EFFECTS OF CASPUREA AS CRUDE PROTEIN SOURCES ON PERFORMANCE OF THAI NATIVE x BRAHMAN CROSSBRED BEEF CATTLE

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ผลของการใช้แคสพูเรียเพื่อเป็นแหล่งโปรตีนหยาบ ต่อสมรรถนะการผลิตโคเนื้อลูกผสมบราห์มันพื้นเมือง

นายคณิน บรรณกิจ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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คณิน บรรณกิจ : ผลของการใช้แคสพูเรียเพื่อเป็นแหล่งโปรตีนหยาบ ต่อสมรรถนะการผลิตโคเนื้อลูกผสมบราห์มันพื้นเมือง (EFFECTS OF CASPUREA AS CRUDE PROTEIN SOURCES ON PERFORMANCE OF THAI NATIVE x BRAHMAN CROSSBRED BEEF CATTLE) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 210 หน้า.

วัตถุประสงค์ของการศึกษานี้เพื่อศึกษาผลของการแปรรูปโดยการเอ็กซ์ทรูดส่วนผสมระหว่างกากมันสำปะหลังและยูเรีย (แคสพูเรีย) เพื่อเป็นแหล่งโปรตีนหยาบในสูตรอาหารสำหรับโคเนื้อลูกผสมบราห์มันพื้นเมือง โดยแบ่งเป็น 4 การทดลอง ดังนี้

การทดลองที่ 1 เพื่อทำการศึกษาระดับการทดแทนกากถั่วเหลืองด้วยแคสพูเรีย (45% CP) ในสูตรอาหารข้นที่ระดับ 0, 25, 50 และ 75% สัตว์ทดลอง คือโคบราห์มัน-พื้นเมืองรุ่นเพศผู้ จำนวน 4 ตัว (อายุประมาณ 1 ปี น้ำหนัก เฉลี่ย 154.7±26.8 กิโลกรัม) ตามแผนการทดลอง แบบ 4 x 4 Latin square design ผลการทดลอง พบว่า ปริมาณการกินได้วัตถุแห้งทั้งหมด (P>0.05) ไม่มีผลกระทบเนื่องจากระดับแคสพูเรียที่เพิ่มขึ้น ส่วนความสามารถในการย่อยได้วัตถุแห้ง ความเข้มข้นของกรดไขมันระเหยง่ายทั้งหมด พบว่าลดลงทั้งแบบเส้นตรง (linearly, P<0.01) และแบบเส้นโค้ง (quadratically, P<0.01) เมื่อระดับแคสพูเรียเพิ่มขึ้น นอกจากนี้ ความเข้มข้นแอมโมเนีย-ไนโตรเจนในกระเพาะหมัก ความเข้มข้นยูเรียในกระแสเลือด เพิ่มขึ้นแบบเส้นตรง (linearly, P<0.01) ตามระดับที่เพิ่มขึ้นของแคสพูเรีย ส่วนปริมาณแบคทีเรีย และปริมาณโปรโตซัว ลดลงในแบบเส้นตรง (linearly, P<0.01) ปริมาณการดูดซึมของไนโตรเจน (g/d) ลดลงแบบเส้นตรง (linearly, P<0.01) ส่วนปริมาณการเก็บกักของไนโตรเจน (%N intake) พบว่ามีแนวโน้มลดลง (linearly, P=0.08) เนื่องจากปริมาณการทดแทนกากถั่วเหลืองด้วยแคสพูเรียที่เพิ่มขึ้น ดังนั้นจึงสามารถสรุปได้ว่าแคสพูเรียสามารถทดแทนกากถั่วเหลืองได้ที่ระดับ 50% โดยไม่กระทบต่อประสิทธิภาพการผลิต

การทดลองที่ 2 ศึกษาระดับทดแทนโปรตีนหยาบทั้งหมดในสูตรอาหารข้นด้วย Caspurea (45% CP) ในโคบราห์มัน-พื้นเมืองรุ่นจำนวน 12 ตัว น้ำหนักเฉลี่ย 200±36 กิโลกรัม อายุประมาณ 1 ปี ได้ทำการแบ่งกลุ่มออกเป็น 4 กลุ่ม ตามแผนการทดลอง แบบ Randomized complete block design (RCBD) ก่อนทำการสุ่มให้ได้รับอาหารทดลอง ที่มีระดับการทดแทนโปรตีนหยาบทั้งหมดในสูตรอาหารข้น ด้วย Caspurea ที่ระดับ 0, 35 และ 70% ผลการทดลอง พบว่า ปริมาณการกินได้วัตถุแห้งทั้งหมด ไม่มีความแตกต่างกันทางสถิติ ส่วนความสามารถในการย่อยได้วัตถุแห้ง และความสามารถในการย่อยได้อินทรีย์วัตถุ ลดลงทั้งแบบเส้นตรง (linearly, P<0.05) และแบบเส้นโค้ง (quadratically, P<0.05) ตามระดับการทดแทนที่เพิ่มขึ้นของแคสพูเรีย ความเข้มข้นแอมโมเนีย-ไนโตรเจนในกระเพาะหมัก และ ความเข้มข้นยูเรียในกระแสเลือด เพิ่มแบบเส้นตรง (linearly, P <0.01) ส่วนความเข้มข้นกรดไขมันระเหยง่ายทั้งหมดในกระเพาะหมัก ลดลงแบบเส้นโค้ง

(quadratically, $P<0.05$) ปริมาณแบคทีเรีย และปริมาณโปรโตซัวลดลงแบบเส้นตรง (linearly, $P<0.01$) ความเข้มข้นยูเรียในกระแสเลือด เพิ่มแบบเส้นตรง (linearly, $P<0.01$) ที่ 0 ชั่วโมง อัตราการเจริญเติบโตเฉลี่ยต่อวันลดลงทั้งในแบบเส้นตรง (linearly, $P<0.01$) และแบบเส้นโค้ง (quadratically, $P<0.01$) ตามระดับของโปรตีนจากแคสพูเรียที่เพิ่มขึ้น ดังนั้นจึงสามารถสรุปได้ว่าแคสพูเรียสามารถทดแทนโปรตีนหยาบทั้งหมดในอาหารข้นได้ที่ระดับ 35% โดยไม่กระทบต่อประสิทธิภาพการผลิต

การทดลองที่ 3 ศึกษาการใช้ปริมาณโปรตีนจากแคสพูเรียที่ระดับ 35% ของโปรตีนหยาบทั้งหมดในสูตรอาหารข้นโดยอาหารข้นมีระดับของโปรตีนที่ไม่ถูกย่อยสลายในกระเพาะหมัก (ruminal undegradable protein, RUP) แตกต่างกันที่ระดับ 30, 35, 40 และ 45% สัตว์ทดลอง คือ โคบราห์มัน-พื้นเมืองรุ่นเพศผู้ จำนวน 4 ตัว (อายุประมาณ 1 ปี น้ำหนัก เฉลี่ย 175.5±18.6กิโลกรัม) ตามแผนการทดลอง แบบ 4 x 4 Latin square design รอบละ 21 วัน ผลการทดลอง พบว่า ปริมาณการกินได้วัตถุแห้งทั้งหมด และความสามารถในการย่อยได้วัตถุแห้ง เพิ่มแบบเส้นตรง (linearly, $P<0.05$) ตามระดับของ RUP ที่เพิ่มขึ้น ความเข้มข้นแอมโมเนีย-ไนโตรเจนในกระเพาะหมัก ลดลงแบบเส้นตรง (linearly, $P<0.01$) ตามระดับของ RUP ที่เพิ่มขึ้น แต่มีความเข้มข้นเพิ่มขึ้นเมื่อระดับ RUP เพิ่มเป็น 45% (quadratically, $P<0.01$) ในขณะที่ความเข้มข้นของกรดไขมันระเหยง่ายทั้งหมดในกระเพาะหมัก เพิ่มแบบเส้นตรง (linearly, $P<0.01$) แต่มีความเข้มข้นลดลงเมื่อระดับ RUP เพิ่มเป็น 45% (quadratically, $P<0.01$) นอกจากนี้ปริมาณแบคทีเรีย และปริมาณโปรโตซัว เพิ่มขึ้นเมื่อระดับ RUP เพิ่มเป็น 40% แต่เมื่อ RUP เพิ่มเป็น 45% พบว่ามีปริมาณลดลง (quadratically, $P<0.05$) แต่ปริมาณเก็บกักไนโตรเจนมีแนวโน้มเพิ่มขึ้นเมื่อระดับ RUP เพิ่มเป็น 40% แต่เมื่อ RUP เพิ่มเป็น 45% พบว่ามีแนวโน้มลดลง (quadratically, $P=0.1$) ส่วนความเข้มข้นยูเรียในกระแสเลือด ที่ 3 และ 6 ชั่วโมงหลังให้อาหารพบว่า ลดลงตามระดับของ RUP ที่เพิ่มขึ้น แต่มีความเข้มข้นเพิ่มขึ้นเมื่อระดับ RUP เพิ่มเป็น 45% (quadratically, $P<0.05$) ดังนั้นจึงสามารถสรุปได้ว่าระดับ RUP ที่ระดับ 40% สัตว์มีแนวโน้มประสิทธิภาพการผลิตเพิ่มขึ้น

การทดลองที่ 4 เพื่อทำการศึกษาผลของแคสพูเรียที่อัดเม็ดร่วมกับแหล่งโปรตีนจากพืชที่แตกต่างกันต่อประสิทธิภาพการผลิต สัตว์ทดลอง คือ โคบราห์มัน-พื้นเมืองรุ่นเพศผู้และเพศเมีย จำนวน 8 ตัว (อายุประมาณ 1 ปี น้ำหนัก เฉลี่ย 185.5±24.4 กิโลกรัม) ตามแผนการทดลอง แบบ double 4 x 4 Latin square design รอบละ 21 วัน ก่อนทำการสุ่มให้ได้รับอาหารทดลอง ที่แคสพูเรียมีส่วนผสมแหล่งโปรตีนจากพืชที่แตกต่างกัน 4 ชนิด คือ 1) แคสพูเรียปกติ (กลุ่มควบคุม) 2) แคสพูเรีย+ใบกระถิน 3) แคสพูเรีย+ใบมันสำปะหลัง 4) แคสพูเรีย+กากถั่วเหลือง ซึ่งทุกแบบมีระดับโปรตีนเท่ากัน คือ 45% CP ระดับ RUP 40% ผลการทดลอง พบว่า ปริมาณการกินได้ทั้งหมด ความสามารถในการย่อยได้วัตถุแห้ง กรดไขมันระเหยง่ายทั้งหมด ปริมาณแบคทีเรีย และปริมาณโปรโตซัวในกระเพาะหมักไม่มีความแตกต่างต่างกันทางสถิติ ส่วนความเข้มข้นของแอมโมเนีย-

ไนโตรเจนในกระเพาะหมัก ($P<0.05$) มีค่าต่ำสุด ในกลุ่มแคสพูเรีย+กากถั่วเหลือง ($P<0.05$) ปริมาณการดูดซึมของไนโตรเจน และปริมาณเก็บกักไนโตรเจน ไม่มีความแตกต่างกันทางสถิติ อย่างไรก็ตาม ความเข้มข้นยูเรียในกระแสเลือดในกลุ่มแคสพูเรียปกติมีค่าสูงที่สุด ($P<0.05$) ดังนั้นจึงสามารถสรุปได้ว่าแคสพูเรียที่มีส่วนผสมแหล่งโปรตีนจากพืช สัตว์มีแนวโน้มประสิทธิภาพการผลิตเพิ่มขึ้น

ดังนั้นจากทั้ง 4 การทดลองสามารถสรุปได้ว่าแคสพูเรีย (45% CP) สามารถทดแทนกากถั่วเหลืองได้ 50% และทดแทนโปรตีนหยาบทั้งหมดในสูตรอาหารข้นได้ 35% โดยคำนวณให้สูตรอาหารมีระดับ RUP 40% รวมถึงการปรับปรุงคุณภาพโปรตีนและความน่ากินของแคสพูเรียโดยอัดเม็ดร่วมกับแหล่งโปรตีนจากพืชคือ ใบมันสำปะหลัง และใบกระถินสามารถเพิ่มความสามารถในการย่อยได้ ผลผลิตสุดท้ายจากกระบวนการหมัก ประสิทธิภาพกระบวนการหมัก และประสิทธิภาพการผลิตของสัตว์ได้

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

KANIN BUNNAKIT : EFFECTS OF CASPUREA AS CRUDE PROTEIN SOURCES ON PERFORMANCE OF THAI NATIVE x BRAHMAN CROSSBRED BEEF CATTLE. THESIS ADVISOR : ASST. PROF. PRAMOTE PAENGKOUM, Ph.D., 210 PP.

CASSAVA PULP / UREA / CASPUREA / STAREA / BEEF CATTLE

The aim of this study is to examine the effects of Caspurea on productive performance of Thai Native x Brahman beef cattle. This research includes 4 experiments.

Experiment 1 : Four yearling Thai Native x Brahman beef cattle with an average body weight (BW) of 154.7±26.8 kg were used in a 4 x 4 Latin square arrangement. The treatments were the four levels of Caspurea replacement for soybean meal in concentrate at 0, 25, 50 and 75%. The results showed that the dry matter (DM) digestibility, total volatile fatty acids (TVFA), bacteria and protozoa populations decreased linearly ($P<0.01$) while ruminal NH_3-N and blood urea nitrogen (BUN) concentration increased linearly ($P<0.01$) and quadratically ($P<0.01$) with the increasing levels of Caspurea. The nitrogen (N) retention tended to increase in 50% replacement diet ($P=0.05$), whereas that in 0, 25 and 50% replacement diet treatments was not different. It could be concluded that Caspurea could replace 50% of soybean meal in the diet without any negative effect on productive performances.

Experiment 2 : Twelve yearling Thai Native x Brahman beef cattle with an average body weight (BW) of 200±36 kg were used in a randomized complete block design (RCBD). The treatments were the three levels of crude protein from Caspurea replacement for total crude protein in concentrate at 0, 35 and 70%. The

results showed that increasing levels of CP replacement from Caspurea caused decrease in DM and OM digestibility linearly ($P<0.05$) and quadratically ($P<0.05$). With increasing the level of CP replacement from Caspurea, the ruminal NH_3-N and BUN concentrations increased ($P<0.05$) while the TVFA decreased (quadratically, $P<0.05$). The bacteria and protozoa populations also decreased linearly ($P<0.01$) as the level of CP replacement from Caspurea increased. Average daily gain (ADG) decreased linearly ($P<0.01$) as the level of CP replacement from Caspurea increased. It could be concluded that crude protein (CP) from Caspurea could replace 35% of the total crude protein in concentrate without any negative effect on productive performances.

Experiment 3 : Four yearling Thai Native x Brahman beef cattle with an average body weight (BW) of 175.5±18.6 kg were used in a 4 x 4 Latin square arrangement. The treatments were the four levels of rumen undegradable protein (RUP) in concentrate at 30, 35, 40 and 45%. The results showed that the DM intake, OM digestibility and TVFA increased linearly ($P<0.05$) while the level of RUP increased. Moreover, the ruminal NH_3-N ($P<0.01$) and BUN linearly decreased, whereas at 45% RUP the ruminal NH_3-N concentration increased (quadratically, $P<0.01$). The bacteria and protozoa populations also increased as the level of RUP increased. However, the bacteria and protozoa populations decreased quadratically ($P<0.05$) when the level of RUP changed from 40 to 45%. The N retention (g/d) tended to increase with increasing the level of RUP. However, the N retention tended to decrease at the level of 45% RUP. It could be concluded that RUP level at 40% in concentrate had positive effects on productive performances.

Experiment 4 : Eight yearling Thai Native x Brahman beef cattle with an average BW weight of 195.5±24.4 kg were used in double 4 x 4 Latin square

arrangement. The treatments were Caspurea with different plant protein sources (extrusion-processed mixture of cassava pulp and urea with plant protein) as follows : 1) normal Caspurea (Control), 2) Caspurea+leucaena leaf, 3) Caspurea+cassava leaf, 4) Caspurea+soybean meal. The results showed that the DM intake, DM digestibility, TVFA, bacteria and protozoa populations were not affected by those plant protein sources ($P>0.05$). The ruminal NH_3-N and BUN concentrations of beef fed on Caspurea+soybean meal were lower ($P<0.05$) than those of other treatments.

In conclusion, the results from the four experiments indicated that 50% of the replacement diet with Caspurea for SBM or 35% of the replacement diet from Caspurea for total CP in concentrate did not affect productive performances. In addition, the diets containing 40% RUP improved digestibility, rumen fermentation, N balance and BW change. Moreover, an extrusion-processed mixture of Caspurea with the plant protein sources had positive effects on improving the performance of Thai Native x Brahman beef cattle.

School of Animal Production Technology

Academic Year 2007

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Co-advisor's Signature

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Kanin Bunnakit

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LIST OF ABBREVIATIONS

AA	=	Amino acid
a	=	Rapidly soluble fraction, the intercept
ADF	=	Acid detergent fiber
b	=	Potentially degradable fraction, the fermentation of the insoluble fraction
BUN	=	Blood urea nitrogen
BW	=	Body weight
c	=	Rate of degradation of *b* fraction, rate of gas production
CH_4	=	Methane
CO_2	=	Carbon dioxide
CP	=	Crude protein
DAPA	=	Diamino pimelic acid
DM	=	Dry matter
G	=	Gram
H	=	Hour
Kg	=	Kilogram
L	=	Liter
LL	=	Leucaena leaf
M	=	Molar
Mg	=	Milligram
Ml	=	Milliter

LIST OF ABBREVIATIONS (Continued)

mM	=	Millimolar
N	=	Nitrogen
NDF	=	Neutral detergent fiber
NH_3-N	=	Ammonia nitrogen
OM	=	Organic matter
P	=	Probability
PEG	=	Polyethylene glycol
RDP	=	Ruminal degradable protein
RUP	=	Ruminal undegradable protein
SEM	=	Standrad error of means
SBM	=	Soybean meal
t	=	Time
TVFAs	=	Total volatile fatty acid
y	=	gas production at *t*

CHAPTER I

INTRODUCTION

1.1 Rationable of the Study

Urea has been utilized as a supplemental nitrogen source for beef cattle rations when natural protein supplements are expensive. However, urea is utilized less efficiently than natural plant proteins such as soybean meal for beef cattle performance. Utilization of urea rations for milk production has been improved by use of a mixture of gelatinized starch and urea, processed through an extruder cooker. Rumen ammonia concentrations were reduced when this mixture was incubated with rumen microorganisms (Helmer et al., 1970). Reduced consumption of high urea rations and loss of dietary nitrogen resulting from rapid hydrolysis in the rumen have prompted researchers to seek new means of improving NPN utilization in ruminant rations (Thompson et al., 1972). Bartley et al. (1968) have attempted to reduce the solubility and to improve the acceptance of urea by cooking a grain-urea mixture at high temperatures. *In vitro* and that the product (Starea) equals soybean meal (SBM) as a nitrogen supplement for ruminants. However, problems in feeding urea to beef cattle are intensified by emphasis on high-concentrate rations to increase production and to provide necessary energy. A study with dairy cows receiving various levels of urea in a high concentrate ration showed that high-urea levels (20-40% of the total ration nitrogen) depressed both milk yield and efficiency (Huber and Sandy, 1965).

Nitrogen balance studies suggested that milk production efficiency decreased with added urea because of decreased nitrogen retention (Huber et al., 1967). Moreover, palatability of concentrate mixtures containing urea lessened with higher feed intake (Van Horn et al., 1967). Previous studies with rumen-fistulated cattle (Stiles et al., 1970) indicated that Starea, an intimate mixture of gelatinized starch and urea, improved utilization of urea nitrogen and palatability of urea-containing rations. Studies with an expansion-processed mixture of grain starch and urea (Starea) indicated that ammonia was released slower from this product both *in vivo* and in *vitro* than when a non-heated control mixture was used and, additionally, this product was superior to urea as a nitrogen supplement for growing-fattening cattle (Deyoe et al., 1968; Helmer et al., 1970). Reported here is work to determine if Starea has a similar effect on urea utilization and feed intake of growing-fattening cattle. Starea was compared with soybean meal or as a protein natural plant proteins supplement.

The purpose of this study was to determine the effect of supplemental nitrogen in the form of Caspurea [extrusion-processed mixture of cassava pulp starch and urea (Starea)] on performance of Thai Native x Brahman crossbred beef cattle.

1.2 Research objectives

1.2.1 To study the effect of Caspurea as a protein source replacement for soybean meal on productive performance of Thai Native x Brahman crossbred beef cattle.

1.2.2 To study the effect of Caspurea as a protein source replacement for the total dietary protein on productive performance of Thai Native x Brahman crossbred beef cattle.

1.2.3 To study the effect of Caspurea as a protein source with rumen undegradable protein levels on performance of Thai Native x Brahman beef cattle.

1.2.4 To study the effect of an extrusion-processed mixture of cassava pulp and urea with natural plant protein and pelleting at high temperatures (Caspurea/Starea) as a protein source on performance of Thai Native x Brahman beef cattle.

1.3 Research hypothesis

1.3.1 Caspurea used as a protein source did not affect productive performance of Thai Native x Brahman crossbred beef cattle.

1.3.2 Caspurea used as a protein source replacement for the total dietary protein did not affect productive performance of Thai Native x Brahman crossbred beef cattle.

1.3.3 Utilization of Caspurea for beef production has been improved by the rate of degradation of rumen undegradable protein.

1.3.4 Utilization of Caspurea has been improved by extrusion-processed mixture with natural plant protein.

1.4 Scope and limitation of the study

Thai Native x Brahman beef cattle from Suranaree University's dairy farm and small-holder farmers in Phuwieng, Khon Kaen province, were used in the studies of the effect of Caspurea as a protein source on feed intake, blood metabolites, ruminal fermentation, digestibility, microbial protein synthesis and growth rate of Thai Native x Brahman beef cattle.

1.5 Expected results

1.5.1 To obtain the effects of Caspurea as a protein source replacement for soybean meal on feed intake, blood metabolites, ruminal fermentation, digestibility, microbial protein synthesis and growth rate of Thai Native x Brahman beef cattle.

1.5.2 To obtain the effects of Caspurea as a protein source replacement for the total dietary protein on performance of Thai Brahman x Native crossbred beef cattle, and to know maximizing NPN use in feeding systems based on agro-industrial by-products.

1.5.3 To obtain the effects of Caspurea as a protein source with rumen undegradable protein levels on productive performance of Thai Native x Brahman beef cattle.

1.5.4 To obtain the effects of an extrusion-processed mixture of cassava pulp and urea with natural plant protein and pelleting at high temperatures (Caspurea/Starea) as a protein source on performance of Thai Native x Brahman beef cattle.

1.6 References

Bartley, E.E., Deyoe, C. W., Pfost, H. B., Anstaett, F. R., and Sung, A. C. (1968). An improved urea product for ruminants. **Anim. Nutri. and Health.** May: 10-13.

Deyoe, C. W., Bartley, E. E., Pfost, H. B., Boren, F. W. B., Perry, H. B., Anstaett, F. R., Helmer, L., Stiles, D., Sung, A. C., and Meyer, R. (1968). An improved urea product for ruminants. (Abstr.). **J. Anim. Sci.** 27: 1163.

Helmer, L. G., Bartley, E. E., and Dyoe, C. W. (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Huber, J. T., and Sandy, R. A. (1965). Response of dairy cows fed unlimited corn silage to three levels of urea and grain. (Abstr.) **J. Anim. Sci.** 24 : 887.

Huber, J. T., Sandy, R. A., Polan C. E., Bryant, C. T., and Blaser, R. E. (1967). Varying levels of urea for dairy cows fed corn silage as the only forage. **J. Dairy Sci.** 50 : 1241-1246.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Van Horn, H. H., Foreman, C. F., and Rodriguez, J. D. (1967). Effect of high-urea supplementation on feed intake and milk production of dairy cows. **J. Dairy Sci.** 50 : 709-716.

CHAPTER II

REVIEW OF THE LITERATURE

2.1 The used of crop residue and agro-industrial by product

Although the natural pastures are often not available for ruminants in the dry season, crop residues are present in large amounts and can be useful for ruminants in the dry season, especially sugarcane top (Chinh et al., 2000), rice straw (Agbagla-Dohnani et al., 2001) and corn stover (Hindrichsen et al., 2001). Crop residues have the advantage of being nutritionally valuable even in the dry season, when feeding ruminants in tropical countries is most critical (Mgheni et al., 2001). However, crop residue has some drawbacks such as high content of cell wall, low protein content and low utilization rate (Hindrichsen et al., 2001).

The deficient nutrients may critically affect the growth of rumen microbes which ferment the feed (Leng, 1997). The addition of foliage from tree leaves or supplementation with seed meals or even urea can improve the utilization of low quality roughages, mainly through the supply of nitrogen to the rumen microbes. In addition, Wanapat et al. (1997) reported that cassava hay might be a good source of protein supplement for ruminants in the dry season, particularly on a small-holder dairy farms. The abundantly available crop residues are rice straw, sugarcane tops, corn stovers, cassava leaves as well as other leguminous crop residues (Wanapat and Pimpa, 1999). Crop residues, generally are not morphological, cytological or chemical uniform, either between different crop species and cultivars or between the residues of the same cultivars grown and harvested under different environmental conditions

(Egan, 1992). In general, crop residues especially rice straw have poor nutritive value, but its high availability as a crop by-products together with the opportunity to improve its utilization by ruminants has to led to research in this area. Its can be characteristically summarized as low digestibility, low protein content, poor palatability and bulky. There are various methods to improve feed utilization such as by physical processing e.g. grinding, chemical or biological pretreatment (Chen et al., 1996), supplement with N (Siulawa and Simuko, 2005) and fungal treatment (Poppi et al., 1995). However, chemical treatment are the most promising, especially urea treated rice straw. Agro-industrial by-product represent a vast animal feed resource, which is and yet largely unexploited (Scarr, 2005). Agro-industrial by-products are derived from processing of particular crops or animal products. Included in this category are materials like molasses and brewer's wastes (Siulapwa and Simukko, 2005). This by products can be classified as energy, protein and combined energy/protein sources (Egbunnike and Ikpi, 2005). There are several documentations on utilization of agro-industrial by product as animal feed, which included potato pulp (Hanada et al., 2004), cassava pulp (Skunmun et al., 2004), oil seed cake (Woods et al., 2003), pineapple wastes (Prachyalak et al., 1996), tomato pomance (Satchaphan et al., 1998). Recently, Nitipot and Sommart (2003) suggested that cassava starch industrial by- products (casava pulp) had the high potential to use as novel energy feed source for ruminants.

2.2 Feed evaluation

In vitro gas production technique

The association between fermentation and gas production has long been known. In an early, researcher attempted to record gas production directly via the rumen cannula in sheep. However, this technique was too difficult excute and of poor

gas production technique to evaluation nutritive value of feedstuffs (Menke et al., 1979; Menke and Steingass, 1988; Blummel and Ørskov, 1993; Tessema and Baars, 2004). *In Sacco* (Ørskov and McDonald, 1979) and *in vitro* (Tilley and Terry, 1963) methods have been employed to characterize rumen fermentation kinetic of feedstuff. However, *in sacco* technique have proved to be time consuming, expensive, needed fistulated animal (Mathis et al., 2001), in some case in accurate (Murray, 1993) and cannot be undertaken routinely (Rymer and Givens, 1998; Mathis et al., 2001). Recently, the *in vitro* gas production technique was proposed to use for determined fermrntation kinetics of ruminant feed (Menke et al., 1979; Menke and Steingass, 1988; Blummel and Ørskov, 1993). It has provided better predictions of the *in vivo* digestibility than other technique (Khazaal et al., 1993). Moreover, *in vitro* gas production method is more efficient than *in sacco* method in evaluating the effects of tannins or other anti-nutritional factors (Makkar et al., 1995b). In addition, the *in vitro* gas method can better monitor nutrient-anti-nutrient and nutrient-anti-nutrient interactions (Makkar et al., 1995b). The technique is gaining popularity because it is a low cost, highly reproducible and easy method of obtaining a dynamic description of the nutritive value of feed stuffs, while at the same time allowing for more samples to be analyzed (Herrero et al., 1996). However, several gas measuring techniques and *in vitro* gas methods are in use by several groups. The *in vitro* gas method based on syringes appears to be the most suitable for use in developing countries (Blummel et al., 1997). One of several method Menke and Steingass (1988) was modified the gas production tecnique describes the kinetics of fermentation based on the exponential model: $P=a+b(1-e^{-ct})$ of (Ørskov and McDonald, 1979), where P, describes gas production at time t, *a*, the gas produced (ml) by instantaneous fermentation of the soluble and readily available fraction of feed, *b*, the gas produced (ml) by the

fermentation of insoluble, but slowly fermentable fraction and c, the fractional rate (rate constant) at which gas is produced per hour (%/h).

Factors affecting gas measurements

The gas production technique has advantages and disadvantages (Getachew et al., 1998a). Therefore before attempting to evaluate nutritive value should consider some of factors that affect gas measurement.

Sample size and preparation

Because gas volumes are proportional to the sample size, therefore, the sample size should be suitable for this method. Raab et al. (1983) reported a highly significant linear correlation between the amount of substrate and amount of gas produce at 24 h. Schofield (2000) also reported the gas volume produced by complete digestion from g of fiber is approximately 350 ml. Digestibility measurement will therefore require the use of either small samples or equipment that is able to handle large gas volumes. In the method of Menke et al. (1979), fermentation is conducted in 100 ml capacity calibrated glass syringes containing the feed stuff (200 mg) and a buffered rumen fluid (30 ml.) In addition Blummel et al. (1997) and Makkar et al. (1995a) modified the method further by increasing the amount of sample from 200 mg to 500 mg and increased pH of incubation medium; consequently the fermentation is inhibited. If the amount of gas production exceeds 90 ml, in the 30 ml. Buffer rumen fluid system and 130 ml, in the 40 ml. Buffer rumen fluid system, the amount of feed being incubated should be reduced (Getachew et al., 1998b). Gerson et al. (1988) reported the suitable of particle should be ranged form 0.1-0.2 mm to 1.0-2.0 mm on *in vitro* gas production. Therefore substrates should be milled using a 1 mm screen to allow more

precise sampling (Oliverla, 1998) and to discount the particle size effect on the grounds that chewing and rumination in the animal will produce a result similar to grinding (Schofield, 2000). Moreover, Menke and Steingass (1988) reported that the *in vitro* essay of feed stuffs in rumen fluid required a careful drying to avoid over heating. Rymer and Givens (1998) also observed that high temperature dried grass negatively affects the production profile.

