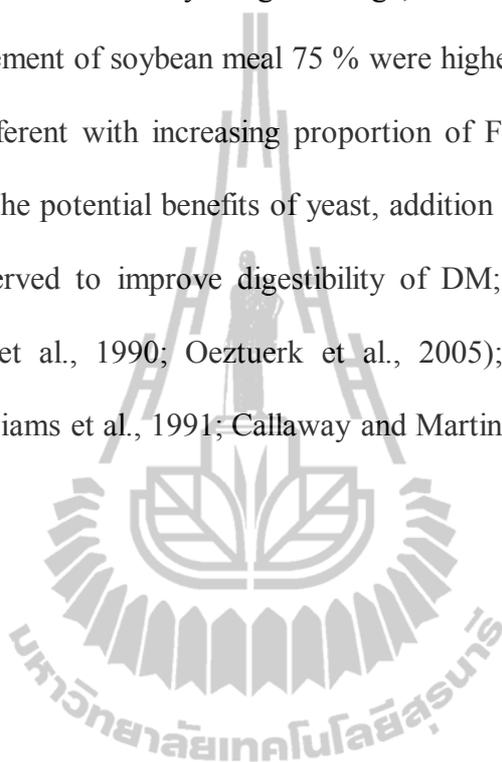


Table 4.2 Voluntary feed intake of fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Concentrate dry matter intake									
g/day	360.0 ^{ab}	384.0 ^b	352.9 ^{ab}	348.4 ^a	359.4 ^{ab}	7.65	ns	ns	*
% BW	1.5	1.5	1.5	1.5	1.5	0.00	ns	ns	ns
gDM/kgBW ^{0.75}	33.2 ^{ab}	33.5 ^b	32.2 ^a	32.8 ^{ab}	33.0 ^{ab}	0.22	ns	ns	ns
Roughage dry matter intake (rice straw)									
g/day	570.5 ^{ab}	580.4 ^b	550.9 ^a	555.3 ^{ab}	559.3	12.4	ns	ns	*
% BW	2.4	2.3	2.3	2.5	2.4	0.07	ns	ns	ns
gDM/kgBW ^{0.75}	52.8	51.3	51.4	53.3	51.7	1.29	ns	ns	ns
Total dry matter intake									
g/day	930.5 ^{ab}	964.4 ^b	903.8 ^a	903.7 ^a	918.7 ^{ab}	16.2	ns	ns	*
% BW	3.9	3.8	3.8	4.0	3.9	0.07	ns	ns	ns
gDM/kgBW ^{0.75}	86.0	84.8	83.6	86.1	84.7	1.26	ns	ns	ns
Ratio Concentrate	39.3	40.3	39.6	39.1	39.0	0.65			
Roughage	60.7	59.7	60.4	60.9	60.4	0.35	ns	ns	ns
Body weight change	4.8 ^b	3.6 ^b	-14.3 ^a	50.0 ^c	7.15	15.8	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P < 0.05$), ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), FCSC=fermented cassava pulp with yeast (*S. cerevisiae*).

4.5.3 Nutrient intake and digestibility

Nutrient intake of meat goats fed with FCSC replacement of SBM 25% in concentrate were higher than those fed treatments between the other. Dry matter digestibility of meat goats fed with FCSC replacement of SBM 25% in concentrate were lower than those fed treatments between the other (0%, and 100%) were affected

significantly ($P>0.05$). The DM, OM and CP digestibility increased quadratically ($P<0.05$) as a consequence of FCSC replacement of SBM. Similarly, NDF and ADF digestibility were not significantly different among treatments (Table 4.3).

Table 4.3 Nutrient intake of fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Nutrient intake (g/day)									
DM	930.5 ^{ab}	964.4 ^b	903.8 ^a	903.7 ^a	918.7 ^{ab}	16.2	ns	ns	*
OM	865.9	875.2	849.1	859.5	851.7	14.7	ns	ns	ns
CP	68.4	69.4	67.8	67.5	66.5	1.20	ns	ns	ns
NDF	545.4	571.8	545.5	555.8	543.6	10.2	ns	ns	ns
ADF	244.5 ^b	250.7 ^b	242.8 ^b	243.6 ^b	227.0 ^a	4.20	**	**	ns
Digestibility (%)									
DM	77.7 ^b	75.5 ^a	76.6 ^{ab}	76.5 ^{ab}	77.7 ^b	0.58	ns	*	ns
OM	81.0 ^b	79.3 ^a	80.2 ^{ab}	80.1 ^{ab}	81.2 ^b	0.49	ns	*	ns
CP	65.3	65.7	68.7	64.6	66.3	0.95	ns	ns	ns
NDF	73.9	71.2	72.2	72.1	73.6	0.72	ns	*	ns
ADF	53.2	48.0	52.0	51.0	51.9	1.27	ns	ns	ns

^{a, b, *} Values on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ^{**} Value on the same row under each main effect with different superscripts differ highly significant ($P<0.01$), ¹contrast effect (L=linear, Q=quadratic and C=cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$).

4.5.4 Nutrient digestibility and energy density (Mcal ME/kg)

The DM, OM, CP, NDF and ADF digestible nutrient intake were not significantly different among treatments. Similarly, energy density are not significantly different ($P>0.05$) (Table 4.4).

Table 4.4 Digestible nutrient intake and energy density of fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Digestible nutrient intake (g/day)									
OM	700.4	692.3	679.7	689.0	690.2	11.6	ns	ns	ns
CP	44.5	45.7	46.6	43.7	43.9	1.01	ns	ns	ns
NDF	402.1	405.6	393.4	401.5	399.3	7.75	ns	ns	ns
ADF	129.5	119.0	125.8	125.0	117.7	3.51	ns	ns	ns
Energy density (Mcal ME/kg)									
	2.80	2.70	2.70	2.70	2.80	0.02	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ^{**} Value on the same row under each main effect with different superscripts differ highly significant ($P<0.01$), ¹contrast effect (L=linear, Q=quadratic and C=cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$).

4.5.5 Ruminal pH and ammonia-nitrogen (NH₃-N)

Ruminal pH and NH₃-N concentration at preliminary feeding 3 and 6 hr post-feeding are presented in Table 4.5. Average rumen pH across treatments prior to feeding was ranged 6.74 to 6.9. Ruminal pH before feeding of goats fed with 0% FCSC replacement of SBM diet were higher than ($P<0.05$) that of 75%, however there

not significantly different with 25, 50, and 100%. Ruminal pH at 3 and 6 post-feeding were not significantly different among treatments. Ruminal pH ranged from 6.5 to 7.3 across treatments ($P>0.05$). Similarly, concentrations of ruminal $\text{NH}_3\text{-N}$ were ranged from 22.8 to 34.2 mg% ($P>0.05$).

Table 4.5 Ruminal pH, and ammonia-nitrogen ($\text{NH}_3\text{-N}$) concentration of meat goats fed fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
pH									
0 h post feeding	7.3 ^b	7.1 ^{ab}	7.0 ^{ab}	7.0 ^a	7.1 ^{ab}	0.08	ns	ns	ns
3 h post feeding	6.7	6.7	6.7	6.6	6.8	0.06	ns	ns	ns
6 h post feeding	6.7	6.5	6.6	6.6	6.6	0.07	ns	ns	ns
Ammonia-N (mg%)									
0 h post feeding	28.2	28.6	26.8	26.9	25.9	0.68	ns	ns	ns
3 h post feeding	33.6	34.2	33.2	31.2	31.4	1.35	ns	ns	ns
6 h post feeding	24.9	25.1	23.5	22.9	23.5	1.18	ns	ns	ns

^{a, b} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ¹contrast effect (L=linear, Q=quadratic and C=cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$), FCSC= fermented cassava pulp with yeast (*S.cerevisiae*).

4.5.6 Plasma urea nitrogen

Plasma urea nitrogen (PUN) concentration of goats fed with 25% FCSC replacement of SBM diet were higher than those of 0, 50, 75, and 100% FCSC replacement of SBM ($P<0.05$) (Table 4.6).

Table 4.6 Plasma urea nitrogen (PUN) concentration of meat goats fed fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCM replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Plasma urea nitrogen (PUN, mg%)									
0 h post feeding	13.5 ^{ab}	16.5 ^b	11.3 ^a	12.4 ^{ab}	11.2 ^a	0.72	**	ns	ns
3 h post feeding	18.5 ^{ab}	22.1 ^b	17.7 ^a	17.7 ^a	17.5 ^a	0.72	ns	ns	ns
6 h post feeding	14.3 ^a	19.2 ^b	14.2 ^a	13.9 ^a	13.5 ^a	0.73	ns	ns	*
Mean	16.1 ^{ab}	19.3 ^b	14.4 ^a	16.7 ^a	14.0 ^a	0.65	*	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), **Value on the same row under each main effect with different superscripts differ highly significantly ($P<0.01$), ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$), FCSC= fermented cassava pulp with yeast (*S.cerevisiae*).

4.5.7 Volatile fatty acids (VFA)

Total volatile fatty acid (TVFA) concentration in rumen fluid ranged from 58.81 to 77.35 mM/L. TVFA of goats fed with 75% FCSC replacement of SBM diets were higher than 100% ($P<0.05$) FCSC, however was not significantly different

with 0% and 25% ($P>0.05$). There were not significantly different among treatments of TVFA at 3 and 6 hour post-feeding (Table 4.7).

Table 4.7 Total volatile fatty acid (TVFA) and average of volatile fatty acids of meat goats fed fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Total volatile fatty acid (mM/L)									
0 h post feeding	61.5 ^{ab}	61.2 ^{ab}	58.8 ^{ab}	74.5 ^b	54.7 ^a	2.54	ns	ns	ns
3 h post feeding	66.8	74.1	77.1	69.2	73.8	2.95	ns	ns	ns
6 h post feeding	77.4	65.9	76.6	73.2	75.4	2.04	ns	ns	ns
Mean	68.0	67.0	68.0	72.3	66.9	1.74	ns	ns	ns
Volatile fatty acids proportion (mole/100 mole)									
Acetic acid (C2)									
0 h post feeding	73.7	74.9	79.1	79.2	75.9	0.88	ns	ns	ns
3 h post feeding	71.5 ^a	77.2 ^{ab}	75.0 ^{ab}	78.1 ^b	76.0 ^{ab}	1.24	ns	ns	ns
6 h post feeding	75.9	76.3	75.8	77.2	71.1	1.13	ns	ns	ns
Mean	74.4	75.1	77.0	78.3	71.9	0.92	ns	ns	ns
Propionic acid (C3)									
0 h post feeding	15.5	12.5	13.4	14.3	12.8	0.69	ns	ns	ns
3 h post feeding	12.9	10.8	18.4	15.0	16.6	1.45	ns	ns	ns
6 h post feeding	17.9 ^b	12.1 ^a	15.5 ^{ab}	15.2 ^{ab}	13.4 ^{ab}	0.91	ns	ns	ns
Mean	16.5	12.2	16.2	14.7	13.6	0.63	ns	ns	ns
Butyric acid (C4)									
0 h post feeding	9.5 ^{ab}	10.9 ^b	8.05 ^{ab}	6.8 ^a	8.0 ^{ab}	0.57	ns	ns	ns
3 h post feeding	8.7 ^a	13.1 ^b	9.6 ^{ab}	7.0 ^a	10.0 ^{ab}	0.95	ns	ns	*
6 h post feeding	8.8 ^b	10.8 ^b	8.6 ^{ab}	6.0 ^a	9.7 ^b	0.51	ns	ns	*
Mean	8.6 ^{ab}	11.4 ^b	7.9 ^a	6.7 ^a	8.8 ^{ab}	0.53	ns	ns	*
C2:C3	4.7	4.3	4.1	4.4	4.3	0.16	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard

error of means, ns=not significantly different ($P>0.05$), FCSC= fermented cassava pulp with yeast (*S.cerevisiae*).

4.5.8 Purine derivatives, microbial N supply

The concentration of urinary purine derivatives (PD) and microbial N supply were shown in Table 4.8. The results showed that total PD and allantoin excretion (mmole/day) of goats fed with 75% FCSC replacement of SBM diets had higher than 0, 25, 50, and 100% FCSC replacement of SBM diets but were not different ($P>0.05$). However, above value were significantly different ($P<0.05$) when expressed in terms of uric acid in urinary PD.

Similarly, microbial N synthesis (g N/day) or microbial protein synthesis (g CP/day) of goats fed 75% FCSC replacement of SBM diets had higher than 0, 25, 50, and 100% FCSC replacement of SBM diets but were not different ($P>0.05$). The efficiency of microbial N supply [g N/kg OM digestible in rumen (OMDR)] was the highest at goats fed 75% FCSC replacement of SBM diets but were significantly different ($P>0.05$).

Table 4.8 Purine derivative (PD) excretion, microbial N supply of meat goats fed fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Purine derivative excretion (mM/day)									
Allantoin	6.57	10.7	4.88	8.28	5.88	1.14	ns	ns	ns
Uric acid	0.76	2.14	0.93	1.82	0.93	0.27	ns	ns	ns
Hydroxanthine	0.62	0.84	0.74	1.32	0.72	0.18	ns	ns	ns
Xanthine	0.89	1.06	0.84	0.97	0.69	0.15	ns	ns	ns
Creatinine	9.12	11.8	11.2	10.4	10.6	1.31	ns	ns	ns
Total PD									
mM/L	8.84	14.7	7.40	12.4	8.21	1.62	ns	ns	ns
mM/BW ^{0.75}	0.80	1.34	0.73	1.10	0.75	0.15	ns	ns	ns
DOMI (gDM/day)									
	700.4	692.3	679.7	689.0	690.2	11.6	ns	ns	ns
Microbial N supply									
g of N/day	5.81	10.9	4.54	8.94	5.29	1.42	ns	ns	ns
g of N/kg of DOMR									
	12.1	23.8	10.3	18.7	11.2	3.00	ns	ns	ns
Microbial crude protein (MCP, g/day)									
	36.3	68.2	28.4	55.9	33.1	8.9	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P < 0.05$), ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), DOMI= digestible organic matter intake, DOMR= digestible organic matter fermented in rumen calculated as $0.65 \times \text{DOMI}$ (ARC, 1984), FCSC=fermented cassava pulp with yeast (*S. cerevisiae*), Microbial N (gN/day) = $0.727X$, $Y = 0.83X + 0.202\text{BW}^{0.75}$.

4.5.9 Nitrogen retention

Nitrogen intake were significantly different ($P>0.05$) and total nitrogen excretion were decreased linearly as a consequence of FCSC replacement of SBM diets. Urine N excretion and N output was the highest at goats fed 75% FCSC replacement of SBM diets ($P<0.05$). Similarly, N absorption and N retention (g/day) were decreased at goat fed 75% and 100% FCSC replacement of SBM diets and were significantly different ($P<0.05$) in the Table 4.9.

Table 4.9 Daily nitrogen balance of meat goats fed fermented cassava pulp with yeast (*S. cerevisiae*) replacement of soybean meal in growing goat diets.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
N intake (g)	10.9	11.1	10.8	10.8	10.7	0.19	ns	ns	ns
N excretion (g)									
Feces	3.9 ^b	3.8 ^b	3.4 ^a	3.8 ^b	3.6 ^{ab}	0.13	ns	ns	ns
Urine	1.1	0.6	0.5	1.3	0.7	0.15	ns	ns	ns
N output (g)	4.9 ^{ab}	4.3 ^{ab}	3.9 ^a	5.1 ^b	4.3 ^{ab}	0.17	ns	ns	*
N absorption (g)	7.1	7.3	7.5	7.0	7.0	0.16	ns	ns	ns
N retention (g)	6.0	6.8	6.9	5.7	6.3	0.22	ns	ns	ns
N retention (%)	55.0 ^{ab}	60.8 ^{ab}	64.1 ^b	51.4 ^a	59.0 ^a	1.60	ns	ns	*

^{a, b, c} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, * Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ** differ highly significantly ($P<0.01$), ns=not significantly different ($P>0.05$), FCSC= fermented cassava pulp with yeast (*S. cerevisiae*).

4.5.10 Ruminal microbial population

4.5.10.1 Direct count methods

The number of protozoa ranged from 2.7 to 7.1 x 10⁵ cells/ml rumen fluid. And as expected, at preliminary feeding and 6 h post feeding even though the effectiveness of FCSC replacement of SBM on protozoa population were not significant different (P>0.05). Protozoa population of 75% FCSC replacement of SBM at 3 hour post feeding decreased cubic (P<0.05), protozoa population lower than 25% FCSC replacement of SBM were significant different (P<0.05). Similarly, number of fungi population in rumen fluid of goats fed 0% and 75% FCSC replacement of SBM in concentrate had higher than 25, 50, and 100% FCSC replacement of SBM at preliminary feeding, 3, and 6 hours post feeding were significant different (P<0.05). In addition, number of bacteria population in rumen fluid of goats fed FCSC replacement of SBM in dietary ranged from 0.7 to 8.0 x 10¹² cells /ml rumen fluid were showed Table 4.10.

Table 4.10 Population of microbial in rumen fluid of direct count methods.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Protozoa count (cells x 10⁵)									
0 h post feeding	6.0	7.1	6.9	5.1	6.0	0.66	ns	ns	ns
3 h post feeding	4.3 ^{ab}	7.0 ^b	5.9 ^{ab}	2.7 ^a	4.3 ^{ab}	0.58	ns	ns	*
6 h post feeding	3.7	5.7	8.0	3.2	5.6	0.77	ns	ns	ns
Mean	4.6	6.6	6.9	3.6	5.3	0.55	ns	ns	ns
Fungi count (cells x 10⁶)									
0 h post feeding	15.0 ^{ab}	12.4 ^a	10.1 ^a	16.8 ^b	10.0 ^a	1.07	ns	ns	*
3 h post feeding	22.0 ^b	14.7 ^{ab}	11.8 ^a	18.6 ^{ab}	11.0 ^a	1.42	*	ns	ns
6 h post feeding	21.6 ^b	13.9 ^{ab}	15.2 ^{ab}	18.9 ^{ab}	11.6 ^a	1.57	ns	ns	ns
Mean	19.6 ^b	13.7 ^{ab}	12.5 ^a	18.1 ^{ab}	10.8 ^a	1.24	ns	ns	*
Bacteria count (cells x 10¹²)									
0 h post feeding	1.1 ^b	0.9 ^{ab}	0.8 ^{ab}	0.7 ^a	0.8 ^{ab}	0.04	ns	ns	ns
3 h post feeding	0.7	0.8	0.7	0.9	0.7	0.04	ns	ns	ns
6 h post feeding	8.0 ^b	8.0 ^b	7.9 ^b	7.2 ^{ab}	6.1 ^a	0.03	**	ns	ns
Mean	0.9 ^b	0.8 ^{ab}	0.8 ^{ab}	0.8 ^{ab}	0.7 ^a	0.03	**	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=linear, Q=quadratic and C=cubic), SEM= Standard error of means, **Value on the same row under each main effect with different superscripts differ highly significantly ($P < 0.01$), ns=not significantly different ($P > 0.05$), FCSC=fermented cassava pulp with yeast (*S. cerevisiae*).

4.5.10.2 Grouping bacteria count by roll tube methods

Table 4.11, the number of cellulolytic bacteria was ranged from 4.2 to 17.4 CFU x 10⁷ in rumen fluid. Goats fed 0%, and 75% FCSC replacement of SBM diets had higher than 25, 50, and 100% FCSC replacement of SBM diets ($P < 0.05$). Similarly, proteolytic bacteria in rumen fluid (CFU x 10⁵) population were

significantly different ($P<0.05$) at difference time sampling. And after feeding 3 hour, ruminal microbial of amylolytic bacteria ($\text{CFU} \times 10^5$) population goat fed 25%, and 75% FCSC replacement of SBM diets had higher than 0, 50, and 100% FCSC replacement of SBM diets ($P<0.05$).

Table 4.11 Population of microbial in rumen fluid of grouping bacteria count by roll tube methods.