Buffer and inoculum

Buffer and inoculums are factor should be considerable during using gas production technique. The buffer should be neutralizing the volatile fatty acids produced during fermentation in order to keep constant pH. Secondly, the buffer should supply all necessary minerals for optimal microbial activity (Oliverla, 1998). The standard buffer has been used base on McDougal's analyzed of sheep saliva and contains both bicarbonate and phosphate. For the quantitative gas measurement using this buffer, it is important that the pH shoud be held with in the range of 6.8-6.2 (Schofield, 2000). At a lower pH, the cellulolytic bacteria become less activity (Russell and Dombrowski, 1980). Cone et al. (1996) also reported exhaustion of buffer when more than 0.5 g corn cob mix was incubated. Therefore, the quantity of feed incubated *in vitro* system must be set in relation to the volume of buffered rumen fluid medium (Getachew et al., 1998a)

The innoculums also have a considerable influence on *in vitro* gas production. Schofield (2000) reported that the composition of rumen fluid will vary from day to day and from animal to animal, and these variations may affect *in vitro* digestion profile. Nagadi et al. (2000) reported that changing the ration of concentrate to hay reduced the initial bacterial concentration and affected the gas production

degradability parameters. Oliverla (1998) recommended to take the rumen fluid before feeding, because it is most constant in its composition and activity. Moreover, Menke and Steingass (1988) also recommended to take rumen fluid mixture from at least two donor animal as this guarantees a greater constancy of activity. Additionally, Mauricio et al. (2001) compared between bovine feces have potential as an alternative inoculums to rumen liquor for *in vitro* gas production technique.

Incubation condition and time of reading

Incubation vessels, i.e. syringe, bottles, should be kept in water bath (Oliverla, 1998); Getachew et al., 2002) or an incubator (Schofield, 2000) at 39 ± 0.5°C. Time of reading can be selected to suit the type of substrate which is being incubated. For forage, it is generally accepted to read after 3, 6, 12, 24, 48, 72 and 96 h but, for concentrate type substrates it may be necessary to take more frequent readings in the first 24 h (Menke and Steingass, 1988).

Methods for measuring gas volume

There are basically two approaches for measuring gas volume : (1) measuring gas collected at atmospheric pressure and its volume determined directly or (2) measuring gas accumulated in fixed volume container, and the volume is calculated from pressure change (Getachew et al., 1998b). There are several methods uses to *in vitro* gas measuring technique. In the earlier work on gas measurement using manometric measuring (El-Shazly and Hungate, 1965: Cited by France et al., 2000). After that the other methods were developed such as Hohenheim gas method or Menke's method (Menke et al., 1979), liquid displacement system (Beuvink et al.,

1992), Manometric method (Waghorn and Stafford, 1993: Cited by Getachew et al., 1998b) and pressure transducer systems (Cone et al., 1996; Brown et al., 2002).

Applications of the *in vitro* gas production technique

After research was success to use of gas production technique as a tool in the field of animal nutrition. The researchers attempt to applied this method for describing nutritive value of feed stuffs. The applications were as following information.

The first application of *in vitro* gas production was to evaluate the energetic value and predict *in vivo* organic matter digestibility (Menke et al., 1979). After those researchers were extended this work to use predict metabolizable energy and *in vivo* digestibility. Macheboeuf et al. (1997) have reported significant correlation between *in vitro* gas measurement and *in vivo* organic matter digestibility. Gas production technique was also employed for evaluation of metabolizable energy. Menke and Steingass (1988) reported a strong correlation between metabolizable energy values measured *in vivo* and predicted from 24 h *in vitro* gas production and crude nutrient as prediction of metabolizable energy (Aiple et al., 1996). The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed, particularly straws (Makkar et al., 1999), mixed diets of lactating cows (Nataraja et al., 1998) and tropical feeds (Krishnamoorthy et al., 1995).

However, Getachew et al. (2002) investigested labolatory variability of an *in vitro* gas production procedure, and calculated ME value of feeds, in several laboratories in different geographical logical locations in the wold. They found that ME values predicted by the gas production technique by laboratories in different parts of the world cannot be considered absolute. Nevertheless, this tecnique has also been used to assess the presence of anti-nutritive compound in different feedstuffs, e.g.

compounds such as the polyphenolic tannins, the terpene or steroid based spooning and nitrogenous alkaloids (Schofield, 2000).

Recently, Makkar et al. (1995a) used a syringe method to evaluate anti-nutritive factor in feedstuffs. After that Getachew et al. (2000) studied the effect of polyethylene glycol (PEG) on *in vitro* degradability of nitrogen and microbial protein protein synthysis from tannin-rich browse and heraceous legumes, they found that tannin-containing feeds incubated in absence of PEG resulted in higher microbial protein synthesis than in the presence of PEG. Hossain and Becker (2002) investigated method to reduce the levels of anti-nutrients in seeds by *in vitro* gas production technique.

Additionally, *in vitro* gas production has been used to predict dry matter intake (Blummel and Ørskov, 1993; Blummel and Becker, 1997) and prediction of ruminal microbial protein efficiencies (Blummel and Lebzein, 2001). Nowadays describes kinetic of fermentation of gas production profiles is the most interested thing. Various models have been used to describe gas production profiles (Getachew et al., 1998a). However, the exponential model of Ørskov and McDonald (1979) seems to be popularity. Nevertheless many researchers attempt to improve model such as multiphase analysis (Groot et al., 1996), tree-phasic model (Cone et al., 1997), steady-flow rumen systems models (Pitt et al., 1999) and mixture simple design (Sandoval-Castro et al., 2002).

2.3 Maximizing use of NPN in feeding systems based on agro-industrial by-products

Feed for ruminants normally includes protein , roughage, and carbohydrates (normally grain) providing calories; various sources of roughage including hay,

pasture, silage, ground cotton burrs. Protein is often supplied by cotton seed meal and soybean meal, in addition to protein found in grain or hay, and typically the feed also contains vitamins, minerals and supplements. Beef cattle and other ruminants foster a population of microorganisms in the forepart of their digestive tract that allows these animals to digest ligno-cellulose materials that are at best poorly digested by non-ruminants. The digestive system of ruminants also allows these animals to utilize non-protein sources of nitrogen to synthesize protein. In ruminants, these NPNs are hydrolyzed to ammonia in the rumen solution and the ammonia is eventually converted to protein through the action of microbial enzymes. Other microbials converted to amino acids which are utilized by the ruminant to from body tissue (NRC, 2001).

Normally, protein sources represent a substantial portion of the cost of feed for ruminants such as beef cattle. Thus, the use of less expensive NPN source is desirable to the extent that such sources can be used as a feed supplement. One such NPN source that has been used is urea. "Urea" as the term is used herein refers to commercial grade urea (46%) (Kearl, 1982). One major problem with the use of urea as a NPN source of nitrogen is ammonia toxicity (Tamminga, 2006; Hammon et al., 2005). When urea is broken down into ammonia in the rumen, the ammonia may be absorbed into the blood stream of the ruminant. The liver will convert the ammonia into urea which may be excreted or reabsorbed into the stomach contents (Van Soest, 1982). However, if the rate of ammonia absorption exceeds the capacity of the liver to convert it to urea, ammonia will accumulate in the animal's blood possibly resulting in ammonia toxicity. Symptoms of ammonia toxicity include tremors, respiratory difficulties and spasms and the animal may ultimately die. Ammonia toxicity normally occurs within a relatively short time after the ruminant consumption of feed containing

a NPN source, e.g., urea, is an important factor. In order to avoid the possibility of ammonia toxicity while using urea as a NPN source of nitrogen in ruminant feed, the use of urea as a feed supplement has been limited to 1% of the total dry weight of the feed (NRC, 1976). The present invention provides a low release non-protein feed supplement that enables the use of a NPN source in ruminant feed than has heretofore been used. The feed supplement of the present invention is formulated to provide for substantially slower release of ammonia from a NPN source, e.g., urea, during anaerobic digestion, thus allowing the use of higher levels of NPN sources in ruminant while avoiding the risk of ammonia toxicity. As used herein, the terminology "slow release" means that the non-protein feed supplement releases ammonia at an average rate of less than 40% of the average rate that urea releases ammonia during the first six hours of *in vitro* testing (Helmer et al., 1970a).

Non-protein nitrogen (NPN) compounds are commonly used in feeding ruminants; their use, especially in countries with limited resources of proteinous feeds, is economical.Various NPN compounds are being used for feeding ruminants, in the first place urea and its derivatives and ammonium salts (Kearl, 1982). In spite of extensive research on factors influencing NPN utilization by ruminants, no appropriate model has been elaborated so far which would take into account all relevant parameters. The extent of NPN utilization depends mainly on the rate of microbial protein synthesis, governed primarily by the availability of substrates (Wallace et al., 1999). An important role is also played by the properties and level of the nitrogenous compound used, the amount, quality and solubility of dietary protein, the nature of dietary protein, the nature of dietary carbohydrates, the productivity of the animals and their protein requirements, adaptation to NPN and other constituents of the ration, the contents of minerals in the diet, the feeding regime, the rate of N recirculation in the organism, etc.(Volden et al., 2002; Fu et al., 2001).

For maximum utilization of NPN by rumen organisms two processes in the rumen should run simultaneously : the degradation of NPN to ammonia, and the fermentation of carbohydrates (Baldwin, 1970) to supply energy for microbial protein synthesis (Nocek and Russell, 1988). Ammonia is relatively rapidly released from NPN, especially from urea in the rumen (Kearl, 1982), as follows;.

$$1 \text{ glucose} \longrightarrow 1.2 \text{ acetate} + 0.4 \text{ propionate} + 0.2 \text{ butyrate} + 1CO_2 + 0.6CH_4 + 0.4\ H_2O$$

$$NH_2 - CO - NH_2 \xrightarrow[\text{Urease}]{H_2O} 2NH_3 + CO_2$$

A number of urea preparations are now being produced with a reduced rate of ammonia liberation. The ways of preparing NPN and supplementing diets composed of home-grown feeds or industrial by-products deficient in protein, depend on local conditions, the availability of these feeds and the nature of the NPN. The most common NPN compounds and NPN supplemented feeds used in feeding ruminants (Kowalczyk et al., 1970) are as follows:

1. Urea, ammonium salts and biuret
   a. mixed with concentrate feeds;
   b. urea or urea-mineral preparations;
   c. liquid supplement consisting of molasses, urea, minerals and vitamins;
   d. NPN supplement to silages or to low-protein green forages during ensiling;
   e. Pellets or briquets of compound feed with a large proportion of ground straw, untreated or treated with alkali and supplemented with NPN.

2. Ammoniated feeds, produced by treating straw, sugar beet pulp, bagasse, citrus pomace, distillery slops, silages or green feeds at the time of ensiling.

The NPN supplement will serve its purpose only if added to feeds with low protein content but rich in readily available energy : molasses, sugar beet pulp, distillery slops and other industrial by-products, whole plants of maize and other green feeds, potatoes, sugar beet, etc. Supplementing these feeds with cheap NPN compounds instead of much more expensive protein-rich feeds such as soybean meal or fish meal is economically justified, as the production effects obtained are similar. The use of those feeds without nitrogen supplement is ineffective and may adversely affect the health of the animals (Kearl, 1982). Moreover, if NPN is used in low-protein/high fiber rations, supplementation with energy-rich feeds or rendering the fiber more digestible by appropriate treatment becomes necessary. Otherwise NPN utilization is poor and may even result in ammonia intoxication (Hammon et al., 2005). With the use of NPN compounds for ruminant feeding a large proportion of protein-rich feeds can be used for feeding other farm animals.

2.4 Historical profile of developmental uses of NPN

Research and discussion on the use of non-protein nitrogen (NPN) in ruminant feeding have continued over 80 years since (Zuntz, 1891: Cited by Kowalczyk et al., 1970) formulated the hypothesis that rumen microorganisms are capable of decomposing cellulose and converting NPN into true protein. In practical animal husbandry the use of urea in feeding increases steadily. In the U.S.A., where it amounted to only 68,000 tons in 1958 (Loosli and McDonald, 1968), it rose from 465,000 to 685,000 tons between 1965 and 1970 (Fonnesbeck et al., 1975) and exceeded 1 million tons in 1973 (Huber, 1975). Urea and other NPN compounds are

also used in large amounts in other countries in America and Europe, including the U.S.S.R. The above figures are convincing argument for the general and extensive use of NPN in feeding ruminants, the more so as the shortage of protein feeds in the world market is conductive to their rational and economic use and also to the search for new sources of feeds.

Protein and non protein nitrogen compound are, along with carbohydrates, fat, vitamins and minerals, the basic constituents of animal diets. In monogastric animals the breakedown of dietary protein in the stomach and small intestine consists mainly in enzymic hydrolysis to amino acids, which are absorbed from the intestine into the blood stream for utilization by the animal (Kearl, 1982).

2.5 Nitrogen Utilization

Kearl (1982) reported that in ruminants, dietary protein is already broken down in the rumen, to a greater or lesser extent depending on its nature, by the action of microbial enzymes. However, this composition does not stop at the hydrolysis level: the liberated amino acids are further degraded by deaminating enzymes to ammonia and the corresponding acids. The extent of protein degradation in the rumen, ranging from 30 to 80%, is primarily a direct function of its solubility (Figure 2.1).

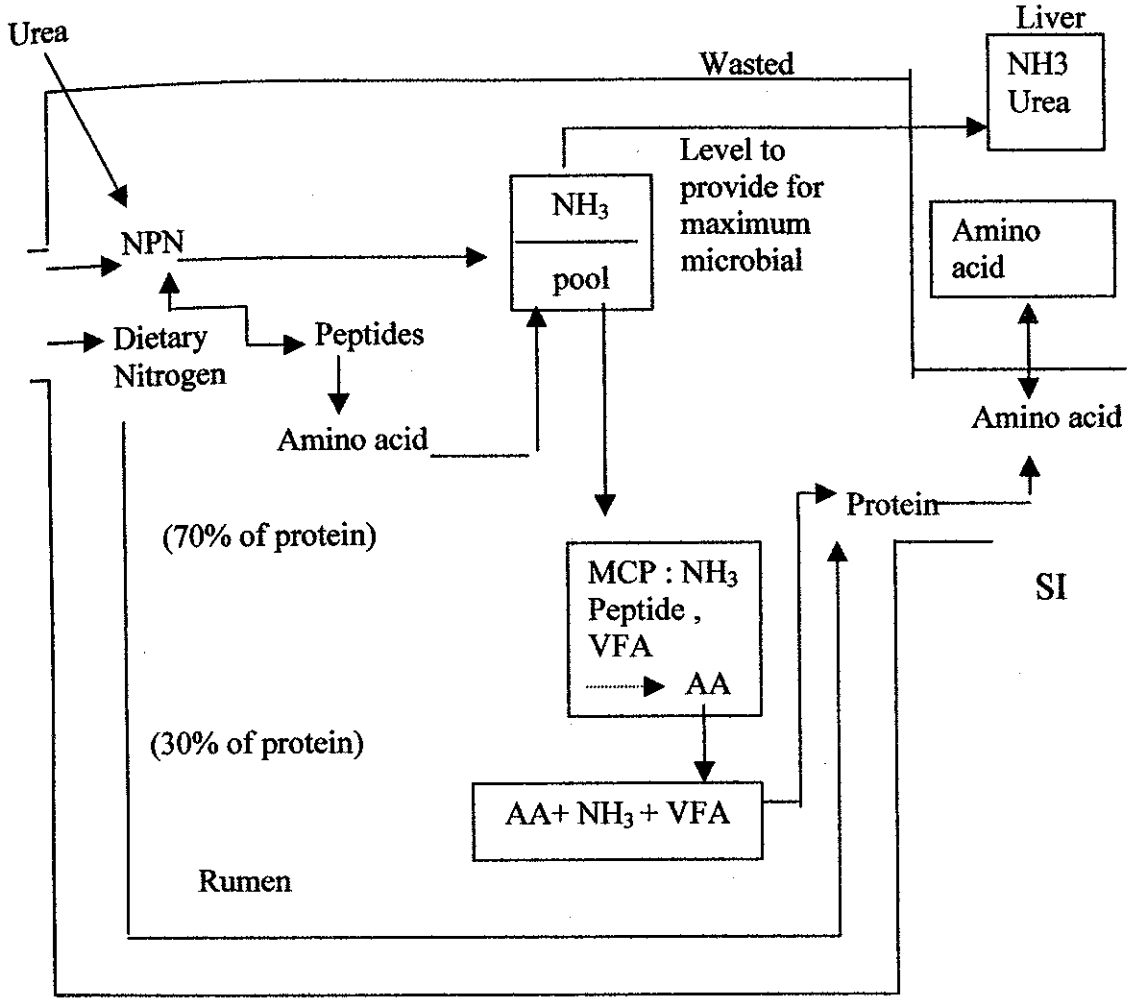

Figure 2.1 Schematic summary of nitrogen utilization by the ruminant (adapted from Kearl, 1982)

Apart from protein, the diet contains also low molecular weight nitrogenous compounds which are usually readily soluble and, consequently, especially susceptible to degradation in the rumen with the liberation of ammonia; the latter enters the ammonia pool of the rumen, which also includes ammonia derived from the breakedown of protein and of urea, the latter transferred from the blood across the rumen wall or entering the rumen with saliva (Van Soest, 1982) (Figure 2.2).

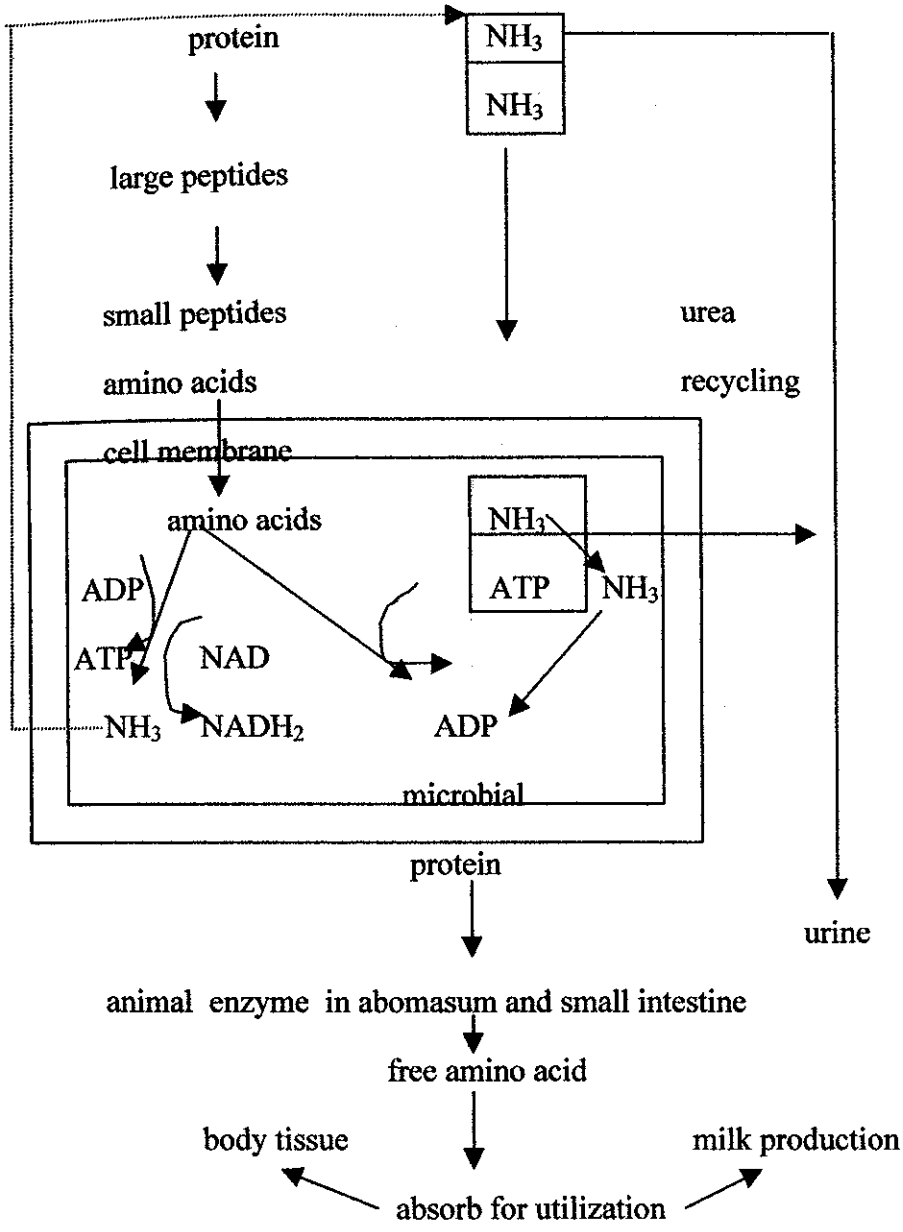

Figure 2.2 Nitrogen utilization by the ruminant

Source : adapted from Van Soest (1982)

Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein (Figure 2.1 and 2.2). In this way a part of dietary protein is converted into microbial protein of high nutritive value.

Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids, have but little effect on the yield of microbial matter (Ørskov et al., 1974). If, however, a certain microoganism causing a different type of fermentation predominated in the rumen, and if that microoganism were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist.

The rate of fermentation is undoubtedly an important factor influencing the economy of microbial growth. With a low rate of fermentation the rate of microbial growth is also reduced; with rapid fermentation the utilization of the ATP formed is impaired.

2.6 Some aspects of bacterial protein synthesis in the rumen

Carbohydrates are the main substrate for rumen bacteria and also the main constituent of the ruminant diet. They are the only substrate from which large amounts of energy can be derived under anaerobic conditions. In contrast to the bacteria for which carbohydrates are the energy source, the growth of those utilizing protein to

cover their energy needs under anaerobic condition is very limited. Fat is not an energy source under the anaerobic conditions of the rumen, but the energy value of its hydrolytic product, glyceral, is similar to that of carbohydrate. Fat also exerts an inhibitory effect on some rumen microorganisms (NRC, 1976) as fallows;.

1) carbohydrate $\xrightarrow{\text{microbe enzyme}}$ volatile fatty acid + keto acid

2) NPN (urea) $\xrightarrow{\text{microbe urease}}$ $NH_3 + CO_2$

3) NH_3 + keto acid $\xrightarrow{\text{microbe enzyme}}$ amino acid

4) amino acid $\xrightarrow{\text{microbe enzyme}}$ microbial protein

5) microbial protein $\xrightarrow{\text{animal enzyme}}$ free amino acid

6) free amino acid $\xrightarrow{\text{abomasum , intestine}}$ absorbed for utilization in animal body tissue

The composition of the rumen microbial population varies according to the diet, but even under stabilized feeding conditions the rumen harbours a great variety of species, and composition of microbial protein is relatively constant. The ratio of nucleic acids to protein in the microbial matter also changes little and is therefore used as an index of the synthesis of bacterial protein in the rumen and of its passage to the abomasum (Smith, 1969). McNaught et al. (1954) found in experiments on rats that the digestibility of bacterial protein ranged from 70 to 75%; Mason and White (1971) obtained similar values. Digestibility values for protozoal protein were higher: 89%

(McNaught et al., 1954). The lower digestibility of bacterial protein is probably due to the resistance of bacterial cell walls to the action of enzymes (Mason and White, 1971). A substantial part, about 40%, of microbial protein produced in the rumen is digested in the abomasum (Kowalczyk et al., 1970).

During recent years the digestion in the rumen of energy–yielding constituents of the ration and the production of microbial protein have been extensively studied. Demeyer et al. (1972) and Thomas (1973) summarized this work and found considerable differences between the results obtained by different workers, due not only to different experimental conditions but also to technical difficulties in estimating the rate of production of microbial matter in the rumen. No satisfactory method has yet been developed for separating dietary and microbial protein. Nucleic acids or 2,6 Diamino pimelic acid (DAPA) are frequently used as indicators of the proportion of microbial protein in the digests, but the amounts of these compounds in individual bacterial species differ and some bacteria contain no DAPA at all. More precise estimates can be obtained with the use of 15N, 32P or 35S labelled compounds. The rate and extent of microbial protein synthesis in the rumen depend on the rate and extent of ammonia liberation, the rate of fermentation of carbohydrates, rumen pH, the rate of absorption of the fermentation products and the number of bacteria. About 20 to 30 g of microbial nitrogen are produced per kg of organic matter digested (Ørskov et al., 1972; Miller, 1973; Thomas, 1973) (Table 2.1).

Table. 2.1 Effects of ruminal undegradable protein on nitrogen excretion and utilization efficiency

References	Solubility/ Degradability CP (%)	Milk (Kg/d)	Ruminal NH_3-N (mg%)	Total N-excretion (%N-intake)	Bact-N (g/d)	BUN mM/l
Volden	HL(34.4%)	32	6	47	370	4
(1999)	HH(47.7%)	30	8	51	415	5
	LL(39.8%)	29	4	45	365	4
Cunningham	17%CP(LRUP)	34	9		225	
et al.	(HRUP)	37	7		230	
(1996)	19%CP(LRUP)	35	11		202	
	(HRUP)	39	7		225	

BUN = Blood Urea nitrogen, HL = High protein, low ruminal degradability, LL = Low protein, low ruminal degradability, LRUP = low ruminal undegradable protein, HRUP = High ruminal undegradable protein

The nitrogen requirement of rumen bacteria on a given diet can be estimated from the amount and digestibility of organic matter digested by the animal, bearing in mind that there should be at least 30 g nitrogen, to be hydrolysed to ammonia in the rumen, per kg of digestible organic matter in the diet (Ørskov, 1976). If the basal diet offered to the animals is deficient in nitrogen but contains large amounts of readily fermentable carbohydrates, it is advisable to supplement the diet with NPN so as to ensure the correct proportions of nitrogen and energy for microbial protein synthesis. The magnitude of the latter is rarely limited by nitrogen deficiency, as nitrogen can

readily be added in form of NPN or, in the first instance by the intake of sufficiently large amounts of rumen-digestible organic matter, mainly carbohydrates (Table 2.2).

Table 2.2 Synchronizing ruminal protein and carbohydrate digestion on microbial protein protein synthesis

References	Treatment	Ruminal NH_3-N (mg/dl)	BUN (mg/dl)	Milk (Kg/d)	VFA mM/l	MC–N (g/Kg OMdigest)
Stoker et al.	NSC : DIP (%)					
(1991)	38 : 13.7	21.2	19.8	37.5	142.6	24.8
	31 : 11.8	15.0	23.4	34.3	121.4	26.4
	24 : 9	8.0	23.7	30.4	99.0	22.0
Herrera –	RDP : NSC (%)					
Seldana et al.	CSM – B					
(1990)	59.5 : 74.4	16.4	14.8	37.4		45.3
	BDG – B					
	43.7 : 69.9	11.4	15.0	34.9		37.1
	CSM – M					
	55.9 : 62.3	12.5	13.6	34.2		35.4
	BDG – M					
	35.4 : 48.3	10.2	13.7	34.6		35.7

CSM : cotton seed meal, BUN= Blood Urea – nitrogen, NSC= Non – structural Carbohydrate, B= Barley, VFA= Volatile Fatty acid, DIP= degradable intake protein, BDG= Brewers dride grains, MC-N= Microbial nitrogen, RDP= Ruminal degradable protein

Supplementing diets of relatively low digestible organic matter content with NPN with is useless, as the bacteria are unable to utilize all the ammonia released in the rumen, the excess being absorbed into the bloodstream, converted to urea and excreted in the urine.

A rapid ingestion of large amounts of readily digestible organic matter and nitrogenous compounds does not by itself provide the proper conditions for efficient bacterial protein synthesis, as in this instance the rate of ammonia release may exceed the rate of protein synthesis and again ammonia will accumulate and be absorbed from the rumen. It will be recirculated to a certain extent to the rumen, but this entails a considerable loss of nitrogen (Nolan et al., 1973; Kowalczyk et al., 1975). It is better, therefore, to offer such diets more frequently but in small amounts. A good example is the use of diets based on molasses and urea, which resulted in fairly good utilization of nitrogen and energy with no accumulation of ammonia or lactic acid in the rumen (Preston and Willis, 1970; Kowalczyk, 1971). Addition of NPN to diets containing large amounts of readily digestible carbohydratesbut small amounts of protein, or protein protected against deamination in the rumen, is particularly advisable, as in such diets there may be a shortage of nitrogen available to the bacteria for growth. A correct synchronization of the rate of carbohydrate fermentation with the rate of amonia release is of particular importance for the efficient utilization of NPN (Figure 2.3). In this respect feeds containing sugars (molasses, sugarcane, sugar beet, etc.) or starch (potatoes, cereals, miaze or cassava) are better suited for supplementation with NPN than those rich in fiber, as the rate of fermentation of sugars or starches matches better the rate of release of ammonia from NPN; the rate of cellulose degradation is slow, and adequate amounts of energy are not supplied in the presence of excess ammonia (Maeng et al., 1997).