Items	FCSC replacement of SBM (%)					SEM	Contrast ¹		
	0	25	50	75	100		L	Q	C
Cellulolytic bacteria ($\text{CFU} \times 10^7$)									
0 h post feeding	8.2	6.6	4.2	4.4	5.7	0.84	ns	ns	ns
3 h post feeding	13.3 ^{ab}	8.3 ^a	9.8 ^a	17.4 ^b	8.6 ^a	2.04	ns	ns	ns
6 h post feeding	16.2	10.4	14.0	15.0	7.7	2.78	ns	ns	ns
Mean	9.1 ^a	13.1 ^b	6.8 ^a	12.3 ^{ab}	6.3 ^a	1.67	ns	ns	ns
Proteolytic bacteria ($\text{CFU} \times 10^5$)									
0 h post feeding	23.8 ^b	18.2 ^{ab}	11.8 ^{ab}	13.4 ^{ab}	6.5 ^a	2.55	ns	ns	ns
3 h post feeding	12.1 ^{ab}	5.4 ^{ab}	4.6 ^a	14.6 ^b	6.6 ^{ab}	1.82	ns	ns	ns
6 h post feeding	16.7	9.1	12.2	15.8	9.2	2.66	ns	ns	ns
Mean	17.0 ^b	11.5 ^{ab}	8.9 ^a	14.6 ^{ab}	7.4 ^a	1.69	ns	ns	ns
Amylolytic bacteria ($\text{CFU} \times 10^5$)									
0 h post feeding	8.5	5.7	6.6	7.6	7.8	0.82	ns	ns	ns
3 h post feeding	9.0 ^a	11.8 ^{ab}	8.6 ^a	20.5 ^b	8.6 ^a	1.97	ns	ns	ns
6 h post feeding	19.7 ^b	14.2 ^{ab}	10.7 ^{ab}	9.8 ^a	14.1 ^{ab}	2.11	ns	ns	ns
Mean	13.5	10.8	7.4	10.8	10.1	1.20	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ¹Contrast effect (L=linear, Q=quadratic and C=cubic), SEM= Standard error of means, **Value on the same row under each main effect with different superscripts differ highly significantly ($P<0.01$), ns=not significantly different ($P>0.05$), FCSC= fermented cassava pulp with yeast (*S. cerevisiae*).

4.6 Discussion

These results indicated the concentrate and roughage intakes were not significantly different, but *S. cerevisiae* cultures increases DM intake by improving ruminal conditions that stimulates ruminal cellulolysis. (Williams, Tait, Innes, and Newbold, 1991; Pinos-Rodríguez, et al., 2008). The addition of *S. cerevisiae* to ruminant diets have been observed to improve digestibility of DM and to increase ruminal bacteria numbers (Dawson, Newman, and Boling, 1990; Oeztuerk, Schroeder, Beyerback, and Breves, 2005).

Supplementation of *S. cerevisiae* alone in the diet of goats has been shown to reduce NH₃-N concentration (Koul, Kumar, Sareen, and Singh, 1998; El-Waziry, Kamel, and Yacout, 2000; Galp, 2006), or kept it unaffected ($P>0.05$) (Corona et al., 1999; Tripathi, Karim, Chaturvedi, and Verma, 2007; Jiang et al., 2008). From the results of Galp (2006a), we can get the averages of ruminal fluid NH₃-N and plasma urea that calculated from 0, 3, and 6 hours post-feeding were 354.0, 308.3 (mmol/L), and 45.50, 43.00 (mg/dL) for control and *S. cerevisiae* treatment group respectively, there were no significant differences. The results of the present study showed that significant raise in NH₃-N was caused by addition of probiotics, these findings were consistent with those of Fadel-Elseed and Abusamra (2007), which reported that *S. cerevisiae* resulted in a numerical increase in ammonia-N concentration. In addition, the present study also found significant raise in PUN, and it agreed with the results of Galp (2006b), which reported that the means of serum urea were 0.53 (8.9), 0.570 (9.5), and 0.57 (9.4) (g/L and mmol/L) for control, 5, and 10 g/d *S. cerevisiae* treatments, respectively, a significant difference was observed. Physiological levels and proportions of rumen VFAs measured in previous studies are variable due to the

large range of diet types, amounts of each feed and animal factors. France and Siddons (1993) summarizes levels of VFA production from different animals and diets. Physiological levels of rumen propionate in dairy cows range from approximately 13 mmol/L on a roughage (hay) diet (France and Siddons, 1993) to 57 mmol/L on a high-concentrate diet (Bauman, Davis, and Bucholtz, 1971). Lindberg (1985) measured allantoin excretion in dairy goats fed varying protein levels and sources and found allantoin excretion to be linearly related ($R=0.83$) to DOM intake.

4.7 Conclusions

The results obtained from this experiment could have a great impact on animal feed especially using local resources-based diets. The present results indicate that cassava pulp fermented can improve CP content and can be made that using cassava pulp fermented by *S. cerevisiae* as the main source of protein to completely replace SBM was beneficial to meat goats in terms of feed intake (75% in concentrate).

4.8 References

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CHAPTER V

EFFECTS OF 75% CASSAVA PULP FERMENTATION
BY *Saccharomyces cerevisiae* ON PROTEIN REPLACEMENT
SOYBEAN MEAL IN CONCENTRATE
DIETS IN GROWING GOATS

5.1 Abstract

Twenty-four male growing goats were randomly assigned to a Randomized Complete Block Design. Dietary treatments were different level of feeding concentrate diet at 1.0, 1.5, 2.0, and 2.5% of body weight (BW). The results, showed that average daily gain, microbial N supply, N retention of meat goats in the group of feeding level at 2.0% BW and 2.5% BW were significantly higher ($P < 0.05$) than those goats fed with feeding levels of 1.0% BW and 1.5% BW. Based on this result the conclusion can be made that using 75% fermented cassava pulp by *Saccharomyces cerevisiae* as the main source of protein to completely replaced soybean meal was beneficial to meat goats in terms of feed intake. The feeding concentrate at levels between 2.0-2.5% BW give highest in the growth of meat goat in this experiment.

5.2 Introduction

In situations where concentrate feed supplements are expensive, farmers should be capable of formulating their own feeds based on available farm resources and their economic viability (Wanapat, 1999). The cassava pulp is by a product from the cassava flour process. Cassava pulp has low protein content and only used as carbohydrate material in the animal feed production (Obloh and Akindahunsi, 2005; Srinorakutara, Kaewvimol, and Saengow, 2006; Ubalua, 2007). In general, cassava pulp is very wet. However, drying is the best way of stored cassava pulp because it can easy to store and it can be stored longer than ferment it in anaerobic condition. Because the protein source for animal feed is growing expensive. So, research has found that cassava pulp is one of good alternative for enriching protein by microbial fermentation (Putipipatkajon and Srinophakun, 1999; Boonraeng, Foo-trakul, Kanlayakrit, and Chetanachitra, 2000). Therefore, cassava pulp is fermented with yeast (*S. cerevisiae*) for protein enrichment before it is used as the high quality animal feed material. The main objective of this study therefore was investigated the effect of fermented cassava pulp by *S. cerevisiae* (FCSC) as protein replacement of soybean meal in concentrate diets in meat goats.

5.3 Objective

This experiment was conducted to determine the effects of replacement of 75% FCSC for soybean meal in concentrate diets on rumen fermentation, carcass quality, and goat productivity.

5.4 Material and Methods

5.4.1 Animal and Management

Twenty-four meat male crossbred (Thai native x Anglo-Nubian) growing goats with weighed 18.0 ± 5.0 kg (mean \pm SD) and aged about 7 months, were purchased from local market at Nakhon Ratchasima province. The starting the experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9×1.4 m²) where the animals could have an easy access to rice straw and clean water *ad libitum*. The pens were cleaned and disinfected before animals were housed. Animals were fed *ad libitum* of rice straw and were fed concentrate twice daily at 08.00 am and 16.00 pm. Experiment period was lasted for 108 days. The first 21 days was used for animal adaptation and following by 90 days for parameters measurement and in the last 7 days for total collection. Feed refusal were weighed daily prior to the morning feeding to determine daily dry matter intake (DMI). Body weight (BW) of each animal was measured weekly immediately before the morning feeding.

5.4.2 Experimental design and treatments

The experiment was taken according in randomized complete block design (RCBD), with 6 goats in each treatment. The body weight was used as block. The dietary treatments were allocated to 4 treatments were as follows 75% fermented cassava pulp replacement for soybean meal in concentrate feeding level at 1.0, 1.5, 2.0, and 2.5% BW.

5.4.3 Sampling methods

The above samples were collected, preserved and analyzed as previously described (Paengkoum, Liang, Jalan and Basery, 2006). The daily offered and left

concentrate and rice straw were weighed (the residues were removed) every morning before offering for the purpose of determination dry matter intake. Body weight of the animals were measured weekly prior to the morning feeding. The rice straw and concentrate were sampled weekly and dried at 60~65°C in hot air oven for determination of dry matter (DM) composition, and followed by grinding through a 1 mm sieve and then kept in tightly covered zip plastic bags to make a pool respectively for further approximate analysis. During the post-experiment week for urinary and fecal samples total collection, the all-day feces and urine (10% H₂SO₄ was used as a preserving reagent, 30 ml/container) were collected and the total amount was recorded down every morning (measured feces weight and urine volume). Subsequently, 15% of the total amounts was sub-sampled to make a pool respectively for each animal, and then was kept at -20°C and in the end was dried prior to chemical composition analysis that aimed to determine digestibility and nitrogen balance. For ruminal fluid samples, they were withdrawn on the last day of the experiment through an esophageal stomach tube following 0, 3 and 6 hour post-morning meal timing. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 ml of the samples were measured well and truly with a pipette into the tubes containing 9 ml 10% formalin (v:v, 9:1) as a preserving reagent and then were closed tightly with screw caps that with butyl rubber lining for checking the counts of ruminal protozoa and bacteria. At the same time, 20 ml of the samples were measured and then put into small plastic bottles containing 5 ml 6 N HCl as a preserving reagent, and then the bottles were closed tightly with screw caps that with butyl rubber lining for determination of ruminal ammonia-N and volatile fatty acids. With that, all samples

were kept at -20°C until further analysis. The blood samples were collected from jugular veins into EDTA containing vacuum tubes and were centrifuged at 3000 r for 5 min to separate plasma from the cells within 20 minutes after sampling. Subsequently the plasma was collected, and then it was stored at -80°C for subsequent analyses of plasma urea nitrogen.