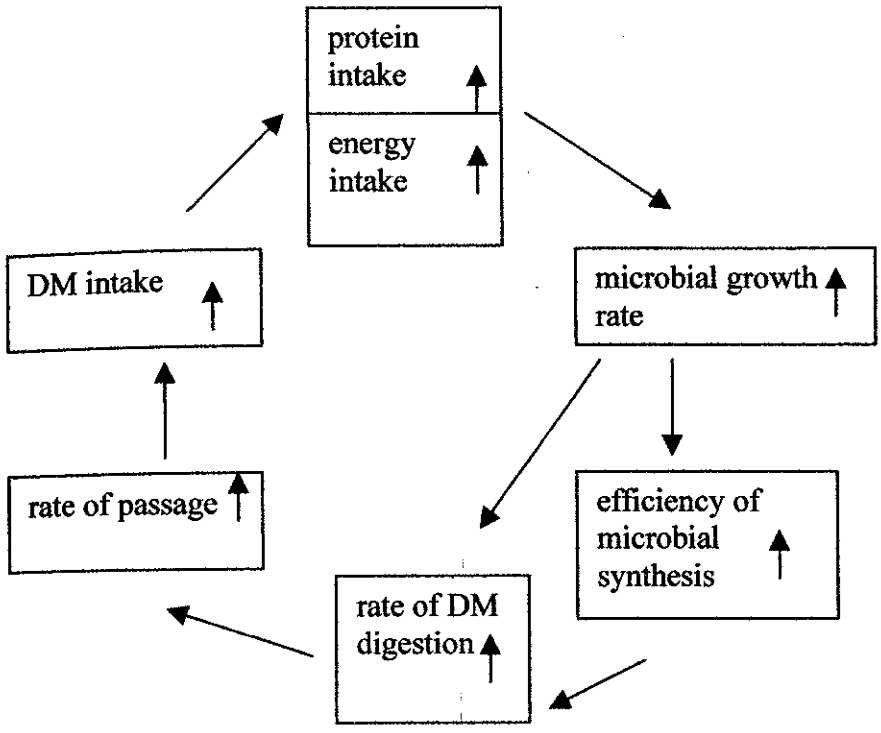

Figure 2.3 Synchronizing ruminal protein and carbohydrate digestion on microbial protein protein synthesis

Source : Nocek and Russell (1988)

Thompson et al. (1972) reported that if NPN is used as a protein replacement, the diets should be supplemented with minerals, especially sulphur, which is a constituent of protein. Sulfur deficiency in the diet can seriously reduce bacterial protein synthesis in the rumen. The N:S ratio should be from 10 to 13.5 : 1 in rations for sheep and from 13.5 to 15.1 in those for cattle. The form in which sulphur is added is of no importance because sulphates, thiosulphates or elementary sulphur alike are converted, in the strongly anaerobic environment of the rumen, to H_2S, which is utilized by rumen bacteria for the synthesis of sulphur amino acids.

2.7 NPN compounds and NPN preparations

NPN compounds used as protein replacements for ruminants should be inexpensive and well utilized by microorganisms and should not adversely affect the health of animals. Table 2.3 lists some of the compounds of possible importance as protein replacement. Apart from these, others which may be of some importance in the feeding of ruminants can be found in the literature.

Table 2.3 Certain Non-Protein Nitrogen Compounds

Compound	**Chemical formula**	**N content(%)**	**Protein equivalent (Nx6.25) (%)**
Urea	$(NH_2)_2CO$	46.7	292
Ammonium lactate	$CH_3CHOHCO_2NH_4$	13	81
Ammonium acetate	$CH_3CO_2NH_4$	18	112
Acetamide	CH_3CONH_2	23.7	148
Glutamine	$NH_2CO(CH_2)_2CHNH_2COOH$	19	119
Glycine	NH_2CH_2COOH	19	119
Ammonium bicarbonate	NH_4HCO_3	17.7	110
Ammonium formate	$HCOONH_4$	19	119
Ammonium sulphate	$(NH_4)_2SO_4$	21.2	132
Biuret(pure)	$NH(CONH_2)_2$	40	252

Source : Loosli and McDonald (1968).

Acetamide, glycine and glutamine are hydrolised at a slower rate than urea, and their nitrogen is efficeintly utilized by ruminants, but their use in practical feeding

is limited by their high price. However, urea is the most preferred NPN source, because of its relatively low cost and high nitrogen content. Biuret is relatively resistant to hydrolysis in the rumen, and a long adaptation period is needed for the microbial population to utilize it efficiently. The conditions for its use and the extent of biuret utilization by the animals are still controversial (Fonnesbeck et al., 1975; Loosli and McDonald, 1968).

In general the amount of NPN substance use in the present supplements will exceed to a greater or lesser degree the amount which could be tolerated by ruminants as a toxicity and palatibility if simply mixed with a starch source and fed directly. It has been found that a ratio of starch source to nitrogen from the NPN substance is advantageously maintained within the range of from about 4 : 0.45 to 1 : 1.80, with the most preferred starch source-NPN nitrogen ratio ranging from about 2 : 0.45 to 1 : 0.90. In the case of the most preferred NPN source, urea, the starch source /NPN nitrogen ratio should range from about 4 : 1 to 1 : 4, most preferably from about 2 : 1 to 1 : 2. (Helmer et al., 1970a; Stiles et al., 1970),

2.8 Urea and urea preparations

From among these nitrogenouse compounds, urea finds widest use in ruminant feeding. The conditions of its effective utilization are also best know (Kowalczyk, 1971). These are:

a. adaptation of the animals, or rather of the rumen microbial population, to urea feeding. The amounts given should be small at the start and increase gradaully throughout the adaptation period, which should be no less than 10 days;

b. careful and uniform mixing with other feeds, as crystalline urea tends to lump and needs grinding. Various substances are added to urea to prevent lumping. Crystalline or granulated urea added to feed mixtures tends to separate during transport and should be redispersed before feeding. Better ways of preventing separation are pelleting of the feeds containing urea or the use of special urea or urea-mineral preparations like Starea;

c. the presence in the diets of adequate amounts of sugars, starch and minerals, especially S, P, Ca and Co. As previously mentioned, when urea is fed with roughages containing no readily digestible carbohydrates, it is poorly utilized. Best results are obtained if all the necessary components, in proportions appropriate for the given type of production, are combined in a balanced feed given to appetite. Under these conditions the animals eat small amounts of feed frequently, and this favours the effective utilization of NPN by rumen microbes.

As compared to other NPN compounds urea is inexpensive, easy to produce and generally available, but it has also certain disadvantages:

a. too rapid hydrolysis, leading to the accumulation of ammonia in the rumen and its absorption into the bloodstream, reducing its utilization for protein synthesis;

b. urea is highly hygroscopic and tends to aggregate (lump) ; this impairs its adequate mixing with other feeds;

c. the unpleasant, bitter taste makes feed mixtures with a high proportion of urea unpalatable and adversely affects voluntary intake, especially by cows.

In the course of studies on the use of urea for feeding cattle and sheep, various methods of addition of urea to feeds and diets have been developed.

2.9 Urea-mineral preparation

Special preparations with a high content of urea have been developed with a view not only to obviating the disadvantages of urea and eliminating the labour-consuming ways of adding it to feeds, but also to improving its utilization by ruminants. Among preparations extensively tested *in vitro* and verified in practical feeding are " Starea" and " Dehy-100" (Helmer et al., 1970a; Stiles et al., 1970), manufactured on a large scale in the U.S.A. according to technologies protected by patents. They contain, apart from 20 to 32% urea, ground cereal grains or dried potatoes ("Starea", "Golden-pro") and luceme meal ("Dehy-100"); 5% bentonite or 0.5% propionic acid acting as moisture absorber or conservant are often added. The advantage of these preparations is to moderate the bitter taste of urea, which therefore may given in fairly large amounts (up to 1 g/kg body weight) with a minimum risk of toxication. During processing at high temperature and pressure, the starch mixed with urea gelatinizes, forming a homogeneous mass ("Starea", "Golden-pro") which decomposes slowly in the rumen, gradually releasing urea and thus supplying the bacteria simultaneously with ammonia and energy for protein synthesis. Under this circumstances there is little accumulation of ammonia in the rumen and, consequently, only a limited absorption of ammonia into bloodstream. These preparations proved beneficial in the feeding of high-yielding dairy cows and replaced soybean meal to a large extent without adversely affecting production.

In other countries, different urea preparations have been developed and tested in practical feeding. In Hungary, satisfactory results have been obtained in fattening lambs and lactating cows with a preparation in meal form, "Urea-Abdukt", based on paraffin oils (50%) and urea (50%). Czechoslovakia, a pelleted preparation "DL-100" has been manufactured with the following composition: urea 31.8% lucerne meal

64%; sodium propionate 0.4%; Na_2HPO_4 1.5%; $CaCO_3$ 1.3% and premix 1%. The preparation was given together with cereal mixture and maize silage to cows with a daily milk yield of 19 liters. There were no differences in milk yield between the cows given "DL-100" and control cows on protein (Kowalczyk et al., 1975).

In Bulgaria, a urea-mineral preparation called "Carbisale" consists of urea 42%; NaCl 34.5%; $Ca_3(PO_4)_2$ 17%; $CaSO_4$ 6%; and trace element mixture 0.5%. The performance of fattening lambs given 15 g of this preparation daily was similar to that of lambs on sunflower meal and better than that of lambs given crystalline urea (Kowalczyk et al., 1975).

In Scotland, a method has been developed of supplementing barley with urea. The grain is soaked in urea solution and dried. The resulting product is similar in quality to other urea preparations (Ørskov et al., 1974).

In Poland several urea preparations are being produced and tested in experiments with sheep, cows and fattening cattle. The pelleted preparation "KBM" contains 13%urea, 30% repeseed meal, 35% ground cereals, 5% molasses, 5% grass meal and 12% minerals. It sould be used especially with home-produced feeds like silages and root crops. In experiments with cows fed on home grown feeds and dried maize (whole plants) supplemented with up to 1 kg "KBM" daily, a yearly milk yield of 4,000 liters was obtained with no oilseed meals.

Another urea preparation, called "Grysik", consists of 32% urea, 42% ground barley and 26% minerals. The throughly mixed constituents are steamed at 1,500; the starch gelatinizes while urea and minerals dissolve in it. The cooled and ground product can be added to concentrate feeds for cattle and sheep. It can also be added, instead of high- protein feeds, to raw potatoes, beet roots or maize silage. The amount added varies depending on the protein deficiency in the diet. In experiments with cows

given 1 g of urea in the form of the "Grysik" preparation per kg body weight, a good daily milk yield was obtained, about 4,000 liters/year, with no signs of intoxication or digestive disorders (Kowalczyk et al., 1975).

2.10 Briquets and pellets with NPN

In recent years the manufacture of balanced feeds for winter and summer feeding in the form of pellets and briquets has been started in different countries, including Denmark, France, G.D.R. and U.K. Apart from cereal grains they contain large amounts (up to 50%) of straw, sometimes previously treated with sodium hydroxide, and urea (up to 2%), ammonia or ammonium carbonate, as well as other feeds. During processing at high temperature and pressure (90-120 degree C), urea condenses partly to biuret, releasing ammonia, which acts on the fibre of the feeds, rendering it more readily digestible. Attemts to feed cows and fattening cattle on such briquets have given encouraging results (Kowalczyk et al., 1975).

2.11 Molasses with urea

Molasses, the main by-product of sugar manufacture, is a good source of readily fermentable carbohydrates. Combined with urea and a small proportion of concentrates and roughages it can provide the basal diet for fattening cattle. (Kowalczyk et al., 1975).

2.12 Ammoniated feeds

Ammoniating is one of the ways of enriching low-protein feeds with nitrogen. Ammonia is released at a slower rate from ammoniated feeds than from urea, and this favours bacterial protein synthesis. The technology of impregnating different feeds

with gaseous ammonia or ammonia solution has been developed and patented . The quantity of ammonia that can be fixed in the feeds depends on their chemical composition and on the physical conditions of the treatment, especially temperature and pressure. Ammonia in ammoniated feeds appears in the form of ammonium salts, amide compounds in pectins, and imidazoles; it can also be bound by sugars and lignin. If processing is carried out at high temperature and pressure, products with higher ammonia contents are obtained, but the nitrogen utilization of such feeds by animals is often poorer than that of feeds treated with ammonia under less drastic conditions although the latter contain less nitrogen. patented (Stiles et al., 1970).

2.13 Sugar beet pulp, citrus pulp and apple pomace

Sugar beet pulp, citrus pulp and apple pomace contain relatively large amounts of pectins which readily bind ammonia, forming a stable compound; the nitrogen content of the ammoniated product increases twice or more. The result of numerous experiments carried out in Poland, with ruminants given different amounts of ammoniated sugar beet pulp, suggest good utilization of nitrogen with this feed. Ammoniated sugar beet pulp is more readily eaten by the animals than untreated pulp, is safe in practical feeding and increases the digestibility of dietary fibre (Loosli and McDonald, 1968).

2.14 Straw

Ammonia treatment of the straw of various cereals doubled its nitrogen content. Straw contains little readily fermentable carbohydrate, and the addition of feeds such as molasses, ground cereals etc., is therefore advisable. Ammoniated maize meal, prepared from whole plants harvested at the milk-wax stage of ripeness,

provided avaluable feed supplemented with minerals it was used as a mono diet for lambs and growing-fattening cattle. Efficiency of feed utilization and live weight gains were better than with untreated maize meal supplemented with rep seed meal. Ammonia treatment of sugarcane bagasse (Davis, 1957), rice hulls and wood wastes (Erlinger and Klofenstein, 1971) has not taken on importance because of the poor ammonia binding capacity of these products, which consequently yielded little improvement of animal performance. Better results were obtained with fattening cattle fed on sugarcane bagasse enriched with molasses and urea (McDowell and Hernandez-Urdaneta, 1975). More work is needed to improve the utilization of the cellulose energy of these high-fiber feeds.

2.15 Ammoniated molasses and distillers slops

The results obtained with feeding ammoniated molasses are less good than those with molasses supplemented with urea; apart from this, there are reports of harmful effects of ammoniated molasses on the health of animals. Its use is therefore not to be not recommended (Bartlett and Brooster, 1958). Distillers' slops (by – products of alcohol production from molasses or potatoes), ammoniated and condensed, gave good results in the feeding of dairy cows and fattening cattle when added to straw or hay (Tillman and Kidwell, 1951).

An ammonium lactate preparation is manufactured from molasses fermented to lactic acid, neutralized with ammonia and condensed. The resulting product, a thick brown syrup, can be added to straw, hay or sugar beet pulp. Sheep and cows fed on diets in which up to 40% of oilseed meal nitrogen was replaced by ammonium lactate showed good live weight gains and milk yield; the milk fat content was slightly higher than with oilseed meals (Kowalczyk, 1971).

2.16 Starea preparation

Starea are produced through an extrusion process wherein a starch bearing food source such as corn, cassava and an NPN substance such as urea are admixed and pelleted (0.76 cm. die) by run through a cooker-extruder at high temperature (90-120° C., moisture about 20%), and pressure to give a chunk-type product. Experience has proven that use of this type of process permits use of the NPN substance at levels which would be extremely toxic if simply mixed with a raw starch source and fed directly, it was discovered that it was essential to add the NPN substance to the starch bearing material initially, whereupon this starch-NPN admixture was extruded. Attempts to add urea subsequent to the extrusion of an NPN-free starch source proved ineffective in giving an equivalent product (Stiles et al., 1970).

2.16.1 Studies on toxicity of Starea

The use of urea by ruminants has been restricted by inefficient conversion of urea nitrogen to microbial protein by toxicity, by palatability and by urea segregation in mixed rations fed in meal form (Briggs, 1967; Chalupa, 1968; Reid, 1953). In a previous study (Helmer et al., 1970b) an expansion-processed mixture of grain starch and urea (Starea) was approximately equal to soybean meal as a protein supplement for lactating cows. Cows fed grain rations supplemented with either soybean meal or Starea consumed more grain and produced more milk than cows fed the same grain ration supplemented with urea. In another study (Helmer et al., 1970a) Starea improved the utilization of urea in vitro. After 4 hours of fermentation *in vitro*, concentration of rumen ammonia was lower with Starea than with unprocessed corn and urea. The lower rumen ammonia was at least in part the result of more efficient utilization of ammonia by its conversion to microbial protein.

2.16.2 Studies on estimating microbial protein in incubated mixtures of starea

Starea, an extrusion-processed mixture of grain or cassava and urea, has increased synthesis of microbial protein *in vitro* (Helmer et al., 1970a) and *in vivo* (Stiles et al., 1970) above that of unprocessed mixtures of grain and urea. Maintaining specific moisture, temperature, and pressure in the extrusion-cooking process is vital for producing high quality Starea (Behnke et al., 1973). As the quantity of microbial protein synthesized from urea in Starea is the primary goal of processing, it is logical to use microbial protein synthesis *in vitro* as a quality test of Starea products.

It is difficult to distinguish between plant and microbial protein in determining the extent of microbial protein synthesis, so many methods to estimate microbial protein synthesis have been attempted. Ellis and Pfander (1965) used nucleic acid synthesis; Walker and Nader (1968) measured radioactive sulfur incorporation; Bucholtz and Bergen (1973) measured phospholipid synthesis ; and Ibrahim and Ingalls (1972) used diaminopimelic aicd and aminoethylphosphonic acid. Many less complex and time conserving methods have been developed. Protein precipitating agents have been used widely. Helmer et al. (1970a) used trichloroacetic acid; and Gil et al. (1973), tungstic acid. Differential centrifugation also has been used to measure microbial synthesis in rumen digesta (Blackburn and Hobson, 1960; Meyer et al., 1967).

2.16.3 Studies on comparison of Starea, Urea and Soybean meal as protein sources for lactating dairy cows

Problems in feeding urea to dairy cows are intensified by emphasis on high-concentrate rations to increase production and to provide necessary energy. A

study with high-producing cows receiving various levels of urea in a high concentrate ration showed that high-urea levels (20-40% of the total ration nitrogen) depressed both milk yield and efficiency (Huber and Sandy, 1965). Nitrogen balance studies suggested that milk production efficiency decreased with added urea because of decreased nitrogen retention (Huber et al., 1967). Palatability of concentrate mixtures containing urea lessened with higher feed intake (Van Horn et al., 1967). Previous studies with rumen-fistulated cattle (Stiles et al., 1970) indicated that Starea, an intimate mixture of gelatinized starch and urea, improved utilization of urea nitrogen and palatability of urea-containing rations. Reported here is work to determine if Starea has a similar effect on urea utilization and feed intake of lactating dairy cows. Starea was compared with urea and soybean meal as a protein supplement.

2.16.4 Studies on utilization of Starea in complete rations for lactating dairy cows

Urea has been utilized as a supplemental nitrogen source for lactating dairy rations when natural protein supplements are expensive. However, urea is utilized less efficiently than natural plant proteins such as soybean meal for milk production (Helmer and Bartley, 1971). It has been suggested that rumen ammonia concentrations accumulate when dry matter of the ration contains more than 13% crude protein (CP) and that ammonia from NPN in these rations is not utilized by rumen microorganisms (Roffler and Satter, 1973). The dry matter of a dairy ration for lactation should contain at least 14% CP, with 15% CP being recommended for cows producing more than 20 kg milk daily and 16% for cows yielding over 30 kg per day (NRC, 1971). Resulted in higher milk production than a ration containing 13.9% CP

(Garder and Park, 1973). Thus the question of replacing part of the protein with NPN is in doubt.

Utilization of urea rations for milk production has been improved by use of a mixture of gelatinized starch and urea, processed through an extruder cooker (Helmer et al., 1970b). Rumen ammonia concentrations were reduced when this mixture was incubated with rumen microorganisms (Helmer et al., 1970a).

2.16.5 Studies on utilization of Starea in rations for steers.

The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia in the rumen and the subsequent observations that proteins or amino acids administered post-ruminally resulted in greater nitrogen retention than when these were administered directly into the rumen (Schelling and Hatfield, 1968) has led to recent attempts to find ways of protecting soluble, high quality, dietary proteins from microbial degradation within the rumen . Decreasing the rumen solubility of casein (Faichney and Weston, 1971) and soybean meal protein (Peter et al., 1971) by treatment with formaldehyde would appear to be a potential method of decreasing rumen degradation of high quality proteins and allowing more dietary protein by bypass the rumen to the abomasum and lower digestive tract.

Studies with an expansion-processed mixture of grain starch and urea (Starea) indicated ammonia was released slower from this product both *in vivo* and *in vitro* than when a non-heated control mixture was used and, additionally, this product was superior to urea as a nitrogen supplement for lactating cows and for growing-fattening cattle (Deyoe et al., 1968; Helmer et al., 1970b).

2.16.6 Studies on utilization of Starea, urea and sulfur in rations for beef cattle.

Reduced consumption of high urea rations and loss of dietary nitrogen resulting from rapid hydrolysis in the rumen have prompted researchers to seek new means of improving NPN utilization in ruminant rations. Bartley et al. (1968) have attempted to reduce the solubility and to improve the acceptance of urea by cooking a grain-urea mixture at high temperatures. *In vitro* and that the product (Starea) equals soybean meal (SBM) as a nitrogen supplement for ruminants. Other workers have attempted to stimulate microbial activity by supplying various nutrients that might be limiting in high urea diets Matrone et al. (1964) observed an invigorating influence of alfalfa meal on rumen microflora and Lowrey and McCormick (1969) state that feed comsumption and gain were increased by the addition of 5% alfalfa meal to high urea diets. Thomas et al. (1951) reasoned from sulfur studies with lambs that sulfur deficiencies may limit NPN utilization. Gooddrich and Tillman (1966) have also demonstrated that the addition of sulfur improved urea utilization and nitrogen retention.

2.16.7 Effect of Starea as a protein source on feed intake

Helmer et al. (1970b) reported that cows receiving either soybean meal or Starea as the protein supplement in their grain ration consumed more grain and produced more milk than those receiving urea. Grain intake was lower ($P<0.05$) for the urea supplemented group in all experimental periods. Differences between the soybean meal and starea supplemented groups were not significant. This finding was similar to those reported by Roman-Ponce et al. (1974) that Urea (U) rations effected lower ($P<0.01$) daily feed intake than soybean meal (SBM) and Starea rations. Jones et al.

(1974) reported that cows fed SBM consumed more DM ($P<0.05$), which may explain the greater body weight gains ($P<0.05$), than those fed other rations. Intakes of were similar between soybean meal and Starea rations. Starea intake also surpassed that from the low protein ration ($P<0.05$). Losses in body weight with the starea ration cannot be explained by milk production or feed consumption. Moreover, there were no differences between rations for apparent digestibility of DM, CP, ADF or cellulose. Roman-Ponce et al.(1974) reported that lower intake for Urea rations agree with some results (Van Horn et al., 1967; Van Horn et al., 1975) though other investigators have reported no feed intake depression by lactating cows using the same or higher urea (Colovos et al., 1967a; Colovos et al., 1967b; Holter et al., 1971; Van Horn and Jacobson, 1971). Starea rations containing the same percentage of urea as Urea rations (1.7%) and leading to slightly greaster urea intakes than the former (275 and 256 g. per day) did not reduce feed intake as compared to SBM rations, which confirms results of Helmer et al.(1970b) with lactating cows and of Schmidt et al. (1973) with steers. Equal responses in milk yield for Starea and SBM rations (higher than Urea rations) agree with Helmer et al. (1970b).

2.16.8 Effect of Starea as a protein source on ruminal pH

Stiles et al. (1970) reported that no differences were observed in rumen pH between the rations. Although the mean pH for the rations was not different, several variables were negatively correlated with pH of the Starea-fed animals. Low pH values appeared to be related to high VFA and high bacterial nitrogen production. H-ion concentration appeared to be influenced more by VFA than by ammonia or lactate production. Moreover, Roman-Ponce et al. (1974) reported that lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with

the Urea opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when Urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with a more acid rumen pH. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns.

2.16.9 Effect of Starea as a protein source on volatile fatty acid

Stiles et al. (1970) reported that the rumen VFA concentration usually peaked 4 hours post-feeding. While the total VFA concentration and that acetic acid were highest for the starea-fed animals. The molar proportions of propionic, iso-butyric and iso-valeric acids were significantly greater for those fed the control ration.This finding was similar to those reported by Roman-Ponce et al. (1974) that Starea and SBM rations result in higher ($P<0.01$) amount of total VFA than urea. Propionate concentration was similar in SBM and Starea rations and higher ($P<0.05$) in Urea rations. Haskins et al. (1967) reported that no relationship of nitrogen source to molar percentages of VFA's but Davis et al. (1957) found more acetate and greater A/P ratios in rumen fluid from SBM than from urea-supplemented cattle. Moreover, the mechanism for the decreased A/P ratios in rumen fluid of steers receiving high sulfur diets may involve a more efficient, sulfur-dependent metabolic pathway. Whanger and Matrone (1966) offered evidence of propionate synthesis via the acrylate pathway from lactate, a compound utilized poorly by animal tissue. This system taps a

supply of energy otherwise deficient in rations which are inadequate in sulfur and provides an additional source of propionate thereby improving the overall efficiency of the diet. However, Holter et al. (1971) also found higher proportions of acetate and butyrate have been depressed compared to controls whereas propionate and total VFA's were increased markedly in rations containing SBM (Davis et al., 1957; Hutjens and Schultz, 1971)

2.16.10 Effect of Starea as a protein source on rumen ammonia nitrogen concentration

Roman-Ponce et al. (1974) reported that rumen ammonia N (NH_3-N) concentration was higher ($P<0.05$) for all rations at 1 h after feeding than 2 h (28.2 and 19.0 mg%). Rumen ammonia was similar for Urea and Starea rations but higher than SBM at 1 h ($P<0.05$) and 2 h ($P<0.01$). In all treatments rumen NH_3-N concentration were higher at 1 h than 2 h (28.2 and 19.0). However, Davis and Stallcup (1967) reported peaks of ammonia concentration in rumen contents 2 or 3 h after feeding either SBM, Urea or SBM+Urea rations. Thompson et al. (1972) found peak rumen NH_3-N for Starea rations 90 min after feeding. Although rumen NH_3-N values for Urea and Starea were not significantly different at either 1 or 2 h, the decline in NH_3-N from 1 to 2 h was 12.8 for Urea and 6.4 mg% for Starea, suggesting a slower hydrolysis for Starea than Urea rations, in agreement with Stiles et al. (1970) and Schmidt et al. (1973). Many workers (as these data confirm) have obtained higher ammonia contents after feeding with Urea than SBM rations (Davis and Stallcup,1964; Freitag et al.,1968; Schmidt et al., 1973; Thompson et al., 1972). Stiles et al. (1970) observed lower rumen ammonia content in the starea-fed cows than urea-fed cows. Others (like these data) failed to find lowering of rumen ammonia with starea as

compared to Urea (Schmidt et al., 1973) but did find a slow decline in rumen ammonia with Starea after feeding which agreed with these results. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns, e.g. lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with the Urea rations having the opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when Urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with more acid rumen pH. Schmidt et al. (1973) reported that at the 1.5 h sampling time, ruminal ammonia levels in animals fed urea or Starea were not different ($P<0.05$), perhaps indicating that Starea was hydrolyzed slower than urea (Stiles et al., 1970). If this were the case, then one would expect ruminal ammonia of the urea fed group to be higher than that of the Starea fed group prior to the initial post-feeding sample. Helmer et al. (1970a) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. Rumen ammonia levels in animal fed urea and Starea were higher than those for animals fed SBM and TSBM until the 3.5 h (urea) and 5.5 h (Starea) sampling times. There were no differences in the levels of rumen NH_3-N in animals fed the SBM or TSBM diets. Moreover, high rumen ammonia has been associated with higher content of SBM or crude protein in rations (Freitag et al., 1968).

2.16.11 Effect of Starea as a protein source on blood ammonia and blood urea concentration

Schmidt et al. (1973) Reported that blood NH_3-N level were only slightly higher when urea was used as a nitrogen source than when SBM, TSBM or Starea were used as the nitrogen sources (1.2 and 1.1 μg NH_3-N/ml blood). An increase in blood NH_3 levels would not be expected since the rumen NH_3 concentrations at the 1 h sampling time were approximately one-half the 30 mM/liter rumen fluid necessary to result in a rise in peripheral blood ammonia. Blood urea nitrogen reached its peak concentration approximately 1 h after the rumen ammonia peak irrespective of supplemental nitrogen source. At 2.5 h, BUN was lower in the animals fed Starea than for those fed urea ($P<0.05$), and except for the 0 and 12 h samples, the levels for the animals fed Urea and Starea were higher than those for SBM and TSBM fed groups ($P<0.05$). These data indicate a slower rate of ammonia release and/or more complete uptake of the NH_3-N for microbial protein synthesis when SBM rather than Urea was the nitrogen source. In contrast, reported by Jones et al. (1974) that the highest ($P<0.05$) blood urea nitrogen was with the SBM ration. Reducing dietary crude protein content resulted in lower BUN.

2.16.12 Effect of Starea as a protein source on microbial protein synthesis

Stiles et al. (1970) reported that the results with expanded grain and Starea are similar to those obtained *in vitro* by Helmer et al. (1970a). They observed that Starea or a mixture of expanded grain plus urea lowered rumen ammonia concentration and increased microbial protein synthesis *in vitro* when compared with a mixture of ground grain plus urea. Starea, when tested *in vitro*, was slightly superior to the expanded grain and urea may result in a product that effects the rate of ammonia

release from urea to make its conversion to microbial protein more efficient. If expansion processed grain is valuable in urea utilization, it would be more logical to process the grain with urea (Starea) than to feed expended grain and urea separately. Starea provides enhanced palatability and reduced segregation of urea in mixed feed. The recovery of urea nitrogen in the Starea supplements processed was 98 %, indicating little if any loss of nitrogen during processing. Caffrey et al. (1967) found that rumen microorganisms from sheep had adjusted to urea-rich diets within 13 to 19 days for *in vitro* and *in vivo* studies, respectively. Moreover, Clark et al. (1973) concluded that urea nitrogen was utilized less efficiently than nitrogen from SBM. Expanded corn plus urea was developed to reduce ruminal ammonia, and this decrease has been observed in *in vitro* studies (Helmer et al., 1970a). The advantageous use of urea is restricted to conditions where rumen ammonia concentrations would be below optimal for rumen bacterial growth (Lampila, 1972), perhaps as low as 7 mg rumen ammonia per 100 ml (Oyaert and Bouckaert, 1960). Using published equations (Satter and Roffler, 1973), They estimate that rumen ammonia in cows fed low protein, Urea Starea and SBM rations were 9.3, 12.2 11.9 and 11.8 mg %. The resistance of protein to attack by rumen microorganisms was correlated negatively with protein solubility (Hendrickx and Martin, 1963).