5.4.4 Experimental location

The experiment was conducted at Suranaree University of Technology's goat farm. The center for Scientific and Technological Equipment Building 1, 2, and 3, Suranaree University of Technology.

5.4.5 Chemical analysis

The daily feed offered, refusal, feces and urine samples were collected, samples and analyzed in similar manner as that described in Chapter IV.

Rumen fluid and plasma samples for all growing goats were collected, samples and analyzed in similar manner as that described in Chapter IV.

The weight gain of growing goats was calculated from the weighing done before and after the 90 days feeding trial.

5.4.6 Statistical methods

All data were subjected were statistically analyzed as a Randomized Complete Block Design (RCBD) using the general linear model procedure of the Statistical Analysis System Institute SAS (1996). The treatment means were statistically compared by Duncan's New Multiple Range Test and Orthogonal Contrast analysis were compare treatment means (Steel and Torrie, 1980).

5.5 Results and Discussion

5.5.1 Feed intake and average daily gain

Ingredients and chemical composition of 75% FCSC replacement soybean meal in concentrate were crude protein (CP) content of concentrate and roughage 13.14 and 2.08% DM respectively. All diets treatments had similar chemical composition. These results indicated the dry matter intake (DMI) and showed that total dry matter intake (g/d) give the highest when growing goats feeding level at 2.5% BW significantly ($P < 0.05$) and improved ruminal parameters that stimulate to nutrient digestibility similarly to Pinos-Rodríguez et al. (2008).

Total DM intake were greater ($P < 0.05$) by increasing with 75% FCSC replacement of SBM in concentrate different feeding level of % BW. The results from this study were similar to those from Paengkoum, Liang, Jelan, and Basery (2006) who indicated that DM intake of goats were greater for goats fed high energy than low energy diets. Similarly, Hwangbo et al. (2009) reported that DMI was not significantly different among diets when Korean black goats were fed with increasing protein levels from 14-20%. However, indigenous goats in tropics fed to appetite have a daily DMI in the range 1.8-4.1% BW equivalent to 40.5-131.1 g/kgBW^{0.75}. For meat breeds, had daily DMI of 1.8-3.8% BW and 40.5-127.3 g/kgBW^{0.75} (Devendra and Burns, 1983). These ranges are wider probably due to the effects of multiple factors on DMI. Therefore, fed to appetite DMI must be used with caution (Sepsibe, 2006).

Initial, average and final body weight, and average daily gain (ADG) in growing goats with 75% FCSC replacement of SBM in concentrate different levels of % BW are presented in Table 5.1. The final weight and ADG of growing goats fed with different ($P < 0.05$) among treatments. The ADG throughout the 90 days of

experiment increasing with 75% FCSC replacement of SBM in concentrate different feeding level of 1.0, 1.5, 2.0, and 2.5% BW were significantly different ($P<0.05$).

Table 5.1 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diet in meat growing goats on feed intake.

Items	Feeding levels (%BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Roughage intake								
g/day	436.5	402.7	402.2	402.0	7.82	ns	ns	ns
% BW	2.3 ^b	2.1 ^{ab}	2.1 ^{ab}	2.0 ^a	0.06	*	ns	ns
gDM/kgBW ^{0.75}	48.3 ^b	44.4 ^{ab}	43.7 ^{ab}	42.7 ^a	0.98	*	ns	ns
Concentrate intake								
g/day	190.5 ^a	289.8 ^b	384.9 ^c	494.2 ^d	26.0	**	ns	ns
% BW	1.0 ^a	1.5 ^b	1.9 ^c	2.4 ^d	0.11	**	ns	ns
gDM/kgBW ^{0.75}	20.8 ^a	31.3 ^b	41.3 ^c	51.6 ^d	2.41	**	ns	ns
Total intake								
g/day	626.9 ^a	692.5 ^a	787.0 ^b	896.1 ^c	26.36	**	ns	ns
% BW	3.3 ^a	3.6 ^b	4.1 ^c	4.5 ^d	0.06	**	ns	ns
gDM/kgBW ^{0.75}	69.1 ^a	75.7 ^b	85.0 ^c	94.2 ^d	2.10	**	ns	ns
Initial BW (kg)	18.3	18.2	17.8	18.0	0.62	ns	ns	ns
Final BW (kg)	19.5 ^a	20.1 ^a	21.8 ^{ab}	22.8 ^b	0.75	**	ns	ns
ADG (g/day)	11.0 ^a	19.0 ^a	43.5 ^b	53.7 ^b	6.76	*	ns	ns

^{a, b, c, d} Value on the same row under each main effect with different superscripts differ significantly ($P<0.05$), ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$), ADG=Average daily gain, * = ($P<0.05$), ** = ($P<0.01$).

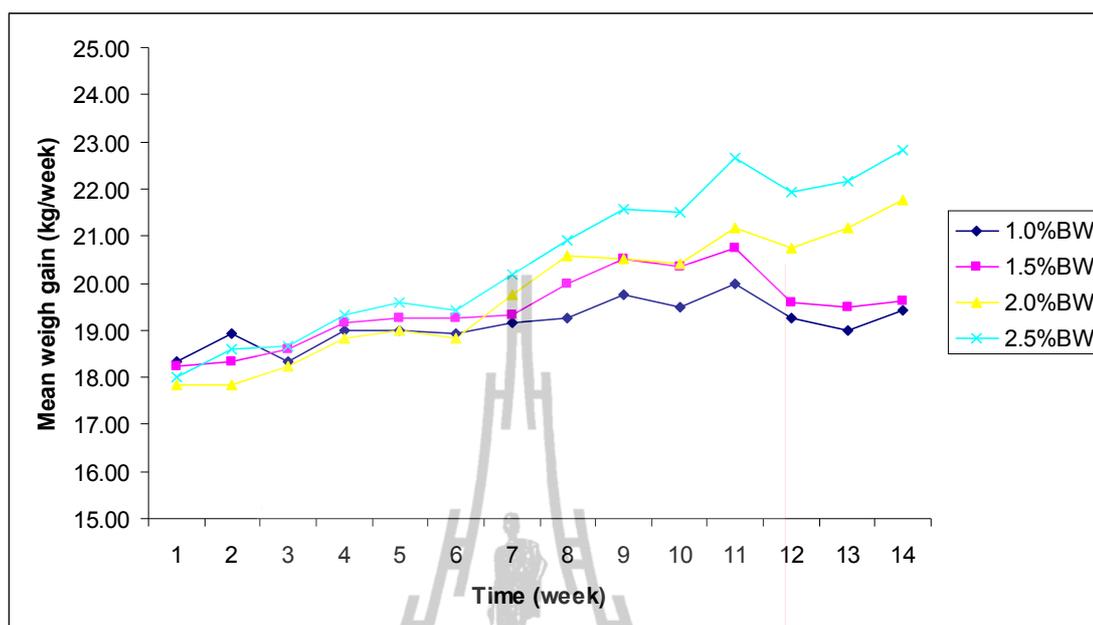


Figure 5.1 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diet in meat growing goats on body weigh gain (kg/week).

The mean body weigh gain (kg/week) growing goats when used 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets. A long-term feeding trial is a technique for the estimation of average dairy gain as proposed by NRC (1981). The result of simple linear regression analysis between time (week) and mean body weigh (kg/week) showed that the y-intercept of equation was 53.9 g/day. Prediction equations generates, considering mean body weight of the growing goat as the dependent variable. From the equations as shown in Table 5.2.

Table 5.2 Prediction equation for body weight gain on growing goats when used 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets.

Feeding levels (% BW)	Regression	R ²	ADG (g/day)
1.0	$y = 0.0777x + 18.549$	0.4897	11.1
1.5	$y = 0.133x + 18.463$	0.5342	19.0
2.0	$y = 0.3062x + 17.465$	0.9459	43.5
2.5	$y = 0.3773x + 17.694$	0.9515	53.9

x = week

5.5.2 Nutrient digestibility

Daily nutrient intakes including dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) are presented in Table 5.3. DM digestibility of goat fed with 75% FCSC replacement of SBM in concentrate were not significant among treatments ($P > 0.05$). Similarly, OM, CP and ADF digestibility increased were not significantly different as a consequence of 75% FCSC replacement of SBM when increased feeding level. NDF digestibility were increased linearly ($P < 0.05$) as were significantly different among treatments ($P < 0.05$).

Table 5.3 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets in growing goats on nutrient digestibility (Total collection).

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Digestibility (%)								
DM	71.4	73.3	73.3	75.3	0.76	ns	ns	ns
OM	74.6	76.6	76.5	78.4	0.65	ns	ns	ns
CP	47.4 ^a	59.9 ^b	66.4 ^{ab}	69.8 ^c	2.27	**	ns	ns
NDF	73.6	75.9	72.9	73.9	0.68	ns	ns	ns
ADF	66.1	65.8	62.5	63.7	0.87	ns	ns	ns
Digestibility nutrient intake (g/day)								
OM	376.0 ^a	412.7 ^a	484.3 ^b	596.6 ^c	21.3	**	ns	ns
CP	26.0 ^a	36.3 ^b	47.3 ^c	62.1 ^d	3.09	**	ns	ns
NDF	192.6 ^a	234.4 ^b	288.9 ^c	350.1 ^d	14.9	**	ns	ns
ADF	208.5 ^a	200.1 ^{ab}	210.1 ^{ab}	241.2 ^b	6.14	**	ns	ns
ME intake (kcal/day)								
	1428.6 ^a	1568.1 ^a	1840.4 ^b	2266.9 ^c	80.9	**	ns	ns
Microbial crude protein (MCP, g/day)								
	48.9 ^a	53.7 ^a	63.0 ^b	77.5 ^c	2.77	**	ns	ns
Energy density (Mcal/kg)								
	2.25 ^a	2.35 ^{ab}	2.35 ^{ab}	2.42 ^b	0.02	**	ns	ns

^{a, b}Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), DM=Dry matter, OM=Organic matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, * = ($P < 0.05$). Microbial crude protein (MCP, g/day) = 0.13x kg DOMI.

5.5.3 Ruminal pH and ammonia nitrogen (NH₃-N)

The ruminal pH were decreased at 3, and 6 hour after feeding and there was not significant ($P > 0.05$). Concentration of rumen NH₃-N were increased at 3, and

6 hour after feeding and there was no significant ($P>0.05$). Ruminal $\text{NH}_3\text{-N}$ increased gradually after feeding and peaked at 6 hour post feeding as show in Table 5.4.

Table 5.4 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets in growing goats on ruminal pH and ammonia nitrogen ($\text{NH}_3\text{-N}$).

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
pH								
0 h post feeding	7.3 ^a	7.6 ^{ab}	7.5 ^{ab}	7.7 ^b	0.08	ns	ns	ns
3 h post feeding	7.1	7.3	7.2	7.4	0.09	ns	ns	ns
6 h post feeding	6.9	7.1	7.0	7.3	0.07	ns	ns	ns
Ammonia-N (mg%)								
0 h post feeding	9.7	12.0	10.0	13.0	0.81	ns	ns	ns
3 h post feeding	17.1	17.8	14.0	22.1	1.97	ns	ns	ns
6 h post feeding	21.9	20.2	17.5	18.1	1.24	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P<0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P>0.05$), ** = ($P<0.01$).

5.5.4 Plasma urea nitrogen (PUN)

The pattern of PUN concentration increased linearly ($P<0.01$) as a consequence of feeding level of % BW at 6 h post feeding increased and peaked at 3 hour post feeding then decreased thereafter when fed 75% FCSC replacement soybean meal in concentrate diets feeding level of % BW increasing were significant difference ($P<0.05$) are presented in Table 5.5.

Table 5.5 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets in growing goats on plasma urea nitrogen (PUN).

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Plasma urea nitrogen (PUN, mg%)								
0 h post feeding	12.8	13.7	13.6	16.5	0.94	ns	ns	ns
3 h post feeding	12.9	17.9	16.4	20.4	1.35	ns	ns	ns
6 h post feeding	12.5 ^a	14.4 ^a	15.0 ^a	21.2 ^b	1.18	**	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), ** = ($P < 0.01$).

5.5.5 Volatile fatty acids and proportion of VFA

Total volatile fatty acids (VFA) concentration at 0, 3, and 6 hour post feeding were shown in Table 5.6. Total VFA were no significant difference ($P > 0.05$). The proportion of acetic acid (mole/100 mole) of growing goats fed 75% FCSC replacement soybean meal in concentrate diets feeding level of 1.0% BW were significantly higher ($P < 0.05$) than those of growing goats fed feeding level of 2.0 and 2.5% BW. However, proportion of acetic acid at 0 and 3 hour post feeding were decreased linearly ($P < 0.01$). The proportion of propionic acid and butyric acid increasing when fed 75% FCSC replacement soybean meal in concentrate diets feeding level of % BW increased were significantly ($P < 0.05$).

Table 5.6 Total volatile fatty acid (TVFA) and average of volatile fatty acids of fed 75% fermented cassava pulp by *S. cerevisiae* replacement of soybean meal in growing goats concentrate diets.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Total volatile fatty acids (mM/L)								
0 h post feeding	52.0	51.0	47.9	42.9	4.19	ns	ns	ns
3 h post feeding	73.1	93.3	66.5	57.9	6.54	ns	ns	ns
6 h post feeding	69.0	60.6	66.3	55.7	2.97	ns	ns	ns
Mean	64.7	68.3	60.2	52.2	3.05	ns	ns	ns
Volatile fatty acids proportion (mole/100 mole)								
Acetic acid (C2)								
0 h post feeding	80.7 ^c	77.7 ^{bc}	76.0 ^b	75.2 ^a	0.59	**	ns	ns
3 h post feeding	77.4 ^b	73.4 ^a	73.6 ^a	72.4 ^a	0.68	**	ns	ns
6 h post feeding	76.9	75.6	75.8	73.4	0.68	ns	ns	ns
Mean	78.3 ^b	75.6 ^a	75.1 ^a	73.7 ^a	0.55	**	ns	ns
Propionic acid (C3)								
0 h post feeding	12.8	14.1	13.6	14.3	0.33	ns	ns	ns
3 h post feeding	15.1 ^a	18.9 ^b	16.7 ^{ab}	18.4 ^b	0.62	ns	ns	ns
6 h post feeding	15.2	16.5	14.9	17.1	0.66	ns	ns	ns
Mean	14.4	16.5	15.1	16.6	0.48	ns	ns	ns
Butyric acid (C4)								
0 h post feeding	6.5 ^a	8.3 ^{ab}	10.4 ^b	10.4 ^b	0.51	**	ns	ns
3 h post feeding	7.6 ^a	7.8 ^a	9.7 ^b	9.1 ^b	0.31	**	ns	ns
6 h post feeding	8.0	7.9	9.5	9.5	0.39	ns	ns	ns
Mean	7.3	8.0	9.9	9.7	0.36	**	ns	ns
C2:C3	5.3	4.6	5.0	4.4	0.16	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), ** = ($P < 0.01$).

5.5.6 Purine derivatives excretion and Microbial N supply

The concentration of urinary purine derivative (PD) excretion, microbial N supply of fed 75% FCSC replacement of soybean meal in concentrate diets growing goats are showed in Table 5.7. Proportion of urinary PD excretion (mM/day), allantoin, uric acid, hypoxanthine and creatinine were non significant difference ($P>0.05$). Total PD were observed with respect increased when fed 75% FCSC replacement of soybean meal in concentrate diets were non significant difference ($P>0.05$) among various feeding level of % BW increased. As a result, microbial N supply (g N/day) increased but the efficiency of microbial N synthesis (g of N/kg of DOMR) decreased were non significant difference ($P>0.05$) among various feeding level of % BW increased. Microbial protein synthesis (g/day) were observed respect increase in growing goats when fed 75% FCSC replacement of soybean meal in concentrate diets were no significant difference ($P>0.05$) among various feeding level of % BW increased. Microbial yield in the rumen depends on many factors, such as the availability sources of carbohydrates and N in rumen. However, microbial protein production may be improved by balancing the overall daily ratio of ruminally available energy and N intake in diet (Devent, Ferret, Calsamiglia, Casals, and Gasa, 2001; Chumpawadee, Sommart, Vongpralub, and Pattarajinda, 2006).

Table 5.7 Purine derivative (PD) excretion and microbial N supply of fed 75% fermented cassava pulp by *S. cerevisiae* replacement of soybean meal in concentrate diets on growing goats.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Purine derivative excretion (mmol/day)								
Allantoin	2.91	3.07	2.15	3.15	0.39	ns	ns	ns
Uric acid	1.09	1.14	1.56	1.58	0.14	ns	ns	ns
Hydroxanthine	0.55	1.03	0.69	1.17	0.18	ns	ns	ns
Xanthine	0.87 ^{ab}	0.56 ^a	1.30 ^b	1.03 ^{ab}	0.13	ns	ns	*
Creatinine	6.17	7.30	12.9	11.4	1.80	ns	ns	ns
Total PD								
mole/L	5.41	5.80	5.69	6.92	0.71	ns	ns	ns
mole/BW ^{0.75}	0.59	0.63	0.57	0.67	0.07	ns	ns	ns
DOMI (gDM/day)	376.0 ^a	412.7 ^a	484.3 ^b	596.6 ^c	21.3	**	ns	ns
Microbial N supply								
g of N/day	3.10	3.40	3.20	4.22	0.62	ns	ns	ns
g of N/kg of DOMR	13.0	13.2	10.1	11.0	2.18	ns	ns	ns
Microbial crude protein (MCP, g/day)								
	19.4	21.3	20.0	26.4	3.89	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), * = ($P < 0.05$), DOMI= digestible organic matter intake, DOMR= digestible organic matter fermented in rumen calculated as $0.65 \times \text{DOMI}$ (ARC, 1984), Microbial N (gN/day) = $0.727X$, $Y = 0.83X + 0.202\text{BW}^{0.75}$.

5.5.7 Nitrogen retention

The nitrogen intake, N excretion, N absorption and N retention of growing goats fed 75% FCSC replacement of soybean meal in growing goats concentrate diets are presented in Table 5.8. Nitrogen intake, N excretion, N absorption and N retention increased linearly ($P < 0.01$) with increasing were significant

difference ($P < 0.05$) among various feeding level of % BW. The result, were observed with increased N intake these effects were likely related to the linear increase ($P < 0.01$). Normally, nitrogen or protein degradability has a major effect on urinary N output because of excess soluble N in the rumen from diets with high ruminal degradability protein (Paengkoum, Liang, Jelan, and Basery, 2006). It has been demonstrated that N excretion through urine increased with increasing CP diet and N intake (Pimpa, Liang, Jelan, and Andullah, 2003).

Table 5.8 Daily nitrogen balance of fed 75% fermented cassava pulp by *S. cerevisiae* replacement of soybean meal in growing goats concentrate diets.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
N intake (g)	6.3 ^a	8.6 ^b	11.2 ^c	14.4 ^d	0.69	**	ns	ns
N excretion (g)								
Feces	3.1	3.2	3.7	4.1	0.17	*	ns	ns
Urine	0.5	0.4	0.3	0.6	0.09	ns	ns	ns
N output (g)	3.6	3.6	3.9	4.7	0.21	ns	ns	ns
N absorption (g)	3.1 ^a	5.4 ^b	7.6 ^c	10.3 ^d	0.60	**	ns	ns
N retention (g)	2.7 ^a	5.0 ^b	7.4 ^c	9.7 ^d	0.59	**	ns	ns
N retention (%)	42.6 ^a	58.3 ^b	65.3 ^b	67.0 ^b	2.62	**	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), * = ($P < 0.05$), ** = ($P < 0.01$).