2.16.13 Effect of Starea as a protein source on milk production

Effect of Starea as a protein source on milk production (Table. 2.4). Helmer et al. (1970b) reported that cows receiving either soybean meal or Starea as the protein supplement in their grain ration consumed more grain and produced more milk than those receiving urea. Grain intake was lower ($P<0.05$) for the urea-supplemented group in all experimental periods. Differences between the soybean meal and Starea-

supplemented groups were not significant. Cows in the soybean group gave more milk ($P<0.05$) than did those in the urea group. Milk production by the Starea group did not differ significantly from either of the two other groups. Although differences in milk production were not significant, the Starea and SBM groups produced significantly more milk than did the Urea group. Since the cows in the urea group lost weight during all three periods, it may be assummed that their milk production was sustained some what by utilization of body stores.

Milk fat was higher in all experimental periods for cows receiving the urea-containing grain, but this difference was significant ($P<0.05$) only in Period 1. Total solids in milk were not affected by source of protein, but differences in milk production made total solids production significantly lower for the Urea group in period 3. The relationship among lactose values was similar to that for total solids. Solid-not-fat were not significantly affected by treatment except during Period 2 when milk of urea group contained significantly less SNF than that from the two other groups. Again, milk production differences influenced production of SNF during Period 3. Milk protein was reduced significantly by Urea feeding during Period 1 and 2 but not during Period 3. Although more total milk protein was reduced in all experimental periods by cows receiving either soybean meal or Starea than by cows receiving urea, the difference was significant only in Period 1.

The reduction in milk fat occurring when cows were fed either soybean meal or Starea proably resulted from increased grain intake. When grain intake of cows receiving soybean meal or Starea approached 13 kg per day, milk fat dropped sharply. The amount of roughage fed was limited; therefore, when daily grain intake exceeded 13 kg, the hay-to-grain ratio probably was too high in grain to maintain milk fat levels. Urea-fed cows, consuming less grain, maintained normal milk fat levels.

Milk protein content and total quantity of milk protein produced were affected by experimental treatment. Combining urea with a more readily available carbohydrate (as in Starea) apparently increased the efficiency of urea's conversion of dietary protein to milk protein. Colenbrander et al. (1967) observed a similar response in conversion of dietary protein to milk protein when expanded grain and ground hay were fed. Hotchkiss et al. (1960) have shown that under feeding depressed milk protein; therefore, reduced grain intake may have contributed to the decrease in milk protein by the urea-fed cows. Jones et al. (1974) reported that there were no significant differences in milk production between either sources of supplemental nitrogen or ration protein. Compared to SBM, reductions in milk yield were 9.3, 1.9, and 4.7% for low protein, urea, and Starea rations. Milk fat percentage was not influenced by ration, but milk protein content was reduced ($P<0.05$) by the low protein ration. Roman-Ponce et al. (1974) reported that milk yield was similar for SBM and Starea rations and higher than in Urea ration ($P<0.01$). No differences were found for milk yield between amounts or forms of bagasse pellets. Starea rations and SBM resulted in low milk fat percents than urea rations (3.3, 3.2 and 3.6; $P<0.05$) which equalized SCM production among these rations. Total solids percent was lower ($P<0.05$) in Starea rations than SBM and Urea rations. Equal responses in milk yield for Starea and SBM rations (higher than Urea rations) agree with Helmer et al. (1970b).

Table 2.5 Effect of Starea as a protein source on milk production

References	Treatment	Milk (Kg / d)	Fat (%)	Protein (%)	Lactose (%)	Solid not fat (%)	Total solid (%)
Helmer et al.	SBM	18.3[b]	3.3[b]	3.5[b]	4.4	8.6	11.8
(1970b)	Urea	14.4[a]	3.9[a]	3.2[a]	4.3	8.1	12.2
	Starea	17.6[b]	3.3[b]	3.4[b]	4.3	8.4	11.8
Jones et al.	Low						
(1974)	protein	23.4	3.3	2.6[a]	4.1	8.1	12.0
	SBM	25.8	3.3	2.8[b]	4.5	8.9	12.5
	Urea	25.3	2.9	2.7[b]	4.2	8.2	12.2
	Starea	24.6	3.0	2.8[b]	4.3	8.7	12.4
Roman-	SBM	14.9	3.3[a]	3.4	-	8.9	12.2[a]
Ponce (1974)	Urea	13.9	3.6[b]	3.2	-	8.7	12.4[a]
	Starea	14.8	3.2[a]	3.3	-	8.8	11.9[b]

[a,b] Means within a colum with different superscripts differ ($P<0.05$), SBM= soybean meal

The positive effect of Urea rations on milk fat percent can be explained by higher proportions of rumen acetate ($P<0.01$) in these rations. Theses results differed from those by Randel et al. (1975) in which crude protein source did not affect rumen VFA, and VFA did not provide an explanation for the low milk fat percent. Association in the rumen has been positive (Colovos et al., 1967a; Davis and Stallcup, 1964; Polan et al., 1968) with positive association also between these factors and increased milk fat percent (Colovos et al., 1967; Polan et al., 1968). An opposite effect of urea on milk fat percent was shown by Holter et al. (1971). They also found higher proportions of acetate and butyrate have been depressed compared to controls whereas

propionate and total VFA's were increased markedly in rations containing SBM (Davis et al., 1957; Hutjens and Schultz, 1971)

Earlier studies showed that a urea ration produced slightly less milk and cows were less persistent compared to a ration compared to a ration comprised of natural plant proteins (Archibald, 1943). Bartlett and Blaxter (1947) observed a slight decrease in milk production to urea. Huber et al. (1967) fed rations were urea supplied 0, 11, 22, 38, or 48% of the ration nitrogen and found a significant decrease in milk yield when urea supplied 22% of the nitrogen and marked depressions at 38 and 48%. For this reason the rations were formulated so that NPN sources would supply no more than 20 to 22% of total ration nitrogen.

Utilization of urea nitrogen or its retention for utilization by animal tissues was slightly inferior to all plant proteins. This may have been due to urea's rapid hydrolysis as reviewed by Helmer and Bartley (1971). Conrad and Hibbs (1968) reported that approximately 32% of the dietary nitrogen was converted to nitrogen of milk and tissue when the ration was comprised of only plant proteins compared to 29 to 32% with high urea, high grain concentrates. In steers urea had to supply more than 20% of the total dietary nitrogen before efficiency of nitrogen metabolism was reduced (Coleman and Barth, 1974).

2.16.14 Effect of Starea as a protein source on body weight change and average daily gain

Helmer et al. (1970b) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. The average change in body weight shows that dairy cows fed urea lost significantly more weight than those

fed soybean meal or Starea. Differences in weight grains were not statistically significant between the soybean meal and Starea fed animals. Body weight change, like milk production, was directly related to grain intake. The unacceptability of the urea-containing ration had so reduced intake that normal production was not maintained even though body reserves were used to meet production and maintenance requirements. On the other hand, Starea and soybean meal rations were consumed in sufficient quantities to support high production and to maintain or increase body reserves.

2.16.15 Effect of Starea as a protein source on toxicity

Stiles et al. (1970) reported that toxicity developed in Animal no. 15 on the second day while she was receiving the urea ration. This animal responded favorably when given 18 liters of 5% acetic acid by way of the rumen fistula. Similarly Animal no. 04 (Urea) was treated on the eleventh day and responded. However, Animals no.14 (Urea-14th day) and no. 05 (Urea-25th day) were treated with acetic but failed to respond. Perhaps that failure was due to a delay in administering acetic acid after fist signs of toxicity were observed. To be effective it appears that acetic acid should be administered within 30 min of first signs of toxicity. The symptoms of toxicity were: dullness, muscle tremors, eyes rolling back into the head, frequent urination, excessive salivation, muscular in coordination, labored breathing and prostration at the approach of the terminal stage. In the time preceding the death of animal no. 14, the venous circulatory system was in a state of collapse (determine by venous puncture). The first toxic symptoms were usually noticed 20 min after urea administration. The results of the experiment indicate that urea in Starea is not so toxic to animals as is urea mixed with rolled sorghum grain. An adaptation to high levels of

urea was also illustrated because animals recently fed urea ingested almost twice as much urea before toxicity occurred as that ingested by non-adapted animals. The concentration of ammonia in the 30-min sample appeared to have a greater bearing on whether toxicity would result than the concentration in samples taken later. If the ammonia concentration approached 100 mg% of rumen fluid in the 30 min sample, toxicity usually resulted. Ammonia concentration often exceeded the 100 mg concentration at later times without evidence of toxicity. On one occasion when Starea was fed, the ammonia concentration rose to 216 mg at 120 min without producing toxicity. Therefore, this studies have shown that Starea retarded urea hydrolysis in the rumen and increased synthesis of rumen microbial protein. Urea contained in Starea was less toxic and more palatable than urea mixed with with ground grain. Lactating cows confirmed many benefits previously demonstrated for Starea and that processing grain starch and urea as in Starea can overcome many of the problems associated with feeding urea to lactating cows. Additional work to determine optimum starch-to-urea ratios for lactation is in progress. Effects of various kinds of starch also will be studied (Stiles et al., 1970).

These examples of the use of NPN compounds for feeding ruminants do not exhaust all the possibilities of NPN use in livestock feeding. The choice of the best method of use depends on local conditions, the supplies of agro-industrial by-products that can be used as feed, and the equipment available. The agro-industrial by-products are largely unbalanced in their chemical composition, but their supplementation with nitrogen will be useful only if they are deficient in nitrogen and at the same time contain an adequate amount of readily available energy for the conversion of dietary nitrogen into bacterial protein in the rumen. Rations composed of such feeds may be supplemented with additional nitrogen in the form of expensive vegetable or animal

protein concentrates or in the form of inexpensive NPN. Replacement of protein with NPN in feeding ruminants would free large amounts of protein for feeding pigs and poultry. Well balanced rations for ruminants, with a correct energy/nitrogen ratio and also containing such other essential nutrients as fibre and minerals, ensure effective conversion of NPN into tissue and milk proteins, as has been proved in numerous experiments with different NPN combinations in all parts of the world (Kowalczyk, 1971). The classical experiment of Virtanen (1966) with cows fed over several years on a synthetic diet containing NPN as the sole source of nitrogen showed that production of to 5,000 liters per year of milk of normal composition was possible; a further increase of milk yield was limited by the insufficient voluntary intake of feed and the capability of bacteria to synthesize protein. The maximum intake of urea in this experiment reached 600 g per cow daily with no adverse effect on health and fertility. With partial (up to 40%) replacement of protein with NPN, an average milk yield exceeding 6,000 liter per year was obtained (Conrad and Hibbs, 1968). Rations for high-yielding cows should be precisely balanced with regard to all nutreints and should be given and libitum, as in this case the intake is spread over a long period during the day, favouring effective conversion of NPN into microbial protein.

2.17 References

Agbagla-Dohnani, A., Noziere, P., Clement, G., and Doreau, M. (2001). *In sacco* degradability, chemical and morphological composition of 15 varieties of European rice straw. **Anim. Feed Sci. Technol.** 94: 15-27.

Aiple, K. P., Steingass, H., and Drochner, W. (1996). Prediction of net energy content of raw materials and compound feeds for ruminants by different laboratory methods. **Arch. Anim. Nutri.** 49: 213-220.

Archibald, J. G. (1943). **Feeding urea to dairy cows.** Massachusetts Agr. Exp. Sta. Bull. 406.

Baldwin, R. L. (1970). Energy metabolism in anaerobes . **American J. Clin. Nutri.** 23: 1508-1513.

Bartlett, S., and Blaxter, K. L. (1947). The value of urea as a substitute for protein in the rations of dairy cattle. I. Field trials with dairy cows. **J. Agr. Sci.** 37:32.

Barlett, S., and Brooster, W. H. Br. (1958). Feeding trials with ammoniated molasses in the diet of young dairy cattle. **J. Agric. Sci.** 50: 60.

Bartley, E.E., Deyoe, C. W., Pfost, H. B., Anstaett, F. R., and Sung, A. C. (1968). An improved urea product for ruminants. **Anim. Nutri. Health.** May: 10-13.

Behnke, K. C., Deyoe, C. W., Bartley, E. E., and Griffel, G. W. (1973). **Results from examing urea containing protein supplements by scanning electron microscropy.** Page 25 in Feedstuffs Nov. 5.

Beuvink, J. M. W., Spoelstra, S. F., and Hogendorp, R. J. (1992). An automated method for measuring time-course of gas production of feedstuff incubated with buffered rumen fluid. Neth. **J. Agri. Sci.** 40: 401-407.

Blackburn, T. H., and Hobson, P. N. (1960). The degradation of protein in the rumen of the sheep and redistribution of protein nitrogen after feeding. **Brit. J. Nutr.** 14: 445.

Bloomfield, R. A., Kearley, E. D., Creach, D. O., and Muhrer, M. E. (1963). Ruminal pH and absorption of ammonia and VFA. **J. Anim. Sci.** 22: 833.

Blummel. M., and Lebzien, P. (2001). Predicting microbial efficiencies of dairy rations by *in vitro* techniques. **Livest. Pro. Sci.** 68: 107-117.

Blummel, M., and Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral detergent fibers as described by *in vitro* gas production and their relationship to voluntary feed intake. **Brit. J. Nutri.** 77: 757-768.

Blummel, M., Markar, H. P. S., Chisanga, G., Mtimuni, J., and Becker, K. (1997). The prediction of dry matter intake of temperate and tropical roughages from *in vitro* digestibility of African roughages in relation to ruminant live weight. **Anim. Feed Sci. Technol.** 69: 131-141.

Blummel, M., and Ørskov, E. R. (1993). Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. **Anim. Feed Sci. Technol.** 40: 109-119.

Briggs, M. H. (1967). **Urea as a protein supplement.** 1st ed. Pergamon Press, Inc., Long Island City, New York.

Brown, V. A., Rymer, C., Agnew, R. E., and Givens, D. I. (2002). Relationship between *in vitro* gas production profiles of forages and *in vivo* rumen fermentation patterns in beef steers fed those forages. **Anim. Feed Sci. Technol.** 98: 13-24.

Bucholtz, H. F., and Bergen, W. G. (1973). Microbial phospholipid synthesis as a marker for microbial protein synthesis in the rumen. **Appl. Microbial.** 25 : 504.

Caffrey, P. J., Hatfield, E. E., Norton, H. W., and Garrigus, U. S. (1967). Nitrogen metabolism in the ovine. I. Adjusment to a urea-rich diet. **J. Anim. Sci.** 26:959-967.

Chalupa, W. (1968). Problems in feeding urea to ruminants. **J. Ainm. Sci.** 27: 207-215.

Chen, X. B., Samaraweera, L., Kyle, K. J., and Ørskov, E. R. (1996). Urinary excretion of purine derivatives and tissue xanthin oxidase (EC 1.2.3.2) activity in buffaloes (*Bubalis bubalis*) with special reference to difference between buffaloes and *Bos taurus* cattle. **Brit. J. Nutr.** 75 : 397-407.

Chinh, B. V., Ly, L. V., Toa, N. H., Hai, N. V., and Ngoc, T. B. (2000). **Study on processing, storing and using sugarcane leaves as ruminant feed.** Workshop-seminar "Making better use of local feed resources" SAREC-UAF, January, 2000.

Clark, J. H., Spahr, S. L., and Derring, R. G. (1973). Urea utilization by lactating cows. **J. Dairy Sci.** 56: 763-169.

Coleman, S., and Barth, K. M. (1974). Nutrient digestibility and N-metabolism by cattle fed rations based on urea and corn silage. **J. Ainm. Sci.** 39:408-415.

Colenbrander, V. F., Bartley, E. E., Morrill, J. L., Deyoe, C. W., and Pfost, H. B. (1967). Feed processing II. Effect of feeding expanded grain and ground hay on milk composition, yield and rumen metabolism. **J. Dairy Sci.** 50:1966

Colovos, N. F., Holter, J. B., Davis, H. A., and Urban, Jr. W. E. (1967a). Urea for lactating dairy cattle. I. Effect of concentrate fiber and urea levels on nutritive value of the ration. **J. Dairy Sci.** 50: 518-523.

Colovos, N. F., Holter, J. B., Davis, H. A., and Urban, Jr. W. E. (1967b). Urea for lactating dairy cattle. I. Effect of concentrate fiber and urea levels on nutritive value of the ration. **J. Dairy Sci.** 50: 523-528.

Cone, J. W., Van Galder, A. H., and Driehuis, F. (1997). Description of gas production profiles with a three-phasic model. **Anim. Feed Sci. Technol.** 66: 31-45.

Cone. J. W., Van Gelder, A. H., Visscher, G. J. W., and Oudshoorn, L. (1996). Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. **Anim. Feed Sci. Technol.** 61: 113-128.

Corad, H. R., and Hibbs, J. W. (1968). Nitrogen utilization by the ruminant. Appreciation of its nutritive value. **J. Dairy Sci.** 51:276.

Coombe, J. B., Tribe, D. E., and Morrison, J. W. C. (1960). Some experimental observations on the toxicity of urea to sheep. **Aust. J. Agr. Res.** 11: 247.

Cunningham, K. D., Cecava, M. J., Honson, T. S. J., and Luden, P. A. (1996). Influence of source and amount of dietary protein on milk yield by cow in early lactation. **J. Dairy Sci.** 79:620-628.

Davis, G. V., and Stallcup O. T. (1964). Influence of dietary nitrogen on nitrogen metabolism in the rumen. **J. Dairy Sci.** 47:1237.

Davis, G. V., and Stallcup, O. T. (1967). Effect of soybean meal, raw soybeans, corn gluten feed, and urea on the concentrate of rumen fluid components at intervals after feeding. **J. Dairy Sci.** 50: 1638-1645.

Davis, R. F., Woodhouse, N. S., Keeny, M., and Beck, G. H. (1957). The effect of various levels of dietary protein upon the volatile fatty acids in the rumen of the dairy cow. **J. Dairy Sci.** 40: 75.

Davis, K. (1957). **Nutritional availability of components livestock feedstuffs.** Gainesville, Florida Agric. Exp. Station. Annual report. p. 63.

Demeyer, D. I., Henderickx, H. K., and Van Nevel, C. J. (1972). About the nitrogen metabolism in the rumen. **International Symposium on rumen Physiology.** Sofia.

Deyoe, C. W., Bartley, E. E., Pfost, H. B., Boren, F. W., Perry, H. B., Anstaett, F. R., Helmer, L., Stiles, D., Sung, A. C., and Meyer, R. (1968). An improved urea product for ruminants. (Abstr.). **J. Anim. Sci.** 27: 1163.

Egan, A. R. (1992). Strategies of feeding and nutrition of dairy cattle based on crop residues. In : The 6th AAAP Animal Science Congress, Bangkok, Thailand. (Eds. Bunyavejchewin, P., S. Sangdid and K. Kangsanet), **The animal Husbandry Association of Thailand.** 167-176.

Egbunnike, G. N., and Ikpi, A. E. (2005). Can agro-indrustrial by products and crop residues save the Nigerian livestock in dustry [online]2005 [cited 2005 Sep 25].Available from:http.//www.ilri.cgiar.org/InfoServ/Webpub/Fulldocs /X5536e/x5536e0y.htm.

Ellis, W. C., and Pfander, W. H. (1965). Rumen microbial polynucleotide synthesis and its possible role in ruminant nitrogen utilization. **Nature.** 205: 974.

Erlinger, L. L., and Klofenstein, T. (1971). Ammoniated acid hydrolized wood residue as a source of nitrogen for ruminants. **J. Anim. Sci.** 41: 1189-1195.

Faichney, G. J., and Weston, R. H. (1971). Digestion by ruminant lambs of a diet containing formaldehyde-treated casein. **Aust. J. Agric. Res.** 22:461.

Fonnesbeck, W. S., Kearl, L. C., and Harris, L. E. (1975). Feed grade biuret as a protein replacement for ruminants. **J. Anim. Sci.** 40: 1151-1156.

France, J., Theodorou, M. K., Lowman, R. S., and Beever, D. E. (2000). **Feed evaluation for animal production: Feed Systems and Feed Evaluation Models**, (Ed, M. K. Theodorou and J. France, pp. 1-9). CABI publishing CAB international Wallingford Oxon OX10 8DE, UK.

Freitag, R. R., Smith, W. H., and Beeson, W. M. (1968). Factors related to utilization of urea vs. protein nitrogen supplemented diets by the ruminant. **J. Anim. Sci.** 27 :478-485.

Fu, C. J., Felton, E. E. D., LehmKuhler, J. W., and Kerley, M. S. (2001). Ruminal peptide concentration required to optimize microbial growth and efficiency. **J. Anim. Sci.** 79:1305-1312.

Gardner, R. W., and Park, R. L. (1973). Protein requirements of cows fed high concentrate rations. **J. Dairy Sci.** 56: 390-397.

Gerson, T., King, A. S. D., Kelly, K. E., and Kelly, W. J. (1988). Influence of particle size and surface area on *in vitro* rates of gas production, lipolysis of triacylglyceral and hydrogenation of linoleic acid by sheep rumen digesta or *Ruminococcus flavefaciens*. **J. Agri. Sci.** 110: 31-37.

Getachew, G., Blummel, M., Makkar, H. P. S., and Becker, K. (1998a). *In vitro* measuring tecniques for assesment of nutritional quality of feeds: a review. **Anim. Feed Sci. Technol.** 72:261-281.

Getachew, G., Blummel, M., Makkar, H. P. S., and Becker, K. (1998b).The *in vitro* gas coupled with ammonia nitrogen measurement for evaluation of nitrogen degradability in low quality roughages using incubation medium of different buffering capacity. **Anim. Feed Sci. Technol.** 77:87-95.

Getachew, G., Crovetto, G. M., Fondivila, M., Krishnamoorthy, U., Sing, B. H., Spaghero, M., Steingass, H., Robinson, P. H., and Kailas, M. M. (2002). Laboratory variation of 24 h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. **Anim. Feed Sci. Technol.** 102: 169-180.

Gil, L. A., Shirley, R. L., and Moore, J. E. (1973). Effect of methionine hydroxy analog on growth, amino acid content, and catabolic products of glucolytic rumen bacteria *in vitro*. **J. Dairy Sci.** 56: 757-765.

Goodrich, R. D., and Tillman. (1966). Effects of sulfur and nitrogen sources and copper levels on the metabolism of certain minerals by sheep. **J. Anim. Sci.** 25: 484.

Groot, J. C., Cone, J. W., Williams, B. A., Debersaques, F. M. A., and Lantinga, E. A. (1996). Multiplephasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feed. **Anim. Feed Sci. Technol.** 64:77-89.

Hammon, D. S., Wang, S., and Dhiman, G. R. (2005). Associations between blood plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactating dairy cows. **Anim. Reprod. Sci.** 89 : 365-430.

Hanada, M., Aibibula, Y., Abdulrazak, O., Muurata, S., and Okamoto, M. (2004). Comparision of potato pulp silage with rolled corn as an energy source of growing Holstein steers. New dimensions and Challenges for Sustainable Livestock Farming VII. In: **Proceeding of 11th Animal production Societies**, 5-9th September 2004, Kuala Lumpur Malaysia; 2004. P. 367-369.

Haskins, B. R., Wise, M. B., Craig, H. B., and Barrick, E. R. (1967). Effect of levels of protein, source of protein and antibiotic on performance, carcass characteristics, rumen environment and liver abscesses of steers fed all-concentrate rations. **J. Anim. Sci.** 26: 430.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25.

Helmer, L. G., Bartley, E. E., Deyoe, C. W., Meyer, R. M., and Pfost, H. B. (1970a). Feed processing. V. Effect of an expansion-processes mixture of grain and urea (Starea) on nitrogen utilization *in vitro*. **J. Dairy Sci.** 53 : 330-335.

Helmer, L. G., Bartley, E. E., and Dyoe, C. W. (1970b). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Hendrickx, H., and Martin. (1963). *In vitro* study of the nitrogen metabolism in the rumen. Compt. Rend. Rech., Inst. Rech. Sci. 2 nd Agri., Bruxelles 31:1. (As cited by Hungate, R. E. 1966. **The rumen and its microbes.** Academic Press, New York.)

Herrera-Saldana, R., Gomez-Alarcon, R., Torabi, M., and Huber, J. T. (1990). Influence of synchronizing protein and starch degradation in rumen on nutrient utilization and microbial protein synthesis. **J. Dairy Sci.** 73: 142-148.

Herrero, M., Murray, I., Fawcett, R. H., and Dent, J. B. (1996). Prediction of *in vitro* gas production and chemical composition of kikuyu grass by near-infrared reflectance spectroscopy. **Anim. Feed Sci. Technol.** 60: 51-67.

Hindrichsen, I. K., Osuiji, P. O., Odenyo, A. A., Madsen, J., and Hvelplund, T. (2001). Effect of supplementation with four multipurpose trees and *Lablab purpureus* on rumen microbial population, rumen fermentation, digesta kinetics and microbial protein supply of sheep and maize stover *ad libitum*. **TSAP Proceeding.** Vol. 28.

Hobson, P. N. (1971). Rumen microorganisms. Progr. Indust. Microbial. 9: 41.

Holter, J. B., Colovos, N. F., Clark, R. M., Koes, R. M., Davis, H. A., and Urban, Jr. W. E. (1971). Urea for lactating cattle. V. Concentrate fiber and urea in a corn silage-high concentrate ration. **J. Dairy Sci.** 54: 1475-1482.

Hossain, M. A., and Becker, K. (2002). *In vitro* rumen degradability of crude protein in seeds from four Sesbania spp. And the effect of treatments designed to reduce the levels of anti nutrients in the seeds. **Anim. Feed Sci. Technol.** 95: 49-62.

Hotchkiss, D. K., Jacobson, N. L., and Cox, C. P. (1960). Effect of various hay:concentrate rations and levels of feeding on production and composition of milk of dairy cows. (Abstr.) **J. Dairy Sci.** 43:872.

Huber, J. T. (1975). Protein and non protein nitrogen utilization in practical dairy rations. **J. Anim. Sci.** 41: 954-960.

Huber, J. T., Sandy, R. A., Polan, C. E., Bryant, C. T., and Blaser, R. E. (1967). Varying levels of urea for dairy cows fed corn silage as the only forage. **J. Dairy Sci.** 50 : 1241-1246.

Huber, J. T., and Sandy, R. A. (1965). Response of dairy cows fed unlimited corn silage to three levels of urea and grain. (Abstr.) **J. Anim. Sci.** 24 : 887.

Hungate, R. E. (1966). **The rumen and its microbes.** Academic Press, London.

Hutjens, M. F., and Schultz, L. H. (1971). Addition of soybeans or methionine analog to high-concentrate rations for dairy cows. **J. Dairy Sci.** 54:1637-1646.

Ibrahim, E. A., and Ingalls, J. R. (1972). Microbial protein biosynthesis in the rumen. **J. Dairy Sci.** 55: 971-978.

Jones, G. M., Stephens, C., and Kensett, B. (1974). Utilization of Starea, Urea, or Soybean Meal in Complete Rations for Lactating Dairy Cows. **J. Dairy Sci.** 58: 689-695.

Khazaal, K., Dentinho, M. T., Riberio, J. M., and Ørskov, E. R. (1993). A comparison of gas production during incubation with rumen contents *in vitro* and nylon bag degradability as predictors of the apparent digestibility *in vivo* and the voluntary feed in take of hays. **Anim. Prod.** 57: 105-112.

Kearl, L. C. (1982). Nutrient requirements of ruminants in developing countries. **Internation feedstuffs institute**, Utah Agricultural experiment station, Utah State University, Logan, Utah, USA.

Kowalczyk, J. (1971). **Nitrogen and carbohydrate metabolism in the rumen of young bulls fed with the urea/molasses diet.** Thesis. Institute of Anim. Physiology and Nutrition , Jabloa near Warsaw.

Kowalczyk, J., Havassy, I., Otwinowska, A., and Kosta, K. (1975). Passage of Intravenously administered 15N urea into the digestive tract and its excretion in the sheep. **Acta physiol. Pol.** 26: 299.

Kowalczyk, J., Ramimirez, A., Geerken, C. M. (1970). Nitrogen and carbohydrate metabolism in the rumen and duodenum of young bulls given diets base on molasses and urea. **Rev. cubana Clenc. Agric.** 4: 181.

Krishnamoorthy, U., Soller, H., Steingass, H., and Menke, K. H. (1995). Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analysis and rumen inoculums studies *in vitro*. **Anim. Feed Sci. Technol.** 52: 177-188.

Lampila, M. (1972). Practical non protein-N-feeding to ruminants. **World Rev. Anim. Prod.** 8:28

Leng, R. A. (1997). Application of Biotechnology to Nutrition Animal in Developing Countries. **FAO Animal Production and Health.** Paper No. 90. Armidale, NSW, Australia.

Loosli, J. K., and McDonald, I. W. (1968). Non protein nitrogen in the nutrition of ruminants. **FAO.**

Lowrey, R. S., and McCormick, W. C. (1969). Factors affecting the utilization of high urea diets by finishing steers. **J. Anim. Sci.** 28: 406-415.