5.5.8 Ruminal microbial population

5.5.8.1 Direct count methods

The number of protozoa was ranged from 5.8 to 10.7 x 10⁵ cells/ml rumen fluid. And as expected, at preliminary feeding and 6 hour post feeding even

though the effectiveness of 75% FCSC replacement of SBM in growing goats on protozoa population were not significant different ($P>0.05$) among various feeding level of % BW. Number of fungi population in rumen fluid of goats fed 75% FCSC replacement of SBM in concentrate at 6 hour post feeding had higher than at preliminary feeding, 0, and 3 hour post feeding were significant different ($P<0.05$) among various feeding level of % BW. In addition, number of bacteria population in rumen fluid of goats fed 75% FCSC replacement of SBM in dietary ranged from 0.9 to 1.2×10^{12} cells/ml rumen fluid. Denvev et al. (2007) reported that yeast culture could consume oxygen in rumen fluid that enters the rumen by coated with feed particles and yeast culture could provide some of the nutrients and co-factors (vitamins B) to rumen bacteria were showed Table 5.9.

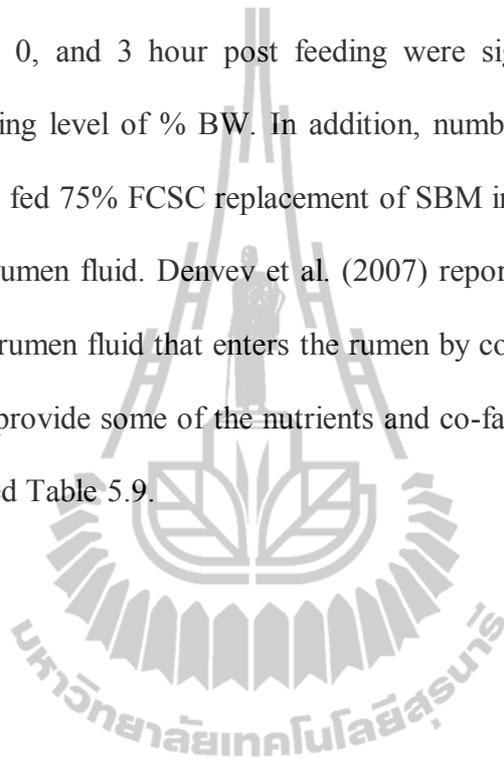


Table 5.9 Population of microbial in rumen fluid of direct count methods.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Protozoa count (cells x 10⁶)								
0 h post feeding	4.9	5.8	12.9	10.5	1.59	ns	ns	ns
3 h post feeding	7.9	7.4	6.7	8.6	0.95	ns	ns	ns
6 h post feeding	4.5	8.7	12.3	10.2	1.59	ns	ns	ns
Mean	5.8	7.3	10.7	9.8	1.20	ns	ns	ns
Fungi count (cells x 10⁶)								
0 h post feeding	9.0 ^a	17.7 ^{ab}	26.5 ^b	22.8 ^b	2.69	*	ns	ns
3 h post feeding	9.5	12.5	13.7	13.9	1.27	ns	ns	ns
6 h post feeding	11.1 ^a	27.0 ^b	25.9 ^b	28.2 ^b	2.85	*	ns	ns
Mean	9.9 ^a	19.0 ^b	22.1 ^b	21.6 ^b	2.00	*	ns	ns
Bacteria count (cells x 10¹²)								
0 h post feeding	0.9	1.0	1.2	0.8	0.07	ns	ns	ns
3 h post feeding	1.4	1.3	1.1	0.9	0.11	ns	ns	ns
6 h post feeding	0.8 ^a	1.5 ^b	1.0 ^{ab}	1.1 ^{ab}	0.10	ns	ns	*
Mean	1.0	1.2	1.1	0.9	0.67	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), * = ($P < 0.05$).

5.5.8.2 Grouping bacteria count by roll tube methods

The average number of cellulolytic bacteria ranged from 0.7 to 1.8 CFU x 10⁸ in rumen fluid increased were not significant different ($P > 0.05$) among various feeding level of % BW increasing. Similarly, proteolytic bacteria in rumen fluid (CFU x 10⁶) population decreased were difference time sampling. And after feeding 3 hours, ruminal microbial of amylolytic bacteria (CFU x 10⁶) population were decreased ($P < 0.05$: linear, quadratic and cubic trends) among various feeding level of % BW increasing are presented in Table 5.10.

Table 5.10 Population of microbial in rumen fluid of grouping bacteria count by roll tube methods.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Cellulolytic bacteria (CFU x 10⁸)								
0 h post feeding	0.6	0.8	1.3	1.6	0.25	ns	ns	ns
3 h post feeding	0.6	0.6	2.4	2.3	0.37	ns	ns	ns
6 h post feeding	0.8	1.0	1.5	1.4	0.21	ns	ns	ns
Mean	0.7	0.8	1.7	1.8	0.18	*	ns	ns
Proteolytic bacteria (CFU x 10⁶)								
0 h post feeding	3.5	3.4	4.1	3.7	0.95	ns	ns	ns
3 h post feeding	2.0	3.4	1.0	0.7	0.55	ns	ns	ns
6 h post feeding	0.9 ^a	3.8 ^b	0.6 ^a	1.4 ^a	0.41	ns	ns	*
Mean	2.1	3.5	1.9	1.9	0.43	ns	ns	ns
Amylolytic bacteria (CFU x 10⁶)								
0 h post feeding	1.8 ^a	1.1 ^a	1.4 ^a	5.3 ^b	0.64	*	*	ns
3 h post feeding	6.1 ^b	1.0 ^a	2.3 ^a	1.1 ^a	0.67	*	*	*
6 h post feeding	3.7	2.3	5.5	1.9	0.86	ns	ns	ns
Mean	3.9 ^b	1.5 ^a	3.1 ^{ab}	2.8 ^{ab}	0.47	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$) , * = ($P < 0.05$).

5.5.9 Meat and carcass performance

As presented in Table 5.11, the living weight, carcass weight, and the weight of free fat alimentary tract and organs were no significant difference ($P > 0.05$) among various feeding level of % BW. The weights of kidney, pelvic, and heart fat (KPH fat) and percentage of KPH fat on carcass growing goat with fed 75% FCSC replacement SBM increased feeding level were greater than significantly ($P < 0.05$). However, carcass performance of growing goats were observed fed 75% FCSC replacement SBM in concentrate diet by feeding level of 1.0, 1.5, 2.0, and 2.5 % BW

with rice straw *ad libitum* were not affected to carcass performance and organs were no significant difference ($P>0.05$).

Table 5.11 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets in carcass growing goats.

Items	Feeding levels (% BW)				SEM	Contrast		
	1.0	1.5	2.0	2.5		L	Q	C
Living weigh (kg)	20.0	20.8	20.0	22.3	1.1	ns	ns	ns
Hot carcass (kg)	8.3	9.0	8.0	10.0	0.58	ns	ns	ns
Cold carcass (kg)	7.75	8.6	7.5	9.5	0.50	ns	ns	ns
Dressing carcass (%)	41.2	42.9	39.9	44.5	0.95	ns	ns	ns
KPH fat(g)	390.0 ^a	600.0 ^{ab}	525.0 ^{ab}	750.0 ^b	67.6	*	ns	ns
LD area (cm ²)	7.4	8.1	7.4	8.2	0.54	ns	ns	ns
Rumen (g)	412.5	450.0	437.5	455.0	34.6	ns	ns	ns
Reticulum (g)	62.5	70.0	75.0	80.0	5.26	ns	ns	ns
Omasum (g)	80.0	107.5	67.5	107.5	8.63	ns	ns	ns
Abomasum (g)	92.5	85.0	60.0	70.0	9.95	ns	ns	ns
Length SI (m)	16.9	16.8	19.0	17.8	0.81	ns	ns	ns
Length LI (m)	4.3 ^a	4.6 ^{ab}	4.3 ^a	5.3 ^b	0.21	*	ns	ns
Length Carcass (cm)	55.8	56.8	55.0	56.5	1.04	ns	ns	ns
Lung (g)	147.5	167.5	160.0	190.0	12.06	ns	ns	ns
Heart (g)	65.0	85.0	72.5	77.5	6.68	ns	ns	ns
Liver (g)	252.5 ^a	295.0 ^{ab}	275.0 ^{ab}	327.5 ^b	20.27	ns	ns	ns
Kidney (g)	40.0	60.0	50.0	52.5	5.13	ns	ns	ns
Spleen (g)	15.0	35.0	22.5	25.0	3.95	ns	ns	ns

^{a, b} Values on the same row under each main effect with different superscripts differ significantly, SEM= Standard error of means, KPH fat=kidney, pelvic, and heart fat, LD area=*Longissimus dorsi* muscle area.

5.5.10 Meat quality traits

A key determinant of meat quality is pH, after slaughter of goat meat are presented in Table 5.12. Meat pH were range 6.1-6.5 and temperature were range

between 34.3-36.6°C significantly difference ($P<0.05$). Sesibe (n.d.) reported to the ultimate pH is determined 24 hours post-slaughter. Good quality meat usually has a pH of 5.4-5.7. The muscle of a living animal has a pH of 7.1.

Sesibe (n.d.) reported to the meat color is an important parameter in meat quality. It can be measured numerically using a colorimeter or subjectively. Several factors affect meat color such as species/breed, age, sex, cut of meat, surface drying of the meat and surface spoilage. Lean color is observed on the inner portion of the flank muscle. The amount of pigments in the muscle increase with animal age, resulting in a darker color. Younger kid goats have a light, grayish pink flank. Goat meat consumers have indicated a preference for meat with lighter color. The color of growing goat when fed 75% FCSC replacement SBM in concentrate diet by feeding level of 1.0, 1.5, 2.0, and 2.5 % BW are showed in Table 5.13. The L^* color for *Longissimus dorsi* muscle, *Semimembranosus* muscle and *Triceps brachii* muscle at pre-chill and 24 hour post-chill when fed 75% FCSC replacement SBM in concentrate diet by feeding level of 2.5, 2.0, 1.5, and 1.0 % BW were decreased respectively were significant difference ($P<0.05$). The a^* color for *Longissimus dorsi* muscle, *Semimembranosus*, and *Triceps brachii* muscle muscle at pre-chill, the a^* color (less redder) had lower than 24 hour post-chill. The a^* color and b^* colour decreasing were significantly difference ($P<0.05$). In addition, meat composition were no significantly ($P>0.05$) are showed in Table 5.14.