Maeng, W. J., Park, H., and Kim, H. J. (1997). The role of carbohydrate supplementation in microbial protein synthesis in the rumen. In Rumen Microbes and Digestive Physiology in Ruminants (Onodera et al., eds.). pp. 107-119. **Japan Sci. Soc.** Press, Tokyo / S. Karger, Basel.

Macheboeuf, M., Jestin, M., Andrieu, J., and Martin-Rosset, W. (1997). Prediction of organic matter digestibility of forages in horses by gas test method. In: *In vitro* technique for measuring nutrient supply to ruminant. **Proceeding of Occasional Meeting of the British Society of Animal Science:** 8-10 July 1997, University of Reading, UK.

Makkar, H. P. S., Aregheore, E. M., and Becker, K. (1999). Effect of saponins and plant extracts containing saponins on the recovery of ammonia during urea-ammoniation of wheat straw an fermentation kinetics of treated straw. **J. Agric. Sci.** (Camb). 132: 313-321.

Markkar, H. P. S., Blummel, M., and Becker, K. (1995a). Formation of complexes between polyvinyl pyrolidones or polyethylene glycols and tannins, and their implication on gas production and true digestibility in *in vitro* techniqes. **Brit J. Nutr.** 73:879-913.

Markkar, H. P. S., Blummel, M., and Becker, K. (1995b). *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. **J. Food. Agric.** 69: 481-493.

Mason, V. C., and White, F. (1971). The digestion of the bacterial mucopeptide constituents in the sheep. I. The metabolism of 2,6 diamino pimelic acid. **J. agric. Sci.** 77: 91-96.

Mathis, C. P., Cochran, R. C., Vanzant, E. S., Abdelgadir, I. E. O., Heldt, S., Olson, K. C., Johnson, D. E., Caton, J., Faulkner, D., Horn, G., Paisley, S., Mass, R., Moore, K., and Halgerson, J. (2001). A collaborative study comparing an *in situ* protocol with single time point enzyme assays for estimating ruminal protein degradability of different forages. **Anim. Feed Sci. Technol.** 93: 31-42.

Matrone, G., Bunn, C. R., and McNeill, J. J. (1964). Investigations of dietary factors in purified diets for ruminants. **J. Nutr.** 84: 215.

Mauricio, R., Owen M. E., Mould, F. L., Givens, I., Theodorou, M. K., France, J., Davies, D. R., and Dhanoa, M. S. (2001). Comparison of bovine rumen liquor and bovine feces as inoculums for an *in vitro* gas production technique for evaluating forages. **Anim. Feed Sci. Technol.** 89: 33-48.

McDonald, I. W. (1948). The absorption of ammonia from the rumen of sheep. **Biochem. J.** 42: 584.

McDowell, R. E., and Hernandez-Urdaneta, A. (1975). Intensive systems for beef production in tropics. **J. Anim. Sci.** 41: 1228-1235.

McNaught, M. L., Oven, E. C., Henry, K. N., and Kon, S. K. (1954). The utilization of non protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa and brewer's yeast for rats. **Biochem. J.** 56: 151.

Menke, K. H., Raab, L., Salewski, A., Steingrass, H., Fritz, D., and Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. **J. Agri. Sci. (Camb).** 93: 217-222.

Menke, K., and Steingrass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. **Anim. Res. and Development.** 28: 7-55.

Meyer, R. M., Bartley, E. E., Deyoe, C. W., and Colenbrander, V. F. (1967). Feed processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. **J. Dairy Sci.** 50: 1327.

Mgheni, D. M., Ndemanisho, E. E., Hvelplund, T., and Weisbjerg, M. (2001). Evaluation of the feeding value of two tropical cereal straw, maize stover, rice straw and their botanical fractions by nylon and mobile bag tecnique. **African. J. Sci. and Technol.** 2: 65-71.

Miller, E. L. (1973). Evaluation of foods as sources of nitrogen and amino acids. **Proc. Nutr. Soc.** 32 : 79-84.

Murray, I. (1993). Forage analysis by near infrared reflectance spectroscopy. (Eds., A. Davies, R. D. Baker, S. A. Grant and A. S. Laildlaw, pp. 285-312), Sward measurment Hanbook. **Br. Grassl. Soc.**

Nagadi, S., Herrero, M., and Jessop, N. S. (2000). The influence of donor animal on the initial bacterial concentration of ruminal on the initial bacterial concentration of ruminal fluid and *in vitro* gas production degradability parameters. **Anim. Feed Sci. Technol.** 87: 231-239.

Nataraja, M. B., Krishnamoorthy, U., and Krishnappa, P. (1998). Assessment of rumen *in vitro* incubation (gas production) technique and chemical analyses by detergent system to predict metabolizable energy content mixed diets of lactating cows. **Anim. Feed Sci. Technol.** 74: 169-177.

NRC, (1976). Urea and other non protein nitrogen compounds in animal nutrition. **National Academy of Sciences**, Washington, U.S.A.

National Research Council. (2001). **Nutrient requirements of dairy cattle.**7th ed. Acad. Sci. Washington, DC.

National Academy of Sciences-National Research Countcil. (1971). **Nutrients requirements of dairy cattle.** No. 3. Washington, DC.

Nitipot, P., and Sommart, K. (2003). Evaluation of ruminant nutritive value of cassava starch industry by products, energy feed sources and roughages using *in vitro* gas production tecnique. In: **Proceeding of Annual Agricultural Seminar for year 2003**, 27-28 January, KKU; 2003. P. 179-190.

Nocek, G. E., and Russell, J. B. (1988). Protein and energy as an integrated system : relationship of ruminal protein and carbohydrate availability to microbial synthesis and production. **J. Dairy Sci.** 71: 2070-2079.

Nolan, J. V., Norton, B. W., and Leng, R. A. (1973). Nitrogen cycling in sheep. **Proc. Nutr. Soc.** 32: 93.

Oliverla, R. M. P. (1998). **Use of *in vitro* gas production tecnique to assess the contribution of both soluble and insoluble fractions on nutritive value of forages** [Master Thesis in Animal Nutrition]. Aberdeen: University of Aberdeen Scotland; 1998.

Ørskov, E. R., and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **J. Agric. Sci.** 92:499-504.

Ørskov, E. R. (1976). Nitrogen digestion and utilization by young and lactating ruminants. **Wld. Rev. Nutr. Diet.** 25, inpress.

Ørskov, E. R., Fraser, C., McDonald, I., and Smart, R. (1974). Digestion of concentrate in sheep. 5. The effect of adding fish meal and urea together to cereal diets on protein digestion and utilization by young sheep. **Brit. J. Nutr.** 31: 89-96.

Ørskov, E. R., Fraser, C., McDonald, I., and Smart, R. (1972). Digestion of concentrate in sheep. 4. The effect of urea on digestion, nitrogen retention and growth in young lambs. **Brit. J. Nutr.** 27: 491-497.

Oyaert, W., and Bouckaert, J. H. (1960). Quantitative aspects of food digestion in the rumen. **Zentr. Veterinamedizin**. 7:929. (As cited by lampila 1972.)

Peter, A. P., Hatfield, E. E., Owens, F. N., and Garrigus, U. S. (1971). Effects of aldehyde treatments of soybean meal on *in vitro* ammonia release, solubility and lamb performance. **J. Nutr.** 101 : 605.

Pitt, R. E., Cross, T. L., Pell, A. N., Schofield, P., and Doane, P. H. (1999). Use of *in vitro* gas production models in ruminant kinetics. **Math. Biosci.** 159: 145-163.

Prachyalak, P., Leeratanchai, U. Wanasitchaiwat, and V. (1996). Using Agricultural by products as basal feed for lactating cows in the dry season. **Annaul Report of Animal Nutrition Division**, 1996. P. 62-69.

Preston, T. R., and Willis, M. B. (1970). **Intensive beef production.** Pergamon Press, Oxford.

Polan, C. E., Huber, J. T., Sandy, R. A., Hall, J. W., Jr., and Miller, C. N. (1968). Urea-treated corn silage as the only forage for lactating cows. **J. Dairy Sci.** 51:1445-1451.

Poppi, D. P., Stuart, R., and McLennan, R. (1995). Protein and energy utilization by ruminants at pasture. **J. Anim. Sci.** 73: 278-290.

Raab, L., Cafantaris, B., Jilg, T., and Menke, K. H. (1983). Rumen protein degradation and biosynthesis: A new method for determination of protein degradation in the rumen fluid *in vivo*. **Brit. J. Nutr.** 50: 569-582.

Randel, P. F., Van Horn, H. H., Wilcox, C. J., Roman-Ponce, H., Marshall, S. P., and Bachman, K. C. (1975). Complete rations for dairy cattle. IV. Comparison of supplemental nitrogen sources by metabolizable protein concept. **J. Dairy Sci.** 58: 1109-1116.

Reid, J. T. (1953). Urea as a protein replacement. A review. **J. Dairy Sci.** 36: 955.

Roffler, R. E., and Satter, L. D. (1973). Using NPN in the dairy cow ration. Page 45 in **Proc. 34th Min. Nutr. Conf.** Bloomington, MN.

Roman-Ponce, H., Van Horn, H. H., Mashall, S. P., Wilcox, C. J., and Randel, P. F. (1974). Complete Rations for Dairy Cattle. V. Interaction of Sugarcane Bagasse Quantity and Form with Soybean Meal, Urea, and Starea. **J. Dairy Sci.** 58: 1320-1327.

Russell, J., and Dombrowski, B. (1980). Effect of pH on efficiency of growth by pure cultures of rumen bacteria in continuous culture. **Applied and Environ. Microb.** 39: 604-610.

Rymer, C., and Givens, D. I. (1998). Relationship between the rates if production of gas and VFA during *in vitro* incubations of high temperature dried grass. In: Proceedings of the **British Society of Animal Science**; 1998 March 22-24, Scarborough.

Sandoval-Castro, C. A., Capetillo-Leal, C., Cetina-Gongora, R., and Ramirez-Aviles, L. (2002). A mixture simture simplex design to study associative effects with an *in vitro* gas production technique. **Anim. Feed Sci. Technol.** 101: 191-200.

Satchaphan, B., Kukkaew, P., Sumamal, W., and Senagate, B. (1998). Use of tomato pomance as animal feed "Use of dry tomace as roughage for steers". **Annual Report of Animal Nutrition Division**, 1998. P. 1-11.

Satter, L. D., and Roffler, R. E. (1973). Using NPN in the dairy cow ration. Page 45 in **Proc. 34th Minn. Nutr. Conf.** Bloomington, MN.

Scarr, M. J. (2005). The optimal use of agro-industrial by products and crop residues in Nigeria [online] 2005 [cite 2005 Sep 25). A vailable from: http://www.fao.org/Wairdocs/ILRI/x5494E/x5494e0k.htm.

Schelling, G. T., and Hatfield, E. E. (1968). Effect of abomasally infused nitrogen sources on nitrogen retention of growing lambs. **J. Nutr.** 96:319.

Schofield, P. (2000). Gas production method: **Farm Animal Metabolism and Nutrition.** (Ed., J. P. F. Dmello), CABI Publishing CAB International Walling ford Oxon OX10 8DE. UK. pp 209-232.

Schofield, P., Pitt, R. E., and Pell, A. N. (1994). Kinetics of fiber digestion from *in vitro* gas production. **J. Anim. Sci.** 72: 2980-2991.

Skunmun, P., Kaewsomprasong, K, and Chamuhnit, W. (2004). Use of ensiled cassava pulp for feeding of buffalo. New dimensions and Challenges for Sustainable Livestock Farming (V.II). In: **Proceeding of 11th Animal Production Societies**, 5-9th September 2004, Kuala Lumpur Malaysia. p. 376-379.

Schmidt, S. O., Jorgensen, N. A., Benevenga, N. F., and Burngart, V. H. (1973). Comparison of soybean meal, formaldehyde treated soybean meal, urea and Starea for steers. **J. Anim. Sci.** 37 : 1233-1237.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Stokes, S. R., Hoover, W. H., Miller, T. K., and Manski, R. P. (1991). Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. **J. Dairy Sci.** 74: 860-870.

Suilapwa, N. J., and Simukko, H. (2005). Status of crop residues and agro-industrial by-products as supplementary animal feed in Zambia-A review [online] 2005 [cited 2005 Sep 25].Available from:http://www.fiuc.org/esap/ZAMB/ZAMB7/ General/cropszambia.php.

Tamminga, S. (2006). The effect of supply of rumen degradable protein and metabolizable protein on negative energy balance and fertility in dairy cows. **Anim. Reprod. Sci.** 96 : 227-239.

Tessema, Z., and Baars, R. M. T. (2004). Chemical composition, *in vitro* dry matter digestibility and degradation of Napier grass (*Pennisetum Purpurreum* (L) Schumach.) mixed with different levels of *Sebania Sesban* (L.) Merr. **Anim. Feed Sci. Technol.** 117: 29-41.

Thomas, P. C. (1973). Microbial protein synthesis. **Proc. Nutr. Soc.** 32: 85.

Thomas, W. E., Loosli, J. K., Williams, H. H., and Maynard, L. A. (1951). The utilization of inorganic sulfates and urea nitrogen by lambs. **J. Nutr.** 43:515.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Tilley, J. M. A., and Terry, R. A. (1963). A two stage technique for the *in vitro* digestion of forage crops. **J. Brit. Grass. Soc.** 18:104-111.

Tillman, A. D., and Kidwell, J. F. (1951). The value of ammoniated condensed distiller's molasses solubles as feed for beef cattle. **J. Anim. Sci.** 10: 943.

Van Horn, H. H., and Jacobson. (1971). Response of lactating cows to added increments of dietary protein and non protein nitrogen. **J. Dairy Sci.** 58:1101-1108.

Van Horn, H. H., Foreman, C. F., and Rodriguez, J. D. (1967). Effect of high-urea supplementation on feed intake and milk production of dairy cows. **J. Dairy Sci.** 50 : 709-716.

Van Horn, H. H., Marshall, S. P., Wilcox, C. J., Randel, P. F., and Wing, J. M. (1975). Complete rations for dairy cattle. III. Evaluation of protein percent and quality, and citrus pulp-corn substitutions. **J. Dairy Sci.** 58: 1101-1106.

Van Soest, P. J. (1982). **Nutritional ecology of the ruminant.** O&B Books, Inc., Corvalis, Oregon, U.S.A.

Virtanen, A. J. (1966). Milk production of cows on protein-free feed. **Science.** 53:1603.

Volden, H., Mydland, L. T., and Olaisen, V. (2002). Apparent ruminal degradation and rumen escape of soluble nitrogen fraction in grass and grass silage administered intraruminally to lactating dairy cows. **J. Anim. Sci.** 2002. 80:2704-2716.

Volden, H. (1999). Effects of level of feeding and ruminally undergraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile and performance of dairy cows. **J. Anim. Sci.** 77: 1905-1918.

Walker, D. J., and Nader, C. J. (1968). Medthod for measuring microbial growth in rumen content. **Appl. Microbial.** 16: 1124-1129.

Wallace, R. J., Atasoglu, C., and Newbold, C. J. (1999). Role of peptides in rumen microbial metabolism. Review. **Asian-Aust. J. Anim. Sci.** 12: 139-149.

Wanapat, M., and Pimpa, O. (1999). Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. **Asian-Aust. J. Anim. Sci.** 12: 904-907.

Wanapat, M., Pimpa, O., Petlum, A., and Boontao, U. (1997). Cassava hay: A new strategic feed for ruminant during the dry season. **Livestock Research for Rural Development.** 9: 1-5.

Whanger, P. D., and Matrone, G. (1966). Metabolism of lactic, succinic and acrylic acids by rumen microorganisms for sheep fed sulfur-adequate and sulfur efficient diets. **Biochimica et Biophysica Acta.** 136:27.

Woods, V. B., Mara, F. P. O., and Moloney, A. P. (2003). The nutritive value of concentrates feedstuffs for ruminant animals. Part I: *In situ* ruminant degradability of dry matter and organic matter. **Anim. Feed Sci. Technol.** 110: 111-130.

CHAPTER III

EFFECT OF CASPUREA AS A PROTEIN SOURCE REPLACEMENT FOR SOYBEAN MEAL IN DIETS ON PERFORMANCE OF THAI NATIVE x BRAHMAN BEEF CATTLE FED UREA-TREATED RICE STRAW AS A ROUGHAGE

3.1 Abstract

This experiment aimed to study the effects of Caspurea (cassava pulp-urea) on productive performance of Thai Native x Brahman beef cattle. Four Thai Native x Brahman beef cattle (approximately 365 days of age and average live weight of 154.7±26.8 kg) were used in a 4 x 4 Latin square arrangement of treatments with 4 periods, each period consisted 21 days. The treatments were levels of Caspurea replacement for soybean meal in concentrate at 0, 25, 50 and 75%. Concentrates were formulated to contain 14% CP and were fed at 2.0% BW. All animals were fed *ad libitum* urea treated rice straw as roughage. The results showed that total dry matter intake (5.5, 5.5, 5.2, 5.3 kgDM/d; $P>0.05$) was not significantly different among dietary treatments. DM digestibility (71.2, 72.1, 71.2, 67.7%; $P<0.01$) was lowest in 75% replacement diet. DM digestibility decreased linearly ($P<0.01$) and quadratically ($P<0.01$) as the level of Caspurea increased. Ruminal ammonia-N concentration (8.5, 8.8, 9.5, 13.3 mg%; $P<0.01$) was highest in

75% replacement diet and increased linearly ($P<0.01$) and quadratically ($P<0.01$) with increasing the level of Caspurea. Moreover, linearly ($P<0.01$) and quadratically ($P<0.01$) decreases in TVFAs (127.4, 123.6, 119.8, 96.4 mM/l; $P<0.01$) observed were (total volatile fatty acid) reflecting increases in level of Caspurea. Increase in level of Caspurea caused linearly ($P<0.01$) decrease in bacteria [2.6, 2.5, 2.5, 2.4 (x 10^{10} cell/ml)] and protozoa populations [2.2, 2.2, 2.1, 1.9 (x 10^{5} cell/ml)]. Nitrogen absorption (65.5, 64.7, 60.6, 57.7 g/d) were also lowest in 75% replacement diet ($P<0.01$), however, among 0, 25 and 50% replacement groups were not differ.With increasing the level of Caspurea, N absorption was decreased. Nitrogen retention (%N intake) (24.1, 23.6, 25.3, 13.6 %N intake) tended to increase in 50 % replacement diet ($P=0.05$) while, in 0, 25 and 50% replacement diet groups were not differ, moreover, N retention tended to decreased linearly ($P=0.08$) as the level of Caspurea increased. Blood urea nitrogen (22.5, 23.2, 23.3, 24.6 mg%; $P<0.01$) was highest in 75% replacement diet and BUN increased linearly ($P<0.01$) with increasing the level of Caspurea. Inconclusion, these results indicated that 50% replacement diet by Caspurea for soybean meal in concentrate has positive effects on Thai Native x Brahman crossbred beef cattle production.

3.2 Introduction

Urea has been utilized as a supplemental nitrogen source for beef cattle rations when natural protein supplements are expensive. However, urea is utilized less efficiently than natural plant proteins such as soybean meal for beef production (Helmer and Bartley, 1971). It has been suggested that rumen ammonia concentrations accumulate when dry matter of the ration contains more than 13% crude protein (CP), thus ammonia from non protein nitrogen (NPN) in these rations is not utilized by

rumen microorganisms (Roffler and Satter, 1973). Utilization of urea rations for milk production has been improved by use of a mixture of gelatinized starch and urea, processed through an extruder cooker (Jittakhot, 1999). Rumen ammonia concentrations were reduced when this mixture was incubated with rumen microorganisms (Helmer et al. 1970). This researches were to replaced for expensive natural plant protein such as soybean meal in the concentrate of Thai Native x Brahman beef cattle.

3.3 Objectives

The objectives of this research were to compare production performance of Thai Native x Brahman beef cattle and utilization of dietary nitrogen with various levels of Caspurea replacement for soybean meal in the concentrate.

3.4 Materials and Methods

Four crossbred (Thai Native x Brahman) male beef cattle were used in the experiment. The animals were randomly assigned in a 4 x 4 Latin square design with four periods, each period consisted 21 days. The dietary treatments were as follows: Caspurea replacement for soybean meal in concentrate at 0, 25, 50 and 75%. All animals were fed *ad libitum* of urea-treated rice straw and were fed concentrate (14% CP) at 2.0% BW, twice daily at 08.00 and 17.00. Each animal was housed in an individaul pen and free access to clean water all times. Daily collection of urine and faeces were made in the last 7 days of each period. Urine of individual animals was collected in 200 ml of 20% H_2SO_4 to keep the final pH of the urine lower than 3 all times in a container. It is essential to acidify the urine to prevent bacterial activity. After recording the weight, urine was diluted 4 times to prevent precipitation of uric

acid during storage. Duplicate urine samples of 50 ml were taken and stored at –20°C until analysis. Daily faeces collected in each period were bulked, mixed and a 5% sub sample was taken. The samples of faeces were oven dried and ground (1 mm. Screen) for determination of DM, ash, OM, NDF, ADF and N content. Rumen fluid and jugular blood were collected on the last day of each period. Ruminal pH was measured immediately after ruminal fluid sampling. Rumen fluid was collected at 0, 3 and 6 h post feeding 5 ml of 6 N HCl was added to 50 ml and jugular blood was collected at 0, 3 and 6 h post feeding and placed into heparinized vacationer tubes and centifuged at 2500 x g for 15 minutes. Both rumen fluid and blood were stored at 5°C until analysis. Liveweights of each animal were measured before feeding at the beginning and at the end of each feeding period (21 d). Urea treated rice straw and concentrate were sampled every two weeks and the composited sample were analyzed for NDF, ADF and ADL contents (Goering and Van Soest,1970), DM, ash and crude protein were determined by the methods of AOAC (1985). Neutral detergent fiber, acid detergent fiber, acid detergent lignin of feeds and faeces were determined by the methods of Georing and Van Soest (1970) and dry matter, ash crude proteins were determined by the methods of AOAC (1985). Rumen fluid TVFA concentration was determined by titration technique of Briggs et al. (1957). Acetic, propionic and butyric acid concentrations were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard Avondale, PA). NH_3 –N contents were determined by the methods of Bromner and Keeney (1965).

Nutritional evaluation of Caspurea for ruminant using *in vitro* gas production tecnique

Caspurea (45% CP, cassava pulp:urea = 85:15; DM basis) is produced through an extrusion process wherein a starch bearing food source such as corn, cassava and an NPN substance such as urea are admixed and pelleted (0.76 cm. die) by runing through a cooker-exturder at high temperature (90-120 degree C., moisture about 20%), and pressure to give a chunk-type product and Control are not produced through an extrusion process. Two NPN feed sources (Caspurea and Control) were ground through a 1 mm screen for the *in vitro* gas production technique.

Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inocculums was removed before the morning feeding by vacuum pump and stomach tube technique into a 2 liters glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as descripbed by Sommart et al. (2000). Mixed rumen fluid inoculums were obtained from two Thai Native x Brahman crossbred male cattle (weightning approximately 200± 5 kg).

The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 60 ml serum bottle (Sommart et al., 2000). The bottles were pre-warmed in a water bath at 39°C for about 1 h prior to injection of 40 ml of rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were stoppered with rubbers stoppers, crimp sealed and incubated in a water bath setting at 39°C.

The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 ml glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 96 h (from 1-12 h, every 3 h from 13-24 h, every 6 h from 25-48 h and every

12 h from 49-96 h) after incubation periods. Amount of cumulative gas volume from 1 to 96 h after incubations were fitted using the equation $y=a+b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979), where y, describes gas production at time t, *a*, the gas produced (ml) by instantaneous fermentation of the soluble and readily available fraction of feed, *b*, the gas produced (ml) by the fermentation of insoluble, but slowly fermentable fraction and *c*, the fractional rate (rate constant) at which gas is produced per hour (%/h).

3.5 Statistical analyses

All data obtained from the experiment were subjected to analysis of variance using Proc. GLM (SAS, 1996), treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torries, 1980) and all data obtained from the experiment were subjected to the General Linear Models (GLM) procedure for orthogonal polynomial analysis of SAS (SAS, 1996).

3.6 Results and Discussion

Gas production characteristics of Caspurea

Gas production characteristics from the fermentation of Caspurea and Control were measured at 1 to 96 h using *in vitro* gas production tecnique adapted to describe the kinetics of fermentation based on the modified exponential model $y=a+b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979). The gas production characteristics are presented in Table 3.1 and Figure 3.1. The intercepts (*a*) were not differ. However, absolute *a* (|*a*|) is used to describe ideally reflects the fermentation of the soluble fraction. The gas volume at asymtote (*b*) described the fermentation of the insoluble fraction. The fermentation of the insoluble fraction were significantly different ($P<0.05$). The

fermentation of the insoluble fractions of Control and Caspurea were 93.2 and 124.8 ml, respectively. The fermentation of insoluble fraction was higher in Caspurea. The results suggest that Caspurea has higher potential for use as ruminant feed when compared to Control. Rate of gas production (*c*) expressed in %/h was not differ. Potential extents of gas production ($|a|+b$) expressed in ml was higher in Caspurea than Control, therefore this data showed that Caspurea improved the utilization of N and OM *in vitro*. For maximum utilization of NPN by rumen organisms two processes in the rumen should run simultaneously: the degradation of NPN to ammonia, and the fermentation of carbohydrates (Baldwin, 1970) to supply energy for microbial protein synthesis (Nocek and Russell, 1988). Ammonia is relatively rapidly released from NPN, especially from urea in the rumen (Kearl, 1982), as follows;.

$$1\ \text{glucose} \longrightarrow 1.2\ \text{acetate} + 0.4\ \text{propionate} + 0.2\ \text{butyrate} + 1CO_2 + 0.6CH_4 + 0.4\ H_2O$$

$$NH_2\text{-}CO\text{-}NH_2 \xrightarrow[\text{Urease}]{H_2O} 2NH_3 + CO_2$$

The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids and CO_2, have little effect on the yield of microbial matter (Ørskov et al., 1974). CO_2 was the end-product of carbohydrate degradation, therefore, the application of *in vitro* gas production (CO_2) was to evaluate the energetic value and predict *in vivo* organic matter digestibility (Menke et al., 1979).

Gas volume

Cumulative gas volumes at 1 to 96 h after incubation are shown in Table 3.1 and Figure 3.1. The results indicate that cumulative gas volume at 96 h after

incubation was significantly different ($P<0.01$). The gas volumes were higher in Caspurea than Control. Sommart et al. (2000) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility had high correlations with gas volume (Sommart et al., 200; Nitipot and Sommart, 2003). Gas volume has also showed a close relationship with feed intake (Blummel and Becker, 1997) and growth rate (Blummel and Ørskov, 1993). The rate of fermentation is undoubtedly an important factor influencing the economy of microbial growth. With a low rate of fermentation, the rate of microbial growth is also reduced. Gas volume in Control group was lower than Caspurea group, it is possible that N from Control group was utilized less efficiently than N from Caspurea. Increase in level of *in vitro* ammonia cumulative caused decrease in substrate degradability microbial growth and activity because when urea too rapid hydrolysis to ammonia into *in vitro* fluid, the ammonia may be accumulate in the fluid possibly resulting in ammonia toxicity.

Table 3.1 Gas production characteristics, gas volume of Caspurea and Control

Parameters	Treatment		SEM	*P*-value
	Control	Caspurea		
Gas production characteristic parameters				
a, ml	-13.2	-14.8	1.00	0.375
b, ml	93.2	124.8	2.57	*
c, %/h	0.04	0.07	0.01	0.168
|*a*|+*b*, ml	106.4	139.6	2.93	*
Gas production (ml/0.5 g DM subtrate)				
96 h	73.6	108.5	2.21	**

SEM = standard error of the mean, $^{*}P<0.05$, ** $P<0.01$, a= the intercept (ml), which ideally reflects the fermentation of the soluble fraction, b= the fermentation of the insoluble fraction (asymtote) (ml), c= rate of gas production (%/h), $|a|+b$= potential extent of gas production (ml)

In order to avoid the possibility of ammonia toxicity while using Caspurea as a NPN source of nitrogen in ruminant feed, the use of Caspurea as a feed supplement has been limited to 1% of DM feed. CO_2 was the end-product of carbohydrate degradation, with a high rate degradation of urea the rate of CO_2 production is also reduced.

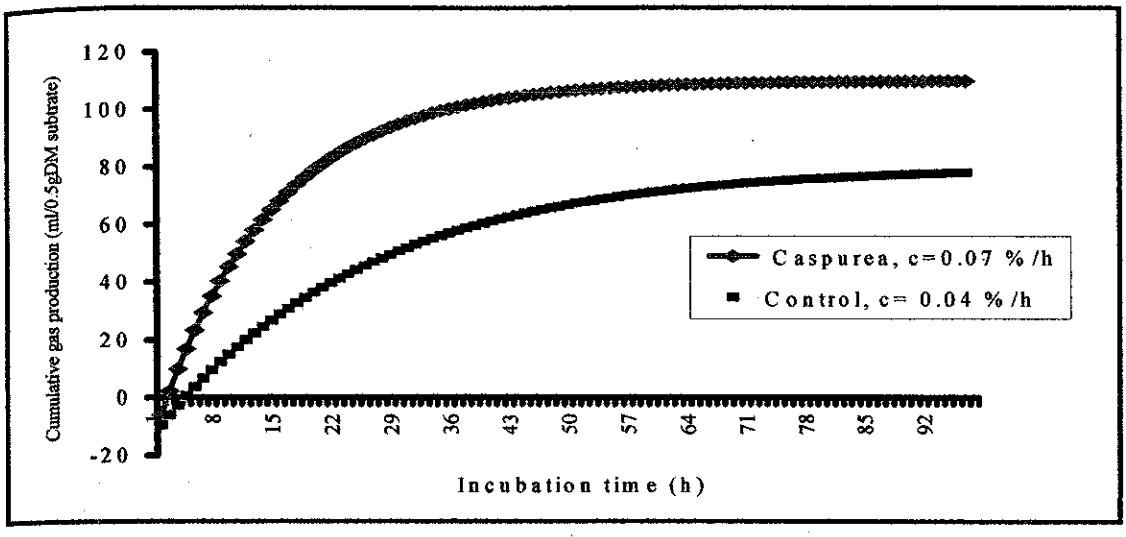

Figure 3.1 Cumulative gas volume estimated by $y=a+b\ (1-e^{-ct})$ (ml/0.5 gDM subtrate) throughout 96 h.

Degradability characteristics

The rapidly soluble fracton (*a* fraction), potentially degradable fraction (*b* fraction), rate of degradation of *b* fraction (*c*) and potential degradation (*a*+*b*) of DM are presented in Table 3.2 and Figure 3.2, and OM are presented in Table 3.3 and Figure 3.3. The rapidly soluble fractions of DM and OM were higher ($P<0.05$) in Caspurea than Control at 3 to 12 h post incubation. The potentially degradable fraction (*b*) of DM and OM were not differ ($P>0.05$). The rate of degradation of the potential degraded (*c*) of DM and OM were also not differ ($P>0.05$) and in this study was lower than those reported by Chumpawadee (2006) for cassava chip. The potential degradation (*a*+*b*) of DM and OM were not differ. This data showed that there were high variations *in vitro* degradability characteristic between N and energy feed sources. Numerous factors effecting *in vitro* degradability were sample size, grinding, diet of host animal, species of animal, sample preparation, incubation time. Chemical composition and processing of feed stuffs also affected degradation characteristics.

DM and OM degradability in Control group was lower than Caspurea group, it is possible increase in level of *in vitro* ammonia cumulative caused decrease in substrate degradability, microbial growth and activity because when urea too rapid hydrolysis to ammonia into *in vitro* fluid, the ammonia may be accumulate in the fluid possibly resulting in ammonia toxicity. CO_2 was the end-product of carbohydrate degradation, with a high rate degradation of urea the rate of CO_2 production and degradability of DM and OM were also reduced.

Table 3.2 *In vitro* DM degradation (IVDMD) characteristics and effective degradability.

Parameters	Treatment		SEM	*P*-value
	Control	Caspurea		
IVDMD characteristic parameters				
a, %	17.7	24.6	2.00	0.136
b, %	59.8	54.1	2.00	0.181
c, %/h	0.06	0.08	0.01	0.293
a+*b*, %	77.5	78.6	2.00	0.735
Effective DM degradability (%)				
3 h	29.2	37.2	1.06	*
6 h	36.4	44.9	1.01	*
9 h	36.6	47.8	1.00	*
12 h	44.1	62.5	1.01	**
24 h	68.0	71.6	1.60	0.249
36 h	68.9	74.1	1.01	0.067
48 h	75.2	76.8	1.01	0.375
72 h	75.2	77.6	1.00	0.232
96 h	77.3	80.5	1.01	0.152

SEM = standard error of the mean, $^{*}P<0.05$, ** $P<0.01$, *a,b,c* are constants in the exponential equation, $P = a+b\ (1-e^{-ct})$ where *a* = the intercept (%), which ideally reflects the fermentation of the soluble fraction, *b* = the fermentation of the insoluble fraction (asymtote) (%), *c* = rate of degradation of fraction *b* (%/h), *a*+*b* = potential degradation (%)

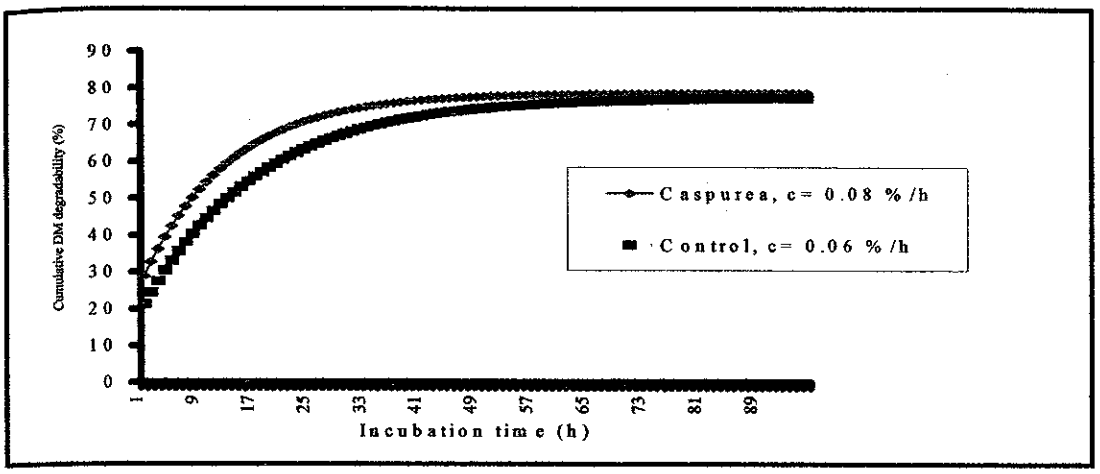

Figure 3.2 Cumulative DM degradability (%) estimated by

$P = a+b\ (1-e^{-ct})$ throughout 96 h.

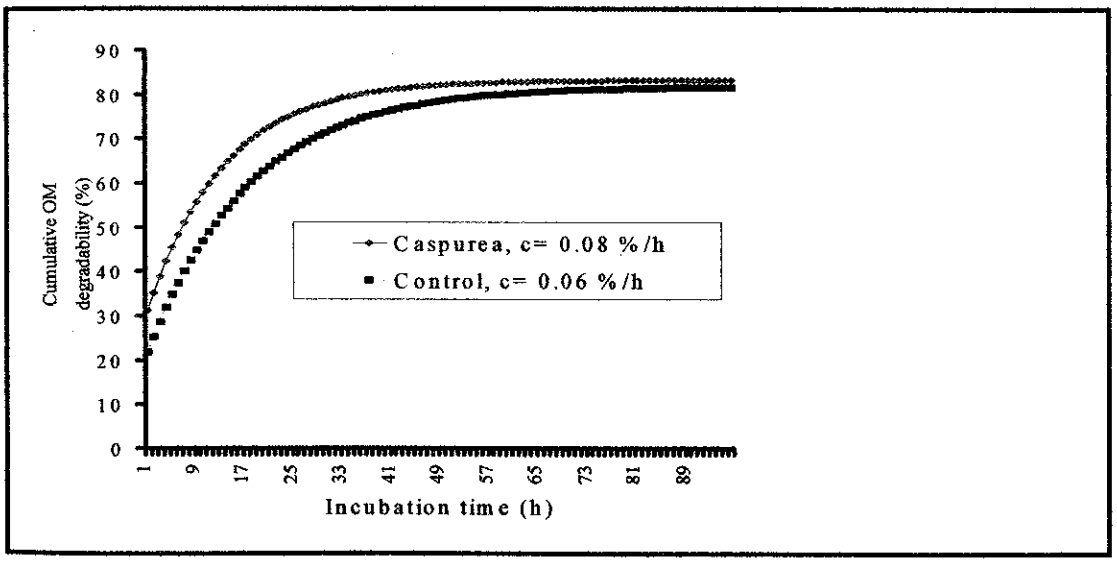

Figure 3.3 Cumulative OM degradability (%) estimated by

$P = a+b\ (1-e^{-ct})$ throughout 96 h.

Effective degradability

The effective degradability of DM are shown in Table 3.2 and Figure 3.4, and of OM are shown in Table 3.3 and Figure 3.5. The effective degradability of DM and

OM indicated that substantial amounts of DM, OM and CP were degraded *in vitro,* thus providing degradable N and OM for *in vitro* microbial synthesis. Effective degradability of Caspurea was higher than Control at 3 to 12 h-post-incubation. This data showed that Caspurea was high biological value and should have been used as a N source for ruminant. It has been suggested that matching supply of energy and N supply *in vitro* may improve microbial growth and activity (Sinclair et al., 1993). For maximum utilization of NPN by rumen organisms two processes in the rumen should run simultaneously: the degradation of NPN to ammonia, and the fermentation of carbohydrates to supply energy for microbial protein synthesis. DM and OM degradability in Control group (unprocessed) was lower than Caspurea group, it is possible increase in level of *in vitro* ammonia cumulative caused not matching supply of energy and N for microbial growth and activity because when urea too rapid hydrolysis to ammonia into *in vitro* fluid, the ammonia may be accumulate in the fluid possibly resulting in ammonia toxicity. With a high rate degradation of urea to ammonia, the rate of degradability of DM and OM is also reduced because high rate degradation of urea results in low efficient incorporation of urea nitrogen into microbial protein. One major problem with the use of urea as a NPN source of nitrogen is ammonia toxicity. The present invention provides a low release non-protein feed supplement that enables the use of a NPN source in ruminant feed than has heretofore been used. The feed supplement of the present invention is formulated to provide for substantially slower release of ammonia from a NPN source, e.g., urea, during anaerobic digestion, thus allowing the use of higher levels of NPN sources in ruminant while avoiding the risk of ammonia toxicity. As used herein, the terminology "slow release" means that the non-protein feed supplement releases ammonia at an

average rate of less than 40% of the average rate that urea releases ammonia during the first six hours of *in vitro* testing (Helmer et al., 1970).

Table 3.3 *In vitro* OM degradation (IVOMD) characteristics and effective degradability.

	Treatment			
Parameters	**Control**	**Caspurea**	**SEM**	***P*-value**
IVOMD characteristic parameters				
a, %	18.2	26.8	2.02	0.093
b, %	63.7	56.5	1.60	0.084
c, %/h	0.06	0.08	0.01	0.293
a+*b*, %	81.8	83.3	1.00	0.400
Effective OM degradability(%)				
3 h	31.3	39.8	1.06	*
6 h	39.4	49.2	1.07	*
9 h	39.5	52.9	1.24	*
12 h	47.2	66.6	1.07	**
24 h	73.1	76.4	1.03	0.148
36 h	75.5	77.6	1.57	0.426
48 h	75.5	81.8	1.04	0.097
72 h	78.7	83.4	0.99	0.078
96 h	81.8	84.5	1.01	0.208

SEM = standard error of the mean, $^{*}P<0.05$, ** $P<0.01$, *a,b,c* are constants in the exponential equation, $P = a+b\ (1-e^{-ct})$ where *a* = the intercept (%), which ideally reflects the fermentation of the soluble fraction, *b* = the fermentation of the insoluble fraction (asymtote) (%), *c* = rate of degradation of fraction *b* (%/h), *a*+*b* = potential degradation (%)

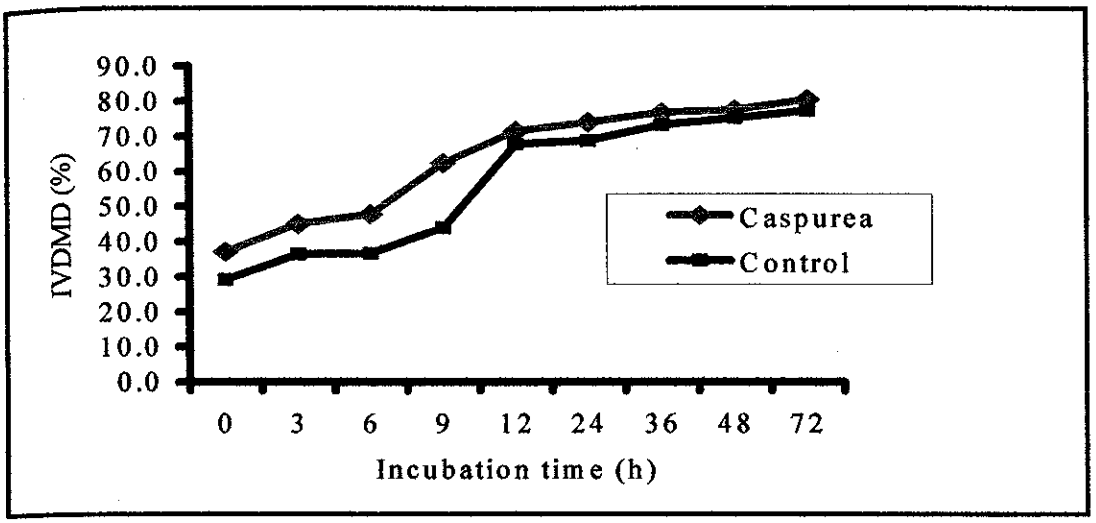

Figure 3.4 Effective *in vitro* DM degradability (%) of Caspurea and Control throughout 72 h.

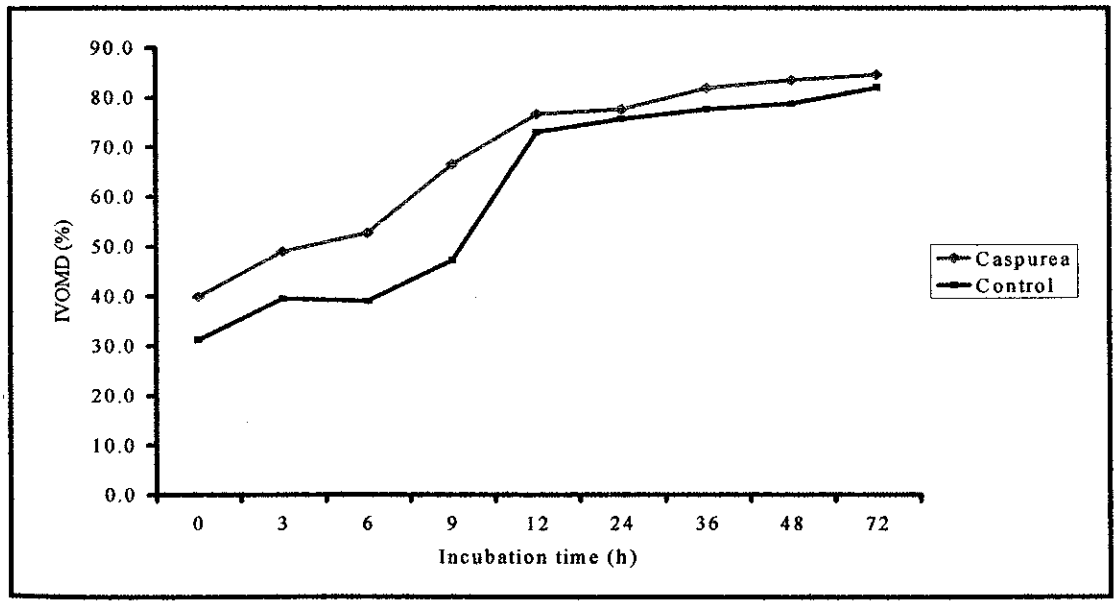

Figure 3.5 Effective *in vitro* OM degradability (%) of Caspurea and Control throughout 72 h.

***In vitro* ammonia nitrogen released characteristics and ammonia nitrogen cumulative**

Ammonia nitrogen released from the fermentation of Caspurea and Control were measured at 3, 6, 9, 12, 24, 36, 48 and 72 h-post-incubation using *in vitro* gas production technique adapted to describe the kinetics of fermentation based on the modified exponential model $P = a+b\ (1\text{-}e^{-ct})$ (Ørskov and McDonald, 1979). The ammonia released characteristics are presented in Table 3.4 and Figure 3.6 and ammonia cumulative released are presented in Table 3.4 and Figure 3.7. The intercept (a) was lower ($P<0.01$) in Control, compared to Caspurea. However, absolute a ($|a|$) is used to describe ideally the fermentation of the soluble N fraction.

The ammonia concentrations at asymtote (b) describe the fermentation of the insoluble N fraction. Ammonia concentrations from the fermentation of the insoluble N fraction of Caspurea and Control were significantly different ($P<0.01$). The fermentation of insoluble N fractions of Caspurea and Control were 80.9 and 30.4 mg%, respectively. The results suggested that Caspurea has higher potential for use as ruminant feed. Rate of ammonia nitrogen release (c) expressed in %/h was higher in Control than in Caspurea. Potential extents of ammonia nitrogen release ($|a|+b$) expressed in mg% was higher in Control than in Caspurea.

Ammonia nitrogen concentrations at 3, 6, 9, 12, 24, 36, 48 and 72 h after incubation are shown in Table 3.4. The results indicated that cumulative ammonia nitrogen concentrations of Caspurea at 12, 24, 36, 48 and 72 h after incubation was lower than Control diet ($P<0.01$). The ammonia nitrogen concentrations were lower in Caspurea. These findings were similar to that reported by Helmer et al. (1970). They observed that Starea or mixture of expanded grain plus urea lowered rumen ammonia concentration and increased microbial protein synthesis *in vitro* when compared with

mixture of ground grain plus urea. Caspurea, when tested *in vitro*, was slightly to the extruded cassava pulp and urea may result in a product that affects the rate releasing of ammonia from urea making its conversion to microbial protein more efficient.

Table 3.4 *In vitro* ammonia-nitrogen released characteristics and NH_3-N cumulative.

	Treatment			
Parameters	**Control**	**Caspurea**	**SEM**	***P*-value**
Ammonia-nitrogen released characteristic parameters				
a, %	-10.8[b]	5.10[a]	1.00	**
b, %	80.9[a]	30.4[b]	1.00	**
c, %/h	0.08	0.07	0.01	0.553
|*a*|+*b*, %	80.9[a]	35.5[b]	1.00	**
Ammonia-nitrogen cumulative (mg %)				
3 h	8.7	8.4	1.00	0.618
6 h	16.8	16.7	0.93	0.963
9 h	21.1	23.8	1.24	0.185
12 h	47.6	19.6	1.07	**
24 h	58.8	28.1	1.03	**
36 h	61.4	32.2	1.57	**
48 h	65.8	35.2	1.04	**
72 h	72.9	36.1	0.99	**

SEM = standard error of the mean, $^{*}P<0.05$, ** $P<0.01$, *a,b,c* are constants in the exponential eqaution, $P = a+b\ (1\text{-}e^{-ct})$ where *a* = the intercept (mg %), which ideally reflects the amonia-nitrogen released of the soluble N fraction, *b* = the amonia-nitrogen released of the N insoluble fraction (asymtote) (%), *c* = rate of amonia-nitrogen released of fraction *b* (%/h), $|a|+b$ = potential amonia-nitrogen released (%)

When urea (NPN sources) from Caspurea and Control is broken down into ammonia *in vitro* for microbial protein synthesis, the ammonia may be diffused into *in vitro* liquid. However, if the rate of ammonia released exceeds the capacity of the microorganisms to convert it to microbial protein, ammonia will accumulate *in vitro,* which is confirmed by the present study.

Stiles et al. (1970) reported that the results with expanded grain and Starea are similar to those obtained *in vitro* by Helmer et al. (1970). They observed that Starea or a mixture of expanded grain plus urea lowered rumen ammonia concentration and increased microbial protein synthesis *in vitro* when compared with a mixture of ground grain plus urea. Starea , when tested *in vitro,* was slightly superior to the expanded grain and urea may result in a product that effects the rate of ammonia release from urea to make its conversion to microbial protein more efficient. If expansion processed grain is valuable in urea utilization, it would be more logical to process the grain with urea (Starea) than to feed expanded grain and urea separately.

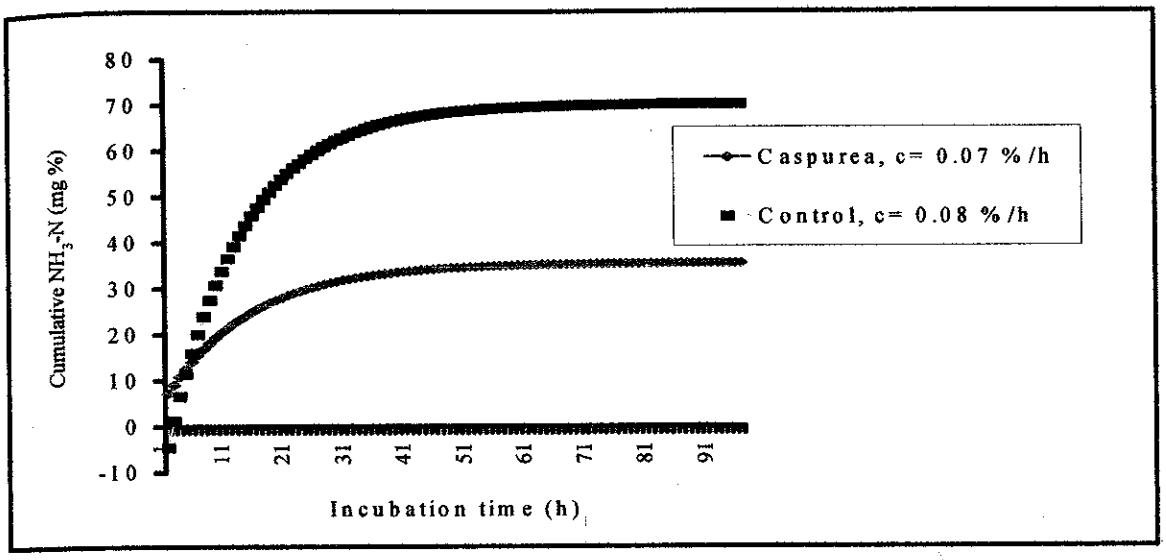

Figure 3.6 *In vitro* NH_3-N (mg%) characteristics released of Caspurea and Control throughout 96 h.

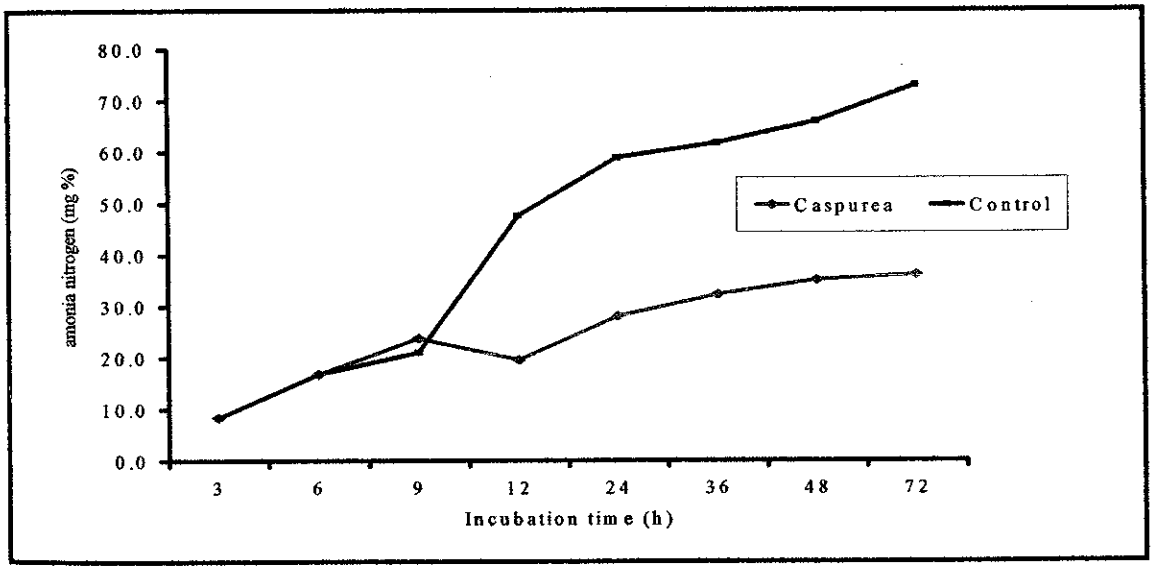

Figure 3.7 *In vitro* ammonia-N (mg%) released of Caspurea and Control throughout 72 h.

Starea provides enhanced palatability and reduced segregation of urea in mixed feed. The recovery of urea nitrogen in the Starea supplements processed was 98%,

indicating little, if any, loss of nitrogen during processing. Caffrey et al. (1967) found that rumen microorganisms from sheep had adjusted to urea-rich diets within 13 to 19 days for *in vitro* and *in vivo* studies, respectively. Moreover, Clark et al.(1973) concluded that urea nitrogen was utilized less efficiently than nitrogen from SBM. Expanded corn plus urea was developed to reduce ruminal ammonia, and this decrease has been observed in *in vitro* studies (Helmer et al., 1970). The advantageous use of urea is restricted to conditions where rumen ammonia concentrations would be below optimal for rumen bacterial growth (Lampila, 1972), perhaps as low as 7 mg rumen ammonia per 100 ml (Oyaert and Bouckaert, 1960). Using published equations (Satter and Roffler, 1973), they estimate that rumen ammonia in cows fed low protein, Urea, Starea and SBM rations were 9.3, 12.2 11.9 and 11.8 mg%. The resistance of protein to attack by rumen microorganisms was correlated negatively with protein solubility (Hendrickx and Martin, 1963).

Effect of Caspurea on beef cattle performance

The composition of the diets is shown in Table 3.5. Variation in CP analyzed within concentrates was small (approximately 14% CP) and were slightly higher than those formulated. The high Caspurea (75 % replacement) rations contained slightly higher NDF than other diets.

The effect of diet on feed intake is shown in Table 3.6. These results indicated that concentrate intake increased quadratically ($P<0.05$) as the levels of Caspurea increased. Total dry matter intake (5.5, 5.5, 5.2, 5.3 KgDM/d; $P>0.05$) was not significantly different among dietary treatments. The results indicated that Caspurea, and intimate mixture of gelatinized starch and urea, improved palatability of urea-

containing rations. These results agree with that reported by Stiles et al. (1970), who found that Starea improved palatability of urea-containing rations.

Table 3.5 Feed formulation and chemical composition of dietary treatment.

	Caspurea : Soybean meal				
Feed stuffs	**0:100**	**25:75**	**50:50**	**75:25**	
Caspurea	0.0	3.8	7.5	11.2	
Cassava pulp	50.0	50.0	50.0	50.0	
Rice bran	15.0	15.0	15.0	15.0	
Soybean meal	18.0	14.2	10.5	6.8	
Palm meal	13.0	13.0	13.0	13.0	
Molasses	1.0	1.0	1.0	1.0	
Urea	0.8	0.8	0.8	0.8	
Sulfur	0.2	0.2	0.2	0.2	
Lime stone	0.5	0.5	0.5	0.5	
Salt	0.5	0.5	0.5	0.5	
Mineral mix	1.0	1.0	1.0	1.0	
Total	100.0	100.0	100.0	100.0	
Chemical composition (%)					**Urea treated rice straw**
DM	90.5	90.2	90.4	89.9	51.5
			% DM		
OM	92.6	91.2	90.6	89.9	90.1
NDF	14.5	15.4	16.2	16.8	69.1
ADF	8.2	8.7	8.9	9.3	46.3
ADL	3.3	3.5	3.6	4.3	8.6
Ash	7.4	8.8	9.4	10.1	9.9
AIA	1.2	1.3	1.5	1.4	1.6
CP	14.1	14.1	14.1	14.0	6.9

DM= dry matter, OM= organic matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, AIA= acid insoluble ash, CP= crude protein

Table 3.6 Effect of dietary treatments on feed intake

Items	Caspurea:Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
Feed intake (kgDM/d)							
Concentrate	1.99	1.98	1.99	2.02	0.01	0.056	0.029
Roughage	3.5	3.5	3.2	3.3	0.14	0.200	0.598
Total intake	5.5	5.5	5.2	5.3	0.14	0.257	0.497
Total intake (%BW)	3.7	3.6	3.4	3.5	0.09	0.083	0.787
Total intake (g/kgBW$^{0.75}$)	127.9	127.5	119.9	124.9	2.93	0.119	0.700

SEM = standard error of the mean, *Orthogonal polynomial contrast L= linear and Q= quadratic

The effect of diet on feed digestibility is shown in Table 3.7. These results indicated the DM and CP digestibility of the diet containing Caspurea at 75% replacement for soybean meal was lower (P<0.01) than other diets. DM digestibility (71.2, 72.1, 71.2, 67.7%) was lowest (P<0.01) in 75% replacement diet. DM digestibility decreased linearly (P<0.01) and quadratically (P<0.01) as the level of Caspurea increased. Moreover, OM and CP digestibility decreased linearly and quadratically (P<0.05) as the levels of Caspurea increased. These data indicated that urea nitrogen from Caspurea was utilized less efficiently than nitrogen from soybean meal (SBM). Increase in level of Caspurea from 50 to 75% caused decrease in digestibility, it is possible that low efficient incorporation of N for microbial growth and activity. When urea is broken down into ammonia in the rumen, the ammonia may be absorbed into the blood stream of the ruminant. The liver will convert the ammonia

into urea which may be excreted or reabsorbed into the stomach contents (Van Soest, 1982). However, if the rate of ammonia absorption exceeds the capacity of the liver to convert it to urea, ammonia will accumulate in the animal's blood possibly resulting in ammonia toxicity. In order to avoid the possibility of ammonia toxicity while using Caspurea as a NPN source of nitrogen in ruminant feed, the use of Caspurea as a feed supplement has been limited to 50% replacement for SBM. Susmel et al. (1989) reported that SBM is very high biological value and have been used as a true protein source for ruminant, it has been suggested that matching supply of energy and N supply in the rumen may improve microbial growth and activity. However, Helmer et al. (1970) reported that cows receiving either soybean meal or Starea as the protein supplement were no differences between rations for apparent digestibility of DM, CP, ADF or cellulose.

Body weight change (0.54, 0.52, 0.55, 0.42 kg/d) was increased ($P<0.05$) by 50% replacement diet, moreover, decrease linearly ($P<0.05$) in BW change reflected increases in level of Caspurea (Table 3.7). Body weight change was lowest when Caspurea replacement for SBM in concentrate at 75% was fed while those 0, 25 and 50% Caspurea replacement for SBM rations showed similar BW. The results indicated that soybean meal has very high biological value for ruminant production, resulting in a decrease in average daily gain when Caspurea level was increased from 50 to 75%. These results agree with the report of Susmel et al. (1989), who found that SBM had the high essential amino acid and biological value for ruminant. Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. The average change in body weight showed that dairy cows fed urea lost significantly more weight than those fed SBM or Starea.