Sesibe (n.d.) reported to the meat color is largely determined by the content of myoglobin and its derivatives. It is normal for meat to change color depending on the presence or absence of air. For instance, exposed meat changes color due to reactions occurring between myoglobin and oxygen. Meat color changes in response to both the quantity of myoglobin it contains, and chemical changes in the

myoglobin itself. The more myoglobin in the meat, the darker the color exhibited. Older sheep contain more muscle myoglobin and hence have darker meat than lambs. Color is also greatly affected by muscle pH. At a high pH, muscle has a closed structure and, hence, appears dark and the meat tends to be tough. Meat color is also affected by diet.

Table 5.12 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets in carcass growing goats on pH, and temperature (°C).

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Meat pH (0 h)								
<i>Longissimus dorsi</i>	6.6	6.7	6.7	7.1	0.10	ns	ns	ns
<i>Triceps brachii</i>	7.2	7.1	7.4	7.4	0.08	ns	ns	ns
<i>Semimembranosus</i>	7.1	6.8	7.2	6.7	0.11	ns	ns	ns
pH (24 h)								
<i>Longissimus dorsi</i>	6.2 ^{ab}	6.1 ^a	6.3 ^b	6.1 ^a	0.03	ns	ns	**
<i>Triceps brachii</i>	6.5 ^b	6.2 ^a	6.3 ^{ab}	6.3 ^{ab}	0.03	ns	*	ns
<i>Semimembranosus</i>	6.3 ^b	6.1 ^a	6.4 ^b	6.3 ^{ab}	0.03	ns	ns	**
Meat Temperature (°C)								
<i>Longissimus dorsi</i>	35.5	35.9	35.7	36.1	0.13	ns	ns	ns
<i>Triceps brachii</i>	35.7	36.1	36.1	36.3	0.13	ns	ns	ns
<i>Semimembranosus</i>	34.3 ^a	35.2 ^a	35.3 ^a	36.6 ^b	0.20	**	ns	ns

^{a, b, *} Values on the same row under each main effect with different superscripts differ significantly (P<0.05) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different (P>0.05), * = (P<0.05), ** = (P<0.01).

Table 5.13 Effects of 75% fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets on meat color of growing goats.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Chill (0 h)								
<i>Longissimus dorsi</i>								
L*	55.8 ^b	46.3 ^a	46.7 ^a	49.8 ^a	1.09	*	**	ns
a*	5.6 ^a	12.7 ^b	12.8 ^b	7.8 ^a	0.69	ns	**	ns
b*	0.4 ^a	2.7 ^b	3.1 ^b	2.1 ^{ab}	0.35	ns	**	ns
<i>Triceps brachii</i>								
L*	62.3 ^b	49.6 ^a	51.0 ^a	47.7 ^a	1.40	*	*	ns
a*	6.2 ^a	9.7 ^b	10.9 ^b	10.4 ^b	0.61	*	ns	ns
b*	2.9	1.1	1.9	1.0	0.48	ns	ns	ns
<i>Semimembranosus</i>								
L*	53.8 ^b	51.0 ^{ab}	49.3 ^a	50.2 ^{ab}	0.72	*	ns	ns
a*	6.5 ^a	9.0 ^b	10.5 ^b	8.8 ^b	0.41	**	**	ns
b*	-2.2 ^a	-0.7 ^a	2.0 ^b	1.6 ^b	0.48	**	ns	ns
Post chill (24 h)								
<i>Longissimus dorsi</i>								
L*	49.4 ^b	46.5 ^a	49.6 ^b	47.3 ^{ab}	0.44	ns	ns	ns
a*	13.6 ^b	11.6 ^{ab}	13.6 ^b	9.9 ^a	0.46	ns	ns	**
b*	5.8 ^{ab}	4.6 ^a	5.91 ^{ab}	6.3 ^b	0.26	*	ns	**
<i>Triceps brachii</i>								
L*	51.3 ^b	51.5 ^b	47.3 ^a	48.0 ^a	0.41	**	ns	**
a*	13.1 ^b	11.1 ^a	12.0 ^a	12.7 ^{ab}	0.21	ns	**	*
b*	5.8 ^b	4.6 ^a	4.9 ^a	4.6 ^a	0.12	**	*	*
<i>Semimembranosus</i>								
L*	49.5 ^b	49.8 ^b	49.6 ^b	47.7 ^a	0.29	*	*	ns
a*	13.8 ^b	12.3 ^a	12.9 ^{ab}	13.0 ^{ab}	0.19	ns	*	ns
b*	5.7	5.3	5.2	4.7	0.25	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), * = ($P < 0.05$), ** = ($P < 0.01$), L*= lightness; 100= white, 0= black, a*= redness (-80= green, 100= red), b*= yellowness (-50= blue, 70= yellow).

Table 5.14 Effects of 75 % fermented cassava pulp by *S. cerevisiae* replacement soybean meal in concentrate diets on carcass quality and carcass composition.

Items	Feeding levels (% BW)				SEM	Contrast ¹		
	1.0	1.5	2.0	2.5		L	Q	C
Drip loss (%)	6.2	5.9	5.7	5.9	0.15	ns	ns	ns
Water holding capacity (%)	4.1 ^b	3.9 ^{ab}	3.2 ^{ab}	2.9 ^a	0.22	*	ns	ns
Carcass composition								
OM (%)								
<i>Longissimus dorsi</i>	97.5	97.6	97.6	95.4	0.47	ns	ns	ns
<i>Tricep brachii</i>	97.6	97.7	97.8	96.7	0.19	ns	ns	ns
<i>Semimembranosus</i>	97.6	97.8	97.6	96.7	0.24	ns	ns	ns
CP (%)								
<i>Longissimus dorsi</i>	21.6	21.6	18.3	18.5	1.14	ns	ns	ns
<i>Tricep brachii</i>	20.6 ^{ab}	19.2 ^a	19.6 ^a	23.3 ^b	1.15	ns	*	ns
<i>Semimembranosus</i>	20.1	22.9	21.3	22.5	0.67	ns	ns	ns
EE (%)								
<i>Longissimus dorsi</i>	0.1 ^a	0.1 ^a	0.4 ^b	0.2 ^b	0.04	*	*	*
<i>Tricep brachii</i>	0.1	0.2	0.3	0.3	0.03	ns	ns	ns
<i>Semimembranosus</i>	0.2	0.1	0.3	0.2	0.03	ns	ns	ns

^{a, b, *} Value on the same row under each main effect with different superscripts differ significantly ($P < 0.05$) ¹contrast effect (L=Linear, Q=Quadratic and C=Cubic), SEM= Standard error of means, ns=not significantly different ($P > 0.05$), * = ($P < 0.05$).

5.6 Conclusions

The results obtained from this experiment could have a great impact on animal feed especially using local resources-based diets. The present results indicate that using 75% cassava pulp fermentation by *S. cerevisiae* replacement soybean meal in concentrate feeding level at 2.0-2.5% BW does beneficial to meat goats in terms of feed intake and ADG.

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CHAPTER VI

OVERALL DISCUSSION AND CONCLUSIONS

This thesis presents three experiments that studied utilization of cassava pulp fermented by yeast (*Saccharomyces cerevisiae*) in diet formulation for meat goats. In addition, 75% fermented cassava pulp by yeast (*S. cerevisiae*) used that replacement protein of soybean meal on ruminal fermentation, nutrient digestibility, and carcass in growing meat goats. The present studies had successfully.

The objective of first study was to develop the simple and reliable methodology of fermented cassava pulp by *S. cerevisiae* (FCSC) on protein enrichment an alternative for feedstuff. At present, many developing countries have been severely suffered by global economic depression that affects the cost increase of raw material in many industries. Consequently, it causes the higher product price throughout the world. The animal feed manufacturing is one of above mentioned industries which get impacted from the economic declination because most essential raw materials are imported (Krongtaew and Srinophakun, 2004). In Thailand, cassava starch is a large and growing industry with about 10 million tons of fresh cassava roots used for the production of starch, generating at least 1 million tons of pulp annually (Sriroth, 1994). The main application for the large quantities of waste material produced each year, after drying, is as a low value animal feed or fertilizer (Sriroth, Chollakup, Chotineeranat, Piyachomkwan and Oates, 2000). There are many attempts to reduce cost of feeds through the utilization of cheap raw materials, such as agro-industrial by-products. Although cassava pulp, the residue obtained after the extraction of starch

from cassava roots, is low in crude protein (CP), it is present in a considerable amount (Suksombat, Lounglawan and Noosen, 2006). The major limitation in the use of cassava pulp for animal feeding is its low protein content. Because of the low protein of cassava products, their use in animal feeding usually requires the supplementation of such diets. Protein enrichment of cassava through less expensive means is therefore desirable. Yeast and Fungal fermentation has been identified as an inexpensive tool for increasing the protein level of substrates in solid state. The yeast *S. cerevisiae* demonstrated the best ability to enrich the cassava peels (Iyayi and Losel, 2001; Kaewwongsa, Paengkoum, Wachirapakorn, and Yuangklang, 2009; Kaewwongsa, Traiyakun, Yuangklang, Wachirapakorn, and Paengkoum, 2011). In these present studies, was prepared and the crude protein of cassava pulp was increase from 3.3 to 31.6% CP in the fermented cassava pulp with 5% *S. cerevisiae* at 5 days period. According to Wanapat, Polyorach, Chanthakhoun, and Sornsongnern (2011) reports that yeast products are beneficial by enhancing dry matter intake and overall animal performance in ruminants (Denvev et al., 2007). Boonnop, Wanapat, Nontaso, and Wanapat (2009); Polyorach, Wanapat, and Sornsongnern (2010) reported that yeast-fermented cassava chip (YEFECAP) was prepared and the crude protein of cassava was increased from 3.4 to 32.5% CP in the YEFECAP. Wainright (1992) and Abu (1997) who reported similar findings using sweet potato in solid state fermentation, fermentation of cereals leads to improvement in protein content. The author reported that fermenting corn meal with the yeast *S. cerevisiae* and *Candida tropicalis* increased the protein content from 7.7 to 8.9% CP. The period between 0-15 days represents the period when the growth of the microorganism is most vigorous. Beyond this period, the microorganism very quickly use up the materials in the medium and

growth is showed down. Adding boosters like malt extract as suggested by Wainright (1992) or molasses at the initial stage can ensure a further increase in protein content of the material being enriched. In study of Jintanawit, Juttupornpong, Markranit, Srimongcholngam and Viwatwongwana (2006) found that ensilaging increased population of lactic acid bacteria and yeasts to maximum at 3 and 5 days after the ensilaging, respectively and then both microbial population decreased until day 28 of ensilaging. An experiment was conducted to evaluate the effect of fermentation on the proximate composition of FCSC for use in composite meat goat feed formulations. In the current study agrees with Oduguwa, Edema, and Ayeni (2007) reported that increase in protein content of the fermented products may be as a result of microbial cell biomass. Moreover, fermentation generally reduced crude fibre content in crop residues especially when fermented with fungi because they possess ability to produce cellulase that degrade ligno-cellulose fibre.