Differences in weight gains were not statistically significant between the SBM and Starea fed animals. On the other hand, Starea and soybean meal rations were consumed in sufficient quantities to support high production and to maintain or increase body reserves.

Table 3.7 Effect of dietary treatments on nutrient intake, digestibility and BW change.

Items	Caspurea:Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
Nutrient intake (kg/d)							
NDF	2.7	2.7	2.5	2.6	0.06	0.436	0.546
CP	0.53	0.53	0.51	0.51	0.01	0.140	0.537
Digestibility (%)							
DM	71.2[a]	72.1[a]	71.2[a]	67.7[b]	0.59	0.005	0.009
NDF	61.8[a]	62.9[a]	61.2[a]	55.1[b]	1.13	0.005	0.021
CP	76.9[a]	76.9[a]	75.3[a]	70.3[b]	0.52	0.001	0.003
Body weight (kg)							
Initial weight	146.1	146.1	146.3	146.0	-	-	-
Final weight	157.5[a]	157.0[a]	157.8[a]	154.8[b]	0.59	0.094	0.033
Body weight change (kg/d)	0.54[a]	0.52[a]	0.55[a]	0.42[b]	0.02	0.014	0.034

[a,b] Means within a row with different superscripts differ ($P<0.05$), SE = standard error of the mean, *Orthogonal polynomial contrast L= linear, Q = Quadratic

Ruminal pH data are showed in Table 3.8 and Figure 3.8. Ruminal pH increased linearly and quadratically ($P<0.01$) as level of Caspurea increased and was highest when Caspurea replacement for SBM in concentrate at 75% was fed.

Ruminal ammonia-N concentrations (8.5, 8.8, 9.5, 13.3 mg%) were highest (P <0.01) in 75% replacement diet and increased linearly (P<0.01) and quadratically (P <0.01) with increasing the level of Caspurea (Table 3.8 and Figure 3.9). The results showed that NH_3-N concentrations were highest when Caspurea replacement for SBM in concentrate at 75% was fed while those 0, 25 and 50% Caspurea replacement for SBM rations were lower (P<0.01) than in 75% replacement. It is possible that true protein was hydrolyzed slower than Caspurea. If this were the case, then one would expect ruminal ammonia of the high NPN (75% Caspurea) fed group to be higher than that of the SBM fed group. It is possible that low efficient capture of N for microbial protein synthesis occured. These data indicated that urea nitrogen from Caspurea was utilized less efficiently than nitrogen from SBM. Increase in level of Caspurea from 50 to 75% caused increase in ammonia in the rumen. It is possible that high level of NPN in Caspurea leading to the accumulation of ammonia in the rumen.

Table 3.8 Effect of dietary treatments on rumen fermentation

Items	Caspurea : Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
pH (hr-post-feeding)							
0	7.0^{c}	7.0^{c}	7.2^{b}	7.3^{a}	0.01	0.001	0.015
3	6.4^{c}	6.5^{b}	6.6^{b}	7.1^{a}	0.01	0.001	0.001
6	6.7^{d}	6.8^{c}	6.9^{b}	7.2^{a}	0.01	0.001	0.001
Mean	6.7^{b}	6.8^{b}	6.8^{b}	7.2^{a}	0.07	0.001	0.066
NH_3-N (mg%)							
0	7.9^{c}	7.5^{d}	9.0^{b}	12.9^{a}	0.08	0.001	0.001
3	9.4^{d}	9.6^{c}	9.8^{b}	13.9^{a}	0.01	0.001	0.001
6	8.1^{d}	8.9^{c}	9.5^{b}	13.2^{a}	0.01	0.001	0.001
Mean	8.5^{c}	8.8^{c}	9.5^{b}	13.3^{a}	0.19	0.001	0.001

^{a,b,c,d} Means within a row with different superscripts differ (P<0.05)

*Orthogonal polynomial contrast, L= linear and Q= quadratic

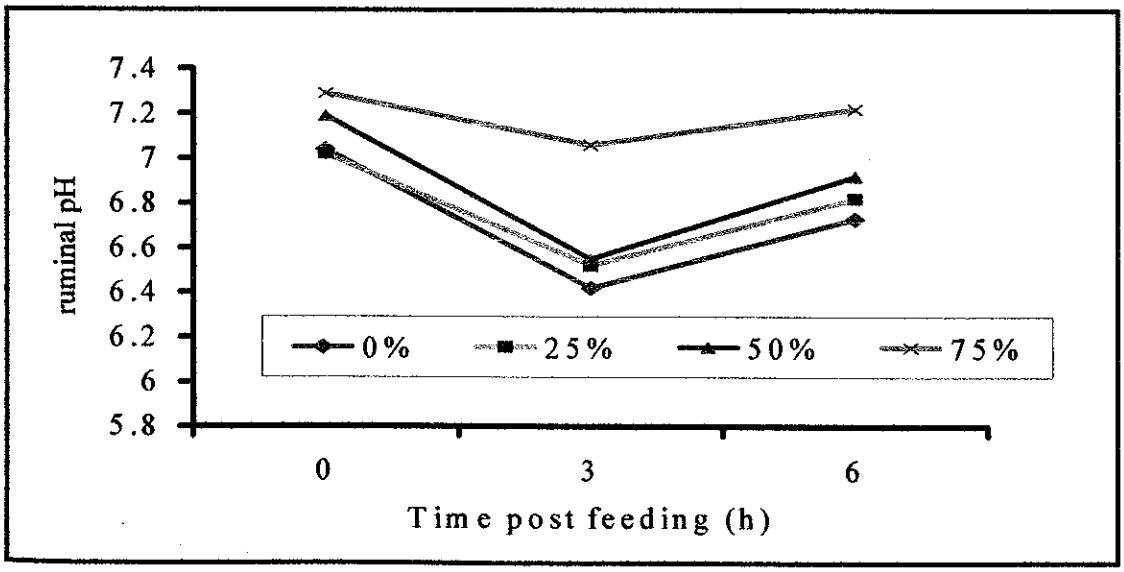

Figure 3.8 Hourly ruminal pH in beef cattle receiving a diet containing four levels of Caspurea

Davis and Stallcup (1967) observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. Reported by Kolver et al. (1998) that decreases in ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis. Moreover, Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea N into microbial protein. Ruminal NH_3-N concentration at 0, 3 and 6 h post feeding found that ruminal NH_3-N concentrations at 3 h post feeding were increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Higher NH_3-N concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feedings decreased indicating a more capture of N for increased microbial protein synthesis (Sinclair et al., 1993).

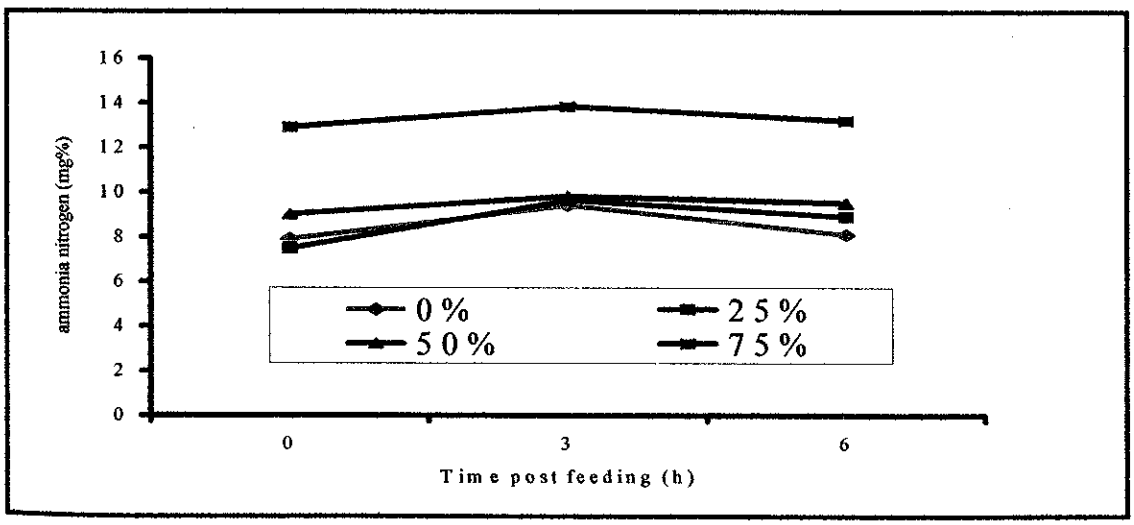

Figure 3.9 Hourly NH_3-N in beef cattle receiving a diet containing four levels of Caspurea

In contrast, Roman-Ponce et al. (1974) reported that rumen ammonia N (NH_3-N) concentration was higher for all rations at 1 h after feeding than 2 h (28.2 and 19.0 mg%). Rumen ammonia was similar for urea and Starea rations but higher than SBM at 1 h and 2 h. However, Davis and Stallcup (1967) reported that peaks of ammonia concentration in rumen contents 2 or 3 h after feeding either SBM, urea or SBM+urea rations. Thompson et al. (1972) found that peak rumen NH_3-N for Starea rations at 90 min after feeding. Although rumen NH_3-N values for urea and Starea were not significantly different at either 1 or 2 h The decline in NH_3-N from 1 to 2 h was 12.8 for urea and 6.4 mg% for Starea, suggesting a slower hydrolysis for Starea than urea rations, which is in agreement with Stiles et al. (1970) and Schmidt et al. (1973). Many workers (as these data confirm) have obtained higher ammonia contents after feeding with Urea than SBM rations (Davis and Stallcup,1964; Freitag et al.,1968; Schmidt et al., 1973; Thompson et al., 1972). Stiles et al. (1970) observed lower rumen ammonia content in the Starea-fed cows than urea-fed cows. Others (like these data) failed to find lowering of rumen ammonia with Starea as compared to Urea (Schmidt et al., 1973) but did find a slow decline in rumen ammonia with Starea after feeding which agreed with these results. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns, e.g. lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with the urea rations having the opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when Urea was fed due to the high concentration of

ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with more acid rumen pH. Schmidt et al. (1973) reported that at the 1.5 h sampling time, ruminal ammonia levels in animals fed urea or Starea were not different, perhaps indicating that Starea was hydrolyzed slower than urea (Stiles et al., 1970). If this were the case, then one would expect ruminal ammonia of the urea fed group to be higher than that of the Starea fed group prior to the initial post-feeding sample. Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different.

Rumen ammonia levels in animal fed urea and Starea were higher than those for animals fed SBM and TSBM until the 3.5 h (urea) and 5.5 h (Starea) sampling times. There were no differences in the levels of rumen NH_3-N in animals fed the SBM diets. Moreover, high rumen ammonia has been associated with higher content of SBM or crude protein in rations (Freitag et al., 1968).

Total VFA concentrations in the rumen are presented in Table 3.9. Total volatile fatty acid (127.4, 123.6, 119.8, 96.4 mM/l; $P<0.01$) were significantly among dietary treatments, decreased linearly ($P<0.01$) and quadratically ($P<0.01$) when the level of Caspurea increased from 50 to 75%. The lowest ($P<0.01$) concentration recorded was on animals fed 75% replacement diet. The low concentration of VFA probably reflecting asynchroneuos diet, resulting in decreased ruminal end product (Kim, 2001). Moreover, Witt et al. (1999) reported that higher VFA concentration might have been related to the microbial population in the same time as optimum pH.

Table 3.9 Effect of dietary treatments on volatile fatty acid concentrations

Items	Caspurea : Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
h-post-feeding							
Acetic acid (mol/100 mol)							
0	68.0	67.0	66.9	65.8	2.37	0.457	0.671
3	69.9	70.0	68.8	71.9	0.75	0.110	0.169
6	72.1	67.9	71.0	72.0	1.45	0.672	0.133
Mean	69.9	68.3	69.0	70.0	1.85	0.981	0.369
Propionic acid (mol/100 mol)							
0	15.0	18.9	14.9	15.0	2.08	0.673	0.367
3	23.0^{ab}	24.9^{a}	25.1^{a}	19.8^{b}	1.44	0.187	0.039
6	22.0^{a}	21.8^{a}	22.0^{a}	18.7^{b}	0.77	0.031	0.095
Mean	20.0	21.9	20.6	17.8	2.36	0.231	0.098
Butyric acid (mol/100 mol)							
0	7.9	7.8	7.1	7.0	0.59	0.733	0.427
3	11.9	10.8	13.9	13.1	0.86	0.133	0.949
6	9.8^{b}	10.9^{ab}	12.0^{a}	12.0^{a}	0.57	0.015	0.398
Mean	9.9	9.8	10.9	11.1	1.43	0.240	0.986
TVFAs (mM/l)							
0	101.2^{a}	100.9^{a}	100.7^{a}	86.1^{b}	0.62	0.001	0.001
3	142.2^{a}	140.4^{a}	139.5^{a}	114.0^{b}	0.66	0.001	0.001
6	139.0^{a}	129.4^{b}	119.2^{c}	89.0^{d}	0.27	0.001	0.001
Mean	127.4^{a}	123.6^{a}	119.8^{a}	96.4^{b}	4.87	0.001	0.050

^{a,b,c} Means within a row with different superscripts differ ($P<0.05$)

*Orthogonal polynomial contrast L= linear and Q= quadratic

Moreover Davis and Stallcup (1967) observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production.

The molar proportions of acetic, propionic and butyric acids at 0, 3 and 6 h post feeding are presented in Table 3.9 and Figure 3.10. VFA concentration at 3 h post feeding was increased similar in all dietary treatments. However, the molar proportions of acetic, propionic and butyric acids (mol/100 mol) were not differ. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Higher VFA concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feedings VFA concentration was decreased indicating a more capture of C-skeleton for increased microbial protein synthesis (Sinclair et al., 1993; Witt et al., 1999). The result agrees with the report of Stiles et al. (1970), who reported that the rumen VFA concentration usually peaked 4 hours post-feeding. While the total VFA concentrations and that acetic acid were highest for the Starea-fed animals. The molar proportions of propionic, iso-butyric and iso-valeric acids were significantly greater for those fed the control ration.This finding was similar to those reported by Roman-Ponce et al. (1974) that Starea and SBM rations result in higher amount of total VFA than urea. Propionate concentration was similar in SBM and Starea rations and higher in urea rations.

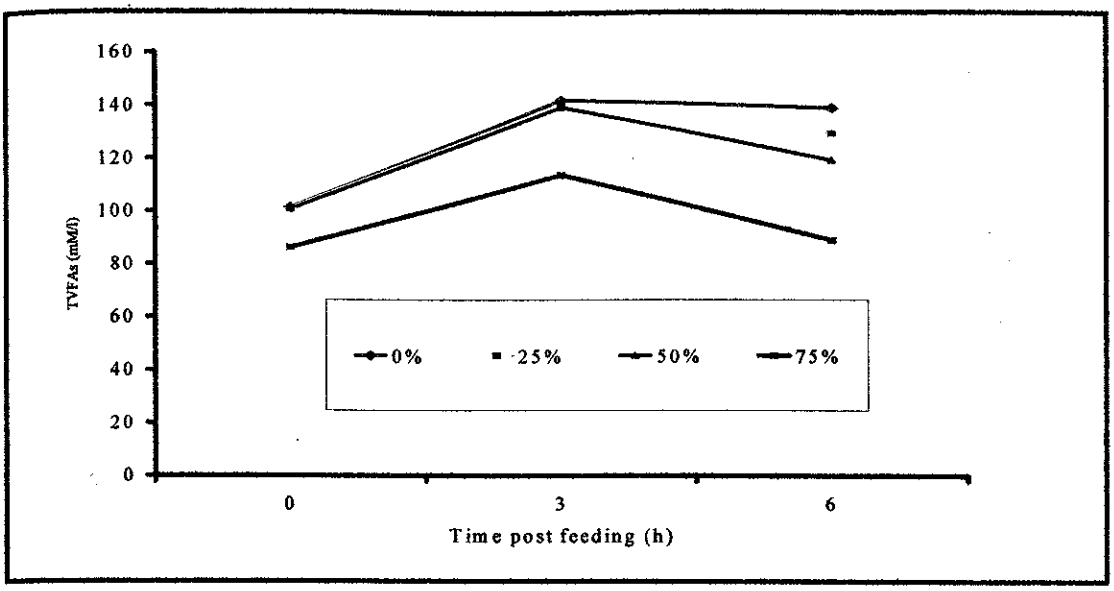

Figure 3.10 Hourly TVFAs (mM/l) in beef cattle receiving a diet containing four levels of Caspurea

Haskins et al. (1967) reported that no relationship of nitrogen source to molar percentages of VFA's, however, Davis et al. (1957) found that more acetate and greater A/P ratios in rumen fluid from SBM than from urea-supplemented cattle. Moreover, the mechanism for the decreased A/P ratios in rumen fluid of steers receiving high sulfur diets may involve a more efficient, sulfur-dependent metabolic pathway. Whanger and Matrone (1966) offered evidence of propionate synthesis via the acrylate pathway from lactate, a compound utilized poorly by animal tissue. This system taps a supply of energy otherwise deficient in rations which are inadequate in sulfur and provides an additional source of propionate thereby improving the overall efficiency of the diet. However, Holter et al. (1971) also found that higher proportions of acetate and butyrate have been depressed compared to controls whereas propionate and total VFA's were increased markedly in rations containing SBM (Davis et al., 1957; Hutjens and Schultz, 1971)

Blood urea nitrogen concentrations are presented in Table 3.10 and Figure 3.11. Blood urea nitrogen (22.5, 23.2, 23.3, 24.6 mg%) was highest ($P<0.01$) in 75% replacement diet than other treatments. BUN increased linearly ($P<0.01$) with increasing the level of Caspurea. These data indicate a slower rate of ammonia release more complete uptake of the NH_3-N for microbial protein synthesis when SBM rather than urea was the N source. These increases reflect the higher concentrations of ammonia in the rumen of these animals indicating that at a high NPN intake in the diet may cause imbalances between N and energy supply to rumen microorganisms and ammonia concentrations increase (Shabi et al., 1998), ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. Moreover, Sinclair et al. (1993) also reported that animals offered energy and nitrogen asynchronous diet had high blood urea nitrogen.

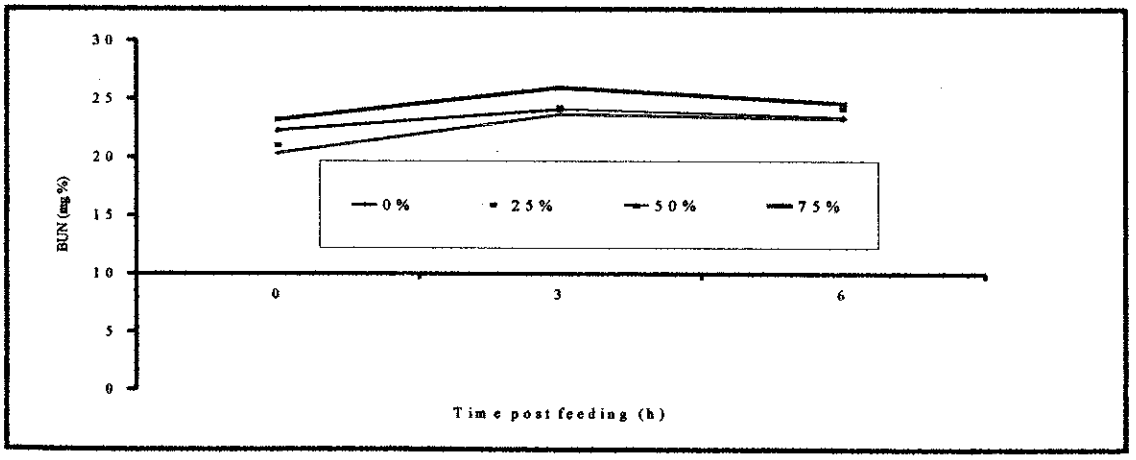

Figure 3.11 Hourly blood urea nitrogen (BUN, mg%) in beef cattle receiving a diet containing four levels of Caspurea

Blood urea nitrogen concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation

was greatest at the 3 h post feeding, rumen ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. however, at the 6 h post feedings BUN was decreased indicating a more excreted in the urine. (Sinclair et al., 1993; Witt et al., 1999). Schmidt et al. (1973) reported that blood NH_3-N level were only slightly higher when urea was used as a nitrogen source than when SBM, TSBM or Starea were used as the nitrogen sources (1.2 and 1.1 μg NH_3-N/ml blood). An increase in blood NH_3 levels would not be expected since the rumen NH_3 concentrations at the 1 h sampling time were approximately one-half the 30 mM/liter rumen fluid necessary to result in a rise in peripheral blood ammonia. Blood urea nitrogen reached its peak concentration approximately 1 h after the rumen ammonia peak irrespective of supplemental nitrogen source. At 2.5 h, BUN was lower in the animals fed Starea than for those fed urea ,and except for the 0 and 12 h samples, the levels for the animals fed urea and Starea were higher than those for SBM and TSBM fed groups. These data indicate a slower rate of ammonia release and/or more complete uptake of the NH_3-N for microbial protein synthesis when SBM rather than urea was the nitrogen source. In contrast, reported by Jones et al. (1974) that the highest blood urea nitrogen was with the SBM ration. Reducing dietary crude protein content resulted in lower BUN.

Table 3.10 Effect of dietary treatments on microbial population and blood urea nitrogen

Items	Caspurea : Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
Blood urea nitrogen (mg%)							
0	20.4^{c}	21.0^{c}	22.3^{b}	23.2^{a}	0.15	0.001	0.647
3	23.7^{c}	24.3^{b}	24.1^{bc}	26.0^{a}	0.10	0.001	0.003
6	23.3^{b}	24.2^{a}	23.5^{b}	24.6^{a}	0.10	0.001	0.438
Mean	22.5^{b}	23.2^{b}	23.3^{b}	24.6^{a}	0.39	0.001	0.451
Bacteria (x 10^{10} cell/ml)	2.6^{a}	2.5^{a}	2.5^{a}	2.4^{b}	0.02	0.001	0.008
Protozoa (x 10^{5} cell/ml)	2.2^{a}	2.2^{a}	2.1^{a}	1.9^{b}	0.04	0.002	0.079

^{a,b,c,d} Means within a row with different superscripts differ ($P<0.05$)

*Orthogonal polynomial contrast L= linear and Q= quadratic

Rumen microbe populations are presented in Table 3.10. Increases in level of Caspurea caused decreased linearly ($P<0.01$) in bacteria and protozoa populations. The lowest ($P<0.01$) population recorded was on animals fed 75% replacement diet. The levels of Caspurea replacement for soybean meal in concentrate had a significant influence upon both total bacteria and protozoa populations ($P<0.01$) which were lowest in 75% replacement diet. It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990). Sinclair et al. (1993) and Kim (2001) reported that higher synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased

microbial protein synthesis. Moreover, Jouaney and Ushida (1999) reported that ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration. Therefore, high soluble N in diets and cassava starch possibly affected the rumen microbe population. Microbial biomass, net 15N incorporation into cells, volatile fatty acid production increased linearly with increasing levels of cassava inclusion in diets (Sommart et al., 2000).

Nitrogen balance study is shown in Table 3.11. Urinary N excretion was similar across treatment, however, fecal N excretion increased linearly and quadratically ($P<0.01$) as level of Caspurea increased and was highest when Caspurea replacement for soybean meal in concentrate at 75% was fed. N absorption decreased linearly ($P<0.01$) as level of Caspurea increased and was lowest when Caspurea replacement for soybean meal in concentrate at 75% was fed. Decreases in N absorption were the results of increased fecal N excretion. Nitrogen absorption (65.5, 64.7, 60.6, 57.7 g/d) were also lowest ($P<0.01$) in 75% replacement diet, however, among 0, 25 and 50% replacement groups were not differ. With increasing the level of Caspurea, N absorption was decreased. Nitrogen retention (%N intake) (24.1, 23.6, 25.3, 13.6% N intake) tended to increase in 50% replacement diet ($P = 0.05$) while, in 0, 25 and 50% replacement diet groups were not differ. Moreover, N retention tended to decreased linearly ($P=0.08$) as the level of Caspurea increased. N excretion increase, indicating that asynchronous diet decrease N capture in the rumen and N utilization in beef cattle (Sinclair et al., 1993).

Table 3.11 Effect of dietary treatments on nitrogen balance

Items	Caspurea : Soybean meal					Contrast*	
	0 : 100	25 : 75	50 : 50	75 : 25	SEM	L	Q
Urine N (g/d)	45.1	44.8	40.2	46.6	4.51	0.999	0.190
Urine N (g/kgBW $^{0.75}$)	1.0	1.0	0.9	1.1	0.06	0.969	0.286
Feces N (g/d)	19.6^{b}	19.3^{b}	19.8^{b}	24.4^{a}	0.63	0.002	0.009
N absorption (g/d)	65.5^{a}	64.7^{a}	60.6^{b}	57.7^{b}	1.12	0.002	0.405
N retention (g/d)	20.5^{a}	19.8^{a}	20.4^{a}	11.2^{b}	2.18	0.088	0.213
N absorption (%N intake)	76.9^{a}	76.9^{a}	75.3^{a}	70.3^{b}	0.52	0.001	0.003
N retention (%N intake)	24.1^{a}	23.6^{a}	25.3^{a}	13.6^{b}	2.85	0.052	0.099

^{a,b} Means within a row with different superscripts differ (P<0.05)

*Orthogonal polynomial contrast L= linear and Q= quadratic

3.7 Conclusions

The results indicated that Caspurea used as a protein source replacement for soybean meal at 25 and 50% in ration were unaffected feed intake, BW change, blood metabolites, rumen microbe populations, end-products of ruminal fermentation, digestibility and nitrogen balance. Therefore, this study suggests that Caspurea would replace for soybean meal not more than 50%. Based on this experiment, Caspurea can be used as a protein source in Thai Native x Brahman beef cattle ration especially when fed on urea-treated rice straw as a roughage.

3.8 References

AOAC. (1985). Oficial Methods of Analysis. **Association of official Chemists,** washington, D.C.

Baldwin, R. L. (1970). Energy metabolism in anaerobes . **American J. Clin. Nutr.** 23: 1508-1513.

Blummel, M., and Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral detergent fibers as described by *in vitro* gas production and their relationship to voluntary feed intake. **Brit. J. Nutr.** 77: 757-768.

Blummel, M., and Ørskov, E. R. (1993). Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. **Anim. Feed Sci. Technol.** 40: 109-119.

Bloomfield, R. A., Kearley, E. D., Creach, D. O., and Muhrer, M. E. (1963). Ruminal pH and absorption of ammonia and VFA. **J. Anim. Sci.** 22: 833.

Briggs, P. K., Hogan, J. F., and Reid, R. L. (1957). The effect of volatile fatty acid, lactic acid, and ammonia on rumen pH in sheep. **Aust. J. Agric. Res.** 8 : 674-710.

Bromner, J. H., and Keeney, D. R. (1965). Steam distillation methods of determination of ammonium, nitrate and nitrite. **Annual. Chem. Acta.** 32:485 - 495.

Caffrey, P. J., Hatfield, E. E., Norton, H. W., and Garrigus, U. S. (1967). Nitrogen metabolism in the ovine. I. Adjusment to a urea-rich diet. **J. Anim. Sci.** 26:959-967.

Chumpawadee, S. (2006). **Synchronizing the rate of degradation of dietary energy and nitrogen release in Brahman-Native crossbred beef cattle ration.** Khon Kaen, Thailand. Graduate School, Khon Kaen University. 297 pp. (Ph. D. Thesis).

Clark, J. H., Spahr, S. L., and Derring, R. G. (1973). Urea utilization by lactating cows. **J. Dairy Sci.** 56: 763-169.

Coombe, J. B., Tribe, D. E., and Morrison, J. W. C. (1960). Some experimental observations on the toxicity of urea to sheep. **Aust. J. Agr. Res.** 11: 247.

Davis, G. V., and Stallcup, O. T. (1967). Effect of soybean meal, raw soybeans, corn gluten feed, and urea on the concentration of rumen fluid components at intervals after feeding. **J. Dairy Sci.** 50 : 1638 – 1645.

Davis, R. F., Woodhouse, N. S., Keeny, M., and Beck, G. H. (1957). The effect of various levels of dietary protein upon the volatile fatty acids in the rumen of the dairy cow. **J. Dairy Sci.** 40: 75.

Freitag, R. R., Smith, W. H., and Beeson, W. M. (1968). Factors related to utilization of urea vs. protein nitrogen supplemented diets by the ruminant. **J. Anim. Sci.** 27 :478-485.

Goering. H. K., and Van Soet, P. J. (1970). **Forage Fiber Analyses** (apparatus, reagents, procedures, and some applications) Agric. Handbook No.379. ARS-USDA, Washington, DC.

Haskins, B. R., Wise, M. B., Craig, H. B., and Barrick, E. R. (1967). Effect of levels of protein, source of protein and antibiotic on performance, carcass characteristics, rumen environment and liver abscesses of steers fed all-concentrate rations. **J. Anim. Sci.** 26: 430.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25 – 32.

Helmer, L. G., Bartley, E. E., and Deyoe, C. W. (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Hendrickx, H., and Martin. (1963). *In vitro* study of the nitrogen metabolism in the rumen. Compt. Rend. Rech., **Inst. Rech. Sci.** 2 nd Agri., Bruxelles 31:1. (As cited by Hungate, R. E. 1966. The rumen and its microbes. Academic Press, New York.)

Herrera-Saldana, R., Huber, J. T., and Poore, M. H. (1989). Influence of varying protein and starch degradabilities on performance of lactating cows. **J. Dairy Sci.** 72 : 1477-1483.

Holter, J. B., Colovos, N. F., Clark, R. M., Koes, R. M., Davis, H. A., and Urban, Jr. W. E. (1971). Urea for lactating cattle. V. Concentrate fiber and urea in a corn silage-high concentrate ration. **J. Dairy Sci.** 54: 1475-1482.