Mašek et al. (2008) explain the effects of yeast in the rumen indicate that supplementation of yeast in the ruminant diet may improve feed intake and an increase rumen content outflow rate by improving the fermentation in the rumen (Williams, Tait, Innes, and Newbold, 1991; Cole, Purdy, and Hutcheson, 1992; Erasmus, Botha, and Kistner, 1992; Robinson and Garrett 1999; Abd El-Ghani, 2004), weight gain (Salamana, Caja, Garin, Albanell, Such, and Casals, 2002), digestion (Wohlt, Finkelstein, and Chung 1991; Jouany, Mathieu, Senaud, Bohatier, Bertin, and Mercier, 1998; Payandeh, and Kafilzadeh, 2007), numbers of anaerobic and cellulolytic bacteria (Newbold, Wallace, Chen, McIntosh, 1995), ruminal pH value (Doreau and Jouany, 1998; Jouany et al., 1998). Similarly, Newbold, Wallace, and McIntosh (1996) demonstrated that Brewer's yeast strains and Baker's yeast strains differed in their

abilities to stimulate critical groups of ruminal microorganisms. Baker's yeast strains had limited ability to bring about stimulation. These studies suggest that care must be taken in selecting *S. cerevisiae* strains for use in yeast culture preparations for ruminants. Such studies also explain some of the variability in production responses, since many of the early studies relied on poorly defined yeast culture supplements and may have used strains with little stimulatory activity (Denvev et al., 2007).

The effects of yeast on fiber digestion were highly variable, with some authors recording increases on the fiber digestion of low quality forages but not recorded any increasing effect. (Gonzalez, Garcia-Bojalil, Mendoza, and Barcena, 1995; Hadjipanayiotou, Antoniou, and Photiou, 1997; Avendano, Enjalbert, Garrett, Moncoulon, Bayourthe, and Chicoteau 1999; Lynch and Martin, 2002) Newbold, Williams, Mckaln, Walker, and Wallacc (1990) showed a possible advantage in using *Aspergillus oryzae* fermentation extract and *Saccharomyces cerevisiae* culture stimulated fiber digestion by ruminal microorganisms. It is assumed that, the yeast supplement may provides factors stimulatory toward proteolytic bacteria. The increase in proteolytic bacteria is more, when high concentrate diets are fed (Williams, Tait, Innes, and Newbold, 1991). Sullivan and Martin (1999) reported that the supplement of a *S. cerevisiae* yeast culture into the diet of dairy cows improved the utilization of lactate and digestion of cellulose. Martin and Nisbet (1992) reported that data observation concurs with the finding that yeast in diets promotes lactate utilization in the rumen.

The optimum ammonia concentration may be defined as that which results in the maximum rate of fermentation or which allows the maximum production of microbial protein per unit of substrate fermented (Mehrez, Ørskov, and McDonald,

1977). Normally, microbial activity, 5-7 mg (Satter and Slyter, 1974) and for maximum nutrient utilization 15-20 mg NH₃-N per 100 ml rumen liquor is required (Perdrix and Leng, 1989). Weisbjerg, Hvelplund, Kristensen, and Stensig (1998) reported that positive effects on microbial activity were obtained with NH₃-N between 10-15 mg/100 ml rumen fluid. Erdaman, Proctor, and Vandersall (1986) and Ørskov (1994) documented that with NH₃-N concentration higher than 24 mg/100 ml, more nitrogen is lost from the rumen and urine. Mutsvangwa, Edwards, Topps, and Paterson (1992) reported higher acetate in animals receiving yeast. Supplementation with yeast culture did not alter carcass characteristics (Mir and Mir, 1994).

From this study it can be concluded that cassava pulp fermentation by *S. cerevisiae* could be improved CP content from 3 to 31.64%. It suggests that FCSC can be replaced the protein source from SBM up to 75% in concentrate feed which compose of rice straw as roughage in growing goats. The results indicate that using 75% FCSC replacement soybean meal in concentrate feeding level at 2.0-2.5% BW.

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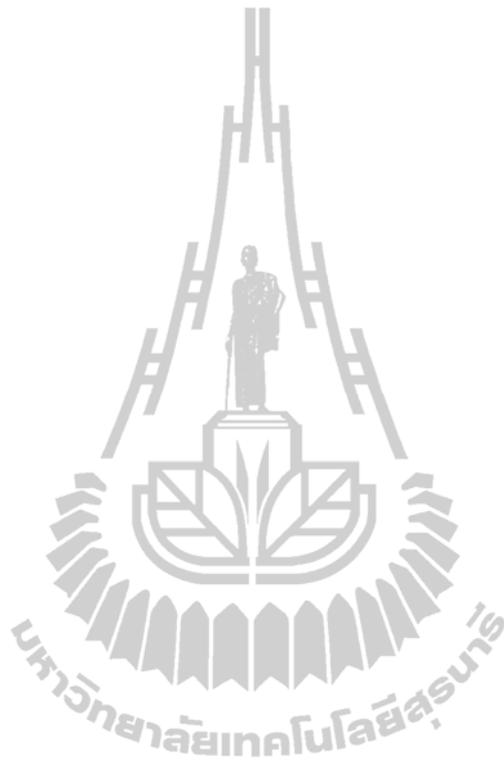
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Determination of plasma urea nitrogen (PUN)

Plasma urea nitrogen (PUN) was determined using a Spectronic R Genesys 5 Spectrophotometer. The principle of plasma urea nitrogen (PUN) determination (Adapted from Preston, Schnakenberg Andw, and Pfander, 1964).

The brief progress for determination of PUN would be shown as followed.

Preparation of reagents:

1) Stock ferric chloride-phosphoric acid reagent

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 15 g
 DI water 30 ml

} + H_3PO_4 (85%) 300 ml, mixed evenly, adjusted to 450 ml with DI water and kept in brown bottle.

2) Acid reagent (preparation should be done shortly before use)

H_3PO_4 (85%) 150 ml + DI water 500 ml + Stock ferric chloride-phosphoric acid reagent 1 ml, mixed evenly and adjusted to 1000 ml with DI water.

3) Color reagent

Diacetyl monoxime 1.7 g
 Thiosemicarbazide 0.3 g
 DI water 100 ml

} mixed evenly and adjusted to 1000 ml with DI water, subsequently filtrated through waterman filter paper and kept in brown bottle.

4) Stock PUN standard (mg/dl)

Urea 214.2 mg + 0.1N HCl 100 ml, mixed evenly and kept in brown bottle at 4°C.

5) Analyzing

The standard was run in triplicate and adopting none but the r_2 over 0.98. The samples and reagents were measured into a 16 x 125 mm² test tube with pipette, and the tubes were closed tightly with a screw cap that with butyl rubber lining, following mixed evenly, supervening by boiling at 80°C until the color changed into pink and cooled them down to normal room temperature in cool water. Within 15 minutes after the preparation, the determination at 540-nanometer wavelength was done with employing of the blank to adjust the spectrophotometer to zero prior to it.

Determination of purine derivatives

The purine derivatives allantoin, uric acid, hypoxanthine and xanthine were analyzed by reverse-phase High Performance Liquid Chromatography (HPLC), which consisted of a multi-solvent delivery system Model 600 E (Waters, USA), an injector Model 712, a multi-wavelength detector Model 490E, set to 205 nm, and a double 4.6×250 mm², C-18 reverse-phase column, according to the technique of Balcells, Guada, and Peiro (1992). The supply of microbial N was then calculated from P using the following factors: digestibility of microbial purines 0.83 and purine-N:total microbial N ratio of 0.116:1.00 (Chen, Chen, Franklin, Ørskov, and Shand, 1992):

$$Y = 0.83X + 0.202 \times BW^{0.75},$$

Where, Y = PD excretion in the urine (mmol/d)

X = PD absorption at small intestine (mmol/d)

BW^{0.75} = Metabolic body weight (kg)

$$\text{Microbial N supply (g/day)} = \frac{(\text{PD} \times 70)}{(0.83 \times 0.116 \times 1,000)} = 0.727 \times X$$

Where, PD = the corresponding amount of microbial purines absorbed (mmol/day)

X = PD absorption at small intestine (mmol/d)

Thus the microbial nitrogen based on total PD (MN_{pd}) can be calculated as follows.



BIOGRAPHY

Walailuck Kaewwongsa was born on December 27th, 1972 in Udonthani Province, Thailand. She finished high school from Kumpawapi School, Udonthani. She graduated Bachelor of Science (Animal Science) from Rajamangala Institute Technology of Bangpra (Surin) in 1993. She received Master of Science in Animal Science from Khon Kaen University in the year 1995. She is now a lecturer at Faculty of Technology, Udon Thani Rajabhat University, Thailand since. In 2005, she was continuing to study for her Doctor of Philosophy in Animal Production Technology, She enrolled at Suranaree University of Technology study Ruminant Nutrition in the School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.