Hutjens, M. F., and Schultz, L. H. (1971). Addition of soybeans or methionine analog to high-concentrate rations for dairy cows. **J. Dairy Sci.** 54:1637-1646.

Jittakot, S. (1999). **Effect of cassarea as a protein replacement for soybean meal on feed intake, blood metabolites, ruminal fermentation, digestibility and microbial protein synthesis in dairy cows fed urea-treated rice straw as a roughage.** Khon Kaen, Thailand. Graduate School, Khon Kaen University. 171 pp. (M. Sc. Thesis).

Jones, G. M., Stephens, C., and Kensett, B. (1974). Utilization of starea, urea, or soybean meal in complete rations for lactating dairy cows. **J. Dairy Sci.** 58: 689-695.

Jouaney, J. P., and Ushida, K. (1999). The role of protozoa in feed digestion. **Asian-Aust. J. Anim. Sci.** 12: 113-126.

Kearl, L. C. (1982). Nutrient Requirements of Ruminants in Developing Countries. **Internation feedstuffs institute**, Utah Agriculcultural experiment station, Utah State University, Logan, Utah, USA.

Kim, C. H. (2001). Effect of different protein sources given synchronously or asynchronously into the rumen of consuming a beef cattle diet high in concentrate on the synthesis of microbial protein. **J. Anim. Feed Sci. Technol.** 43 : 831-840.

Kolver, E., Muller, L. D., Vaga, G. A., and Cassidy, T. J. (1998). Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. **J. Dairy Sci.** 81:2017-2028.

Lampila, M. (1972). Practical non protein-N-feeding to ruminants. **World Rev. Anim. Prod.** 8:28.

Ørskov, E. R., and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **J. Agric. Sci.** 92:499-504.

Ørskov, E. R., Fraser, C., McDonald, I., and Smart, R. (1974). Digestion of concentrate in sheep. 5. The effect of adding fish meal and urea together to cereal diets on protein digestion and utilization by young sheep. **Brit. J. Nutr.** 31: 89-96.

Oyaert, W., and Bouckaert, J. H. (1960). Quantitative aspects of food digestion in the rumen. **Zentr. Veterinamedizin.** 7:929. (As cited by lampila 1972.)

Roffler, R. E., and Satter, L. D. (1973). Influence of ration composition on ruminal ammonia concentration. **J. Dairy Sci.** 56 : 663. (Abstr.)

Roman-Ponce, H., Van Horn, H. H., Mashall, S. P., Wilcox, C. J., and Randel, P. F. (1974). Complete rations for dairy cattle V. Interaction of sugarcane bagasse quantity and form with soybean meal, urea, and starea. **J. Dairy Sci.** 58: 1320-1327.

SAS. (1996). **SAS User's Guide: Statistics.** Version 6. 14th ed Cary, NC: SAS Inst

Satter, L. D., and Roffler, R. E. (1973). Using NPN in the dairy cow ration. Page 45 in **Proc. 34th Minn. Nutr. Conf.** Bloomington, MN.

Schmidt, S. O., Jorgensen, N. A., Benevenga, N. F., and Burngart, V. H. (1973). Comparison of soybean meal, formaldehyde treated soybean meal, urea and Starea for steers. **J. Anim. Sci.** 37 : 1233-1237.

Shabi, K., Arieli, A., Bruckental, L., Aharoni, Y., Zamwel, S., Bor, A., and Tagari, H. (1998). Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dietary cows. **J. Dairy Sci.** 81 : 1991-2000.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R., and Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. **J. Agric. Sci. Camb.** 120: 251-263.

Sommart, K., Parker, D. S., Rowlinson, P., and Wanapat, M. (2000). Fermentation characteristics and microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried Ruzi grass as substrates. **Asian-Aust. J. Anim. Sci.** 13: 1084-1093.

Steel, R. G. D., and Torrie, J. H. (1980). **Principles and Procedures of Statistics with Special Reference to the Biological Sciences.** McGraw Hill, New York.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Susmel, P., Stefanon, B., Mills, C. R., and Candido, M. (1989). Change in amino acid composition of different protien sources after rumen incubation. **Anim. Prod.** 49:375-383.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Whanger, P. D., and Matrone, G. (1966). Metabolism of lactic, succinic and acrylic acids by rumen microorganisms for sheep fed sulfur-adequate and sulfur efficient diets. **Biochimica et Biophysica Acta.** 136:27.

Witt, M. W., Sinclair, L. A., Wilkinson, R. G., and Buttery, P. J. (1999). The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food *ad libitum*. **Brit. Soc. Anim. Sci.** 69: 223-235.

CHAPTER IV

EFFECT OF CASPUREA AS A PROTEIN SOURCE IN CONCENTRATE ON GROWTH PERFORMANCE OF THAI NATIVE x BRAHMAN BEEF CATTLE

4.1 Abstract

This experiment aimed to study the effects of Caspurea (cassava pulp-urea) on growth performance of Thai Native-Brahman beef cattle. Twelve Thai Native x Brahman beef cattle (approximately 365 days of age and average liveweight of 200±36 kg) were used in a randomized complete block design (RCBD). The treatments were levels of Caspurea replacement for total crude protein in concentrate at 0, 35 and 70% . Concentrate were formulated to contain 14% CP and were fed at 1.5% BW. All animals were fed *ad libitum* urea treated rice straw as roughage. The results showed that total dry matter (DM) intake was not significantly different among treatments (5.3, 5.5, 5.1 KgDM/d). Dry matter digestibility (62.6, 64.2, and 56.7%; $P<0.05$) and diet OM digestibility (63.0, 64.6, and 57.2%; $P<0.05$) were lowest in 70% replacement diet. Increase in level of CP replacement from Caspurea caused decreased linearly ($P<0.05$) and quadratically ($P<0.05$) in DM and OM digestibility. Ruminal ammonia-N (NH_3-N) concentration (12.0, 12.1, 16.0 mg%; $P<0.01$) was highest in 70% replacement diet and showed that with increasing the level of CP replacement from Caspurea, ruminal NH_3-N concentration was increased ($P<0.05$). Total volatile fatty acid (76.2, 82.1, 69.1

mM/l) was not significantly different among treatments. Bacteria population [2.16, 2.11, 1.98 (x 10^{10} cell/ml)] and protozoa population [2.06, 2.00, 1.86 (x 10^{5} cell/ml)] were lowest in 70% replacement diet, however, in 0 and 35% replacement diets were not differ. Moreover, bacteria and protozoa populations also decreased linearly (P <0.01) as the level of CP replacement from Caspurea increased. Blood urea nitrogen (20.2, 20.5, 21.8 mg%) was highest (P<0.01) in 70% replacement diet. Average daily gain (0.57, 0.62, 0.24 kg/d) was also lowest (P<0.01) in 70% replacement diet, but at 0 and 35% replacement diets were not differ, moreover, these data showed that ADG decreased linearly (P<0.01) and quadratically (P<0.01) with increasing the level of CP replacement from Caspurea. In conclusion, These results indicated that 35% replacement by crude protein from Caspurea for total crude protein in concentrate has positive effects on Thai Native x Brahman crossbred beef cattle production.

4.2 Introduction

Problems in feeding urea to beef cattle are intensified by emphasis on high-concentrate rations to increase production and to provide necessary energy. A study with high-producing cows receiving various levels of urea in a high-concentrate ration showed that high-urea levels (20 to 40% of the total ration nitrogen) depressed both milk yield and efficiency (Huber and Sandy, 1965). Nitrogen balance studies suggested that milk production efficiency decreased with added urea because of decreased nitrogen retention (Huber et al., 1967). Palatability of concentrate mixtures containing urea lessened with higher feed intake (Van Horn et al., 1967). Previous studies with rumen-fistulated cattle (Stiles, 1969) indicated that Starea, an intimate mixture mixture of gelatinized starch and urea, improved utilization of urea nitrogen and palatability of urea-containing rations. Starea, an extrusion-processed mixture of

grain and urea, has increased synthesis of microbial protein *in vitro* (Helmer et al., 1970) and *in vivo* (Stiles et al., 1970) above that of unprocessed mixtures of grain and urea. Maintaining specific moisture, temperature, and pressure in the extrusion-cooking process is vital for producing high quality Starea (Behnke et al., 1973). Reported here is work to determine if Caspurea has a similar effect on N utilization of lactating dairy cows. Crude protein from Caspurea was replaced for the total dietary protein in concentrate.

4.3 Objectives

The objectives of this study were to compare production performance of Thai Native-Brahman beef cattle and utilization of dietary nitrogen from Caspurea replacement for the total dietary protein in concentrate.

4.4 Materials and Methods

Twelve crossbred (Thai Native x Brahman) beef cattle were used in the experiment. The animals were used in a randomized complete block design (RCBD). The dietary treatments were as follows:

T1= Caspurea as CP in concentrate at 0%

T2= Caspurea as CP source in concentrate at 35%

T3= Caspurea as CP source in concentrate at 70%

All animals were fed *ad libitum* of urea-treated rice straw together with concentrate (14% CP) at 1.5% BW, twice daily at 08.00 and 17.00. Each animal was housed in an individaul pen and free access to clean water all times. Daily collection of faeces were made in the last 7 days of the experiment. The samples were stored at –20°C until analysis. Daily faeces collects in each period were bulked, mixed and a 5%

sub sample taken. The sample of faeces were oven dried and ground (1 mm. Screen) for determination of DM, ash, OM, NDF, ADF. acid insoluble ash (AIA) and N content.

Rumen fluid and jugular blood were collected on the last day of each period. Ruminal pH was measured immediately after ruminal fluid sampling, 5 ml of 6 N HCl was added to 50 ml. Rumen fluid was collected 0 and 4 h post feeding and jugular blood was collected at 0 and 4 h post feeding and placed into heparinized vacationer tubes and centifuged at 2,500 x g for 15 minutes. Both rumen fluid and jugular blood were stored at 5°C until analysis.

Liveweight of animals were measured at the beginning of the trial and every three weeks. Urea treated rice straw and concentrate were sampled every two weeks and the composited samples analyze for NDF, ADF and ADL content (Van Soest ,1970), and CP DM and ash were determined by the methods of AOAC (1985). The AIA content in feed and fecal were used to calculate digestibility (Schneider and Flatt, 1975).

Rumen fluid TVFA concentration was determined by titration technique of Briggs et al. (1957). Acetic, propionic and butyric acids concentration were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard Avondale, PA). NH_3 –N were determined by the methods of Bromner and Keeney (1965). The plasma samples were kept at –20°C until analyzed for blood urea nitrogen (BUN) using BMG's urea reagent (Boehringer Mannheim, Indianapolis, IN).

4.5 Statistical analyses

All data obtained from the experiment were subjected to analysis of variance using Proc. GLM (SAS, 1996) according to a randomized complete block design

(RCBD). Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torries, 1980) and the General Linear Models (GLM) procedure for orthogonal polynomial analysis of SAS (SAS, 1996).

4.6 Results and Discussion

The composition of the diets is shown in Table 4.1. Variation in CP within concentrates was small (approximately 14% CP) and was slightly higher than formulated at 14% CP. The high Caspurea (70% replacement) rations contained slightly higher NDF than 0 and 35% replacement diets.

The effect of diet on feed intake is shown in Table 4.1. These results indicated that concentrate intake decreased quadatically ($P<0.05$) as the levels of Caspurea increased. Although, Caspurea, and intimate mixture of gelatinized starch and urea, improved palatability of urea-containing rations, but in high level of CP from Caspurea at 70% of the total ration CP depressed concentrate intake. However, these result agrees with the report of Stiles et al. (1970), who found that Starea improved palatability of urea-containing rations. Moreover, these results showed that total intake (%BW) increased quadatically ($P<0.05$) as the levels of Caspurea increased.

Table 4.1 Feed formulation and chemical composition of dietary treatment.

	Crude protein Ratio			
	Caspurea : Concentrate			
Ingredient	**0:100**	**35:65**	**70:30**	
Caspurea	0.0	10.9	21.8	
Cassava pulp	40.0	52.1	61.2	
Rice bran	18.0	10.0	4.0	
Soybean meal	21.0	14.0	6.0	
Palm meal	17.0	9.0	3.0	
Molasses	1.0	1.0	1.0	
Sulfur	0.2	0.2	0.2	
Urea	0.1	0.1	0.1	
Lime stone	0.5	0.5	0.5	
Salt (NaCl)	0.5	0.5	0.5	
Mixed mineral	0.8	0.8	0.8	
Total	100.0	100.0	100.0	
Chemical composition (%)				**Urea treated Rice straw**
DM	90.5	89.9	89.4	55.8
	.....% DM.....			
OM	91.8	91.5	91.0	93.6
NDF	15.4	16.8	19.4	65.4
ADF	7.5	9.2	10.4	45.1
ADL	3.1	3.4	4.2	8.3
Ash	8.2	8.5	9.0	6.4
AIA	1.2	1.3	1.5	1.1
CP	14.2	14.2	14.1	7.1

DM: dry matter, OM: organic matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, AIA: acid insoluble ash, CP: crude protein

Table 4.2 Effect of Caspurea on feed intake and nutrient intake

	Crude protein ratio					
	Caspurea:Concentrate				Contrast*	
	0:100	35:65	70:30	SEM	L	Q
Concentrate	2.2	2.5	2.2	0.16	0.104	0.002
Roughage	3.1	3.0	2.9	0.09	0.343	0.909
Total intake	5.3	5.5	5.1	0.18	0.421	0.042
Total intake (%BW)	2.3[b]	2.6[a]	2.5[a]	0.06	0.046	0.064
Total intake (g/kgBW$^{0.75}$)	88.4[b]	99.7[a]	95.3[ab]	2.30	0.078	0.031
Nutrient intake (kgDM/d)						
OM	4.8	5.3	4.8	0.19	0.443	0.005
NDF	2.3	2.4	2.3	0.06	0.602	0.246
CP	0.5[b]	0.6[a]	0.5[b]	0.01	0.154	0.001

SE = standard error of the mean, *Orthogonal polynomial contrast L= linear and Q= quadratic

The effect of diet on feed intake is shown in Table 4.2. These results indicated that total dry matter intake was not significantly different among treatments (5.3, 5.5, 5.1 KgDM/d; $P>0.05$). Total intake (%BW) increased linearly ($P<0.05$) as the levels of Caspurea increased. Moreover, OM and CP intake decreased (quadratically, $P<0.01$) when the level of Caspurea increased from 35 to 70% replacement diet.

The effect of diet on feed digestibility is shown in Table 4.3. These results indicated that DM digestibility (62.6, 64.2, and 56.7%) and OM digestibility (63.0, 64.6, and 57.2%) were lowest ($P<0.05$) in 70% replacement diet. Increase in level of CP replacement from Caspurea caused decreased linearly ($P<0.05$) and quadratically ($P<0.05$) in DM and OM digestibility. Caspurea at 70% of the total ration CP, DM

and OM digestibility was lower than other treatments. Dry matter digestibility decreased linearly ($P<0.05$) as the levels of Caspurea increased. Moreover, OM and CP intake decreased (quadratically, $P<0.05$) when the level of Caspurea increased from 35 to 70% replacement diet. These data indicated that urea N from Caspurea was utilized less efficiently than nitrogen from true protein such as soybean meal. Susmel et al. (1989) reported that SBM has very high biological value and have been used as a true protein source for ruminant, it has been suggested that matching supply of energy and N supply in the rumen may improve microbial growth and activity. However, Helmer et al. (1970) reported that cows receiving either soybean meal or Starea as the protein supplement showed no differences between rations for apparent digestibility of DM, CP, ADF or cellulose.

Table 4.3 Effect of Caspurea on DM, nutrient digestibility and average daily gain.

	Crude protein ratio					
	Caspurea:Concentrate				Contrast*	
	0:100	35:65	70:30	SEM	L	Q
Digestibility (%DM)						
DM	62.6[a]	64.2[a]	56.7[b]	1.21	0.014	0.022
OM	63.0[a]	64.6[a]	57.2[b]	1.20	0.014	0.022
NDF	55.7[ab]	59.0[a]	53.1[b]	1.44	0.246	0.040
CP	71.4[b]	76.6[a]	66.0[c]	1.15	0.017	0.001
Body weight kg)						
Initial weight	187.7	191.2	190.0	-	-	-
Final weight	240.0[a]	247.0[a]	212.3[b]	5.91	0.004	0.009
ADG (kg/d)	0.57[a]	0.62[a]	0.24[b]	0.04	0.001	0.004

SE = standard error of the mean, ADG = average daily gain, *Orthogonal polynomial contrast L= linear and Q= quadratic

Average daily gain (ADG) (0.57, 0.62, 0.24 kg/d) (Table 4.3) was also lowest (P<0.01) in 70% replacement diet, but at 0 and 35% replacement diets were not differ. Moreover, ADG decreased linearly (P<0.01) and quadratically (P<0.01) with increasing the level of CP replacement from Caspurea. Average daily gain was similar for 0 and 35% replacement for the total ration CP. The results indicated that NPN sources would supply no more than 35% of the total ration N, resulting in a decrease in ADG when crude protein from Caspurea levels were increased from 35 to 70%. Average daily gain decreased linearly (P<0.01) as the levels of Caspurea increased. Moreover, OM and CP intake decreased (quadratically, P<0.01) when the level of Caspurea increased from 35 to 70% replacement diet. These results were higher than

the report of Huber et al. (1967) that NPN sources would supply no more than 20-22% of the total ration N , because N source from Caspurea was utilized less efficiently than nitrogen from natural plant protein such as soybean meal. Moreover, Susmel et al. (1989) reported that soybean meal had the high essential amino acid and biological value for ruminant. Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. The average change in body weight shows that dairy cows fed urea lost significantly more weight than those fed soybean meal or Starea. Differences in weight grains were not statistically significant between the soybean meal and Starea fed animals. On the other hand, Starea and soybean meal rations were consumed in sufficient quantities to support high production and to maintain or increase body reserves.

Ruminal pH data are showed in Table 4.4. Ruminal pH was not significantly different ($P>0.05$) among the treatments. Similar, Stiles et al. (1970) reported that no differences were observed in rumen pH between the rations. Although the mean pH for the rations was not different, several variables were negatively correlated with pH of the Starea-fed animals. Low pH values appeared to be related to high VFA and high bacterial nitrogen production. H-ion concentration appeared to be influenced more by VFA than by ammonia or lactate production. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns. Moreover, Roman-Ponce et al. (1974) reported that lower rumen pH observed for SBM and Starea rations resulted in higher VFA

concentrations, with the Urea opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when Urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with a more acid rumen pH.

Rumen NH_3-N concentration are presented in Table 4.4. Ruminal ammonia-N concentration (12.0, 12.1, 16.0 mg%) was highest ($P<0.01$) in 70% replacement diet. Moreover, similar for 0 and 35% replacement diet, perhaps indicating that true protein was hydrolyzed slower than Caspurea. If this were the case, then one would expect ruminal ammonia of the high NPN (70% replacement) fed group to be higher than that of the true protein fed group. It is possible that low efficient capture of N for microbial protein synthesis, because urea N sources from Caspurea was utilized less efficiently than nitrogen from natural plant proteins. Davis and Stallcup (1967) observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. Reported by Kolver et al. (1998) that decreases ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis. Moreover, Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea N into microbial protein. Roman-Ponce et al. (1974) reported that rumen ammonia N (NH_3-N) concentration was higher for all rations at 1 h after feeding than 2 h (28.2 and 19.0 mg%). Rumen ammonia was similar for Urea and Starea rations but higher than SBM at 1 h and 2 h In all treatments rumen NH_3-N concentration were higher at 1 h than 2 h. (28.2 and 19.0). However, Davis and Stallcup (1967) reported peaks of ammonia concentration in rumen contents 2 or 3 h after feeding either SBM, Urea or SBM+urea rations. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia

absorption and that low rumen pH was was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns, e.g. lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with the urea rations having the opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with more acid rumen pH. Schmidt et al. (1973) reported that at the 1.5 h sampling time, ruminal ammonia levels in animals fed urea or Starea were not different, perhaps indicating that Starea was hydrolyzed slower than urea (Stiles et al., 1970). If this were the case, then one would expect ruminal ammonia of the urea fed group to be higher than that of the Starea fed group prior to the initial post-feeding sample. Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. Rumen ammonia levels in animal fed urea and Starea were higher than those for animals fed SBM and TSBM until the 3.5 h (urea) and 5.5 h (Starea) sampling times. There were no differences in the levels of rumen NH_3-N in animals fed the SBM or TSBM diets. Moreover, high rumen ammonia has been associated with higher content of SBM or crude protein in rations (Freitag et al., 1968). Thompson et al. (1972) found peak rumen NH_3-N for Starea rations 90 min after feeding. Although rumen NH_3-N values for urea and Starea were not significantly different at either 1 or 2 h, the decline in NH_3-N from 1 to 2 h was 12.8 for urea and 6.4 mg% for Starea, suggesting a slower hydrolysis for Starea than urea rations, in agreement with Stiles et al. (1970) and Schmidt et al. (1973). Many workers (as these data confirm) have obtained higher ammonia contents after

feeding with urea than SBM rations (Davis and Stallcup,1964; Freitag et al.,1968; Schmidt et al., 1973; Thompson et al., 1972). Stiles et al. (1970) observed lower rumen ammonia content in the Starea-fed cows than urea-fed cows. Others (like these data) failed to find lowering of rumen ammonia with Starea as compared to Urea (Schmidt et al., 1973) but did find a slow decline in rumen ammonia with Starea after feeding which agreed with these results. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA.

Blood urea nitrogen concentrations (Table 4.4) at 0 h after feeding increased linearly and quadratically ($P<0.01$) as level of CP from Caspurea increased. Blood urea nitrogen was higher in the animals fed 70% replacement than other treatments. These data indicate that a slower rate of ammonia release and or more complete uptake of the NH_3-N for microbial protein synthesis when natural plant protein rather than urea was the N source. These increases reflect the higher concentrations of ammonia in the rumen of these animals indicating that at a high NPN intake in the diet may cause imbalances between N and energy supply to rumen microorganisms and ammonia concentrations increase (Shabi et al., 1998), ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. Moreover, Sinclair et al. (1993) also reported that animals offered energy and nitrogen asynchronous diet had high blood urea. Schmidt et al. (1973) reported that blood NH_3-N level were only slightly higher when urea was used as a nitrogen source than when SBM, TSBM or Starea were used as the nitrogen sources (1.2 and 1.1 μg NH_3-N/ml blood). An increase in blood NH_3 levels would not be expected since the rumen NH_3 concentrations at the 1 h sampling time were approximately one-half the 30 mM/liter rumen fluid necessary to result in a rise in peripheral blood ammonia. Blood urea

nitrogen reached its peak concentration approximately 1 h after the rumen ammonia peak irrespective of supplemental nitrogen source. At 2.5 h, BUN was lower in the animals fed Starea than for those fed urea, and except for the 0 and 12 h samples, the levels for the animals fed urea and Starea were higher than those for SBM and TSBM fed groups.

Table 4.4 Effect of dietary treatments on rumen fermentation, microbe population and BUN

	Crude protein ratio					
	Caspurea:Concentrate				**Contrast***	
Items	**0:100**	**35:65**	**70:30**	**SEM**	**L**	**Q**
pH (hr-post-feeding)						
0	6.3	6.3	6.1	0.14	0.355	0.351
4	5.3	5.6	6.2	0.52	0.240	0.850
Mean	5.8	6.0	6.1	0.39	0.352	0.942
NH_3-N (mg%)						
0	10.5^{b}	9.5^{c}	14.5^{a}	0.24	0.001	0.001
4	13.5^{b}	14.5^{c}	17.5^{a}	0.34	0.001	0.050
Mean	12.0^{b}	12.1^{b}	16.0^{a}	1.12	0.002	0.054
Blood urea nitrogen(mg%)						
0	18.4^{b}	18.5^{b}	20.8^{a}	0.46	0.010	0.095
4	22.0	22.5	22.8	0.77	0.502	0.932
Mean	20.2	20.5	21.8	1.11	0.748	0.599
Bacteria (x 10^{10} cell/ml)	2.16^{a}	2.11^{a}	1.98^{b}	0.02	0.002	0.230
Protozoa (x 10^{5} cell/ml)	2.06^{a}	2.00^{a}	1.86^{b}	0.03	0.004	0.306

SE = standard error of the mean, *Orthogonal polynomial contrast L= linear and Q= quadratic

These data indicate a slower rate of ammonia release and/or more complete uptake of the NH_3-N for microbial protein synthesis when SBM rather than Urea was the nitrogen source. In contrast, reported by Jones et al. (1974) that the highest blood urea nitrogen was with the SBM ration. Reducing dietary crude protein content resulted in lower BUN.

Rumen microbe populations are presented in Table 4.4. The levels of crude protein from Caspurea replacement for total crude protein in concentrate had a significant influence upon both total bacteria and protozoa population ($P<0.01$). Bacteria population [2.16, 2.11, 1.98 (x 10^{10} cell/ml)] and protozoa populations [2.06, 2.00, 1.86 (x 10^5 cell/ml)] were lowest in 70% replacement diet, however, in 0 and 35% replacement diets were not differ. Moreover, bacteria and protozoa populations also decreased linearly ($P<0.01$) as the level of CP replacement from Caspurea increased. The lowest population recorded was on animals fed 70% replacement diet ($P<0.01$). It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990). Sinclair et al. (1993) and Kim (2001) reported that higher synchonizing rate of degradation of dietary energy and N release in the rumen beneficially increased microbial protein synthesis. Moreover, Jouaney and Ushida (1999) reported that ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration. Therefore, high soluble N in diets and cassava starch possibly affected the rumen microbe population. Microbial biomass, net 15^N incorporation into cells, volatile fatty acid production increased linearly with increasing levels of cassava inclusion in diets (Sommart et al., 2000).

Table 4.5 Effect of Caspurea on volatile fatty acid concentrations

	Crude protein ratio					
	Caspurea:Concentrate				Contrast*	
Items	**0:100**	**35:65**	**70:30**	**SEM**	**L**	**Q**
h-post-feeding						
Acetic acid (mol/100 mol)						
0	67.7	66.9	67.6	2.10	0.980	0.804
4	70.6	70.2	69.7	1.01	0.514	0.978
Mean	69.2	68.6	68.6	1.18	0.751	0.829
Propionic acid (mol/100 mol)						
0	14.5	17.9	15.5	1.68	0.684	0.198
4	22.5	22.5	24.6	1.70	0.401	0.618
Mean	18.5	20.2	20.1	1.88	0.552	0.685
Butyric acid (mol/100 mol)						
0	6.9	8.5	7.6	0.45	0.295	0.069
4	13.4	12.2	11.7	1.00	0.262	0.782
Mean	10.2	10.3	9.7	1.12	0.747	0.757

SE = standard error of the mean,

*Orthogonal polynomial contrast L= linear and Q= quadratic

Total VFA concentrations in the rumen (76.2, 82.1, 69.1 mM/l) was not different among treatments ($P>0.05$) (Table 4.6). However, total VFA concentrations at 0 and 4 h post feeding decreased quadratically ($P<0.01$) as level of Caspurea increased. The diet level of CP from Caspurea at 70% of the total ration CP caused lower TVFAs than other treatments. The molar proportions of acetic, propionic and butyric acids at 0 and 4 h post feeding (Table 4.5) found that VFA concentration concentration at 4 h post feeding were increased similar in all dietary treatments.

However, the molar proportions of acetic, propionic and butyric acids (mol/100 mol) were not differ. Total volatile fatty acid decreased linearly ($P<0.01$) as the levels of Caspurea increased. Moreover, OM and CP intake decreased (quadratically, $P<0.05$) when the level of Caspurea increased from 35 to 70% replacement diet.

Table 4.6 Effect of Caspurea on TVFA concentrations and C2:C3 ratio

	Crude protein ratio					
	Caspurea:Concentrate				Contrast*	
Items	**0:100**	**35:65**	**70:30**	**SEM**	**L**	**Q**
TVFAs (mM/l)						
0	55.5[b]	68.0[a]	54.0[b]	2.61	0.703	0.006
4	96.9[a]	96.2[a]	84.3[b]	1.60	0.001	0.028
Mean	76.2	82.1	69.1	9.90	0.490	0.287
C2:C3						
0	3.0	3.1	3.1	0.25	0.752	0.827
4	3.2	3.4	3.4	0.21	0.407	0.769
Mean	3.1	3.3	3.3	0.21	0.378	0.687

SE = standard error of the mean, *Orthogonal polynomial contrast L= linear and Q= quadratic, C2= acetic acid, C3= Propionic acid

Stiles et al. (1970) reported that the rumen VFA concentration usually peaked 4 hours post-feeding. While total VFA concentration and that acetic acid were highest for the Starea-fed animals. The molar proportions of propionic, iso-butyric and iso-valeric acids were significantly greater for those fed the control ration.This finding was similar to those reported by Roman-Ponce et al. (1974) that Starea and SBM rations result in higher amount of total VFA than urea. Propionate concentration was

similar in SBM and Starea rations and higher in urea rations. Haskins et al. (1967) reported that no relationship of nitrogen source to molar percentages of VFA's but Davis and Stallcup (1967) found more acetate and greater A/P ratios in rumen fluid from SBM than from urea-supplemented cattle. Moreover, the mechanism for the decreased A/P ratios in rumen fluid of steers receiving high sulfur diets may involve a more efficient, sulfur-dependent metabolic pathway. Whanger and Matrone (1966) offered evidence of propionate synthesis via the acrylate pathway from lactate, a compound utilized poorly by animal tissue. This system taps a supply of energy otherwise deficient in rations which are inadequate in sulfur and provides an additional source of propionate thereby improving the overall efficiency of the diet. However, Holter et al. (1971) also found that higher proportions of acetate and butyrate have been depressed compared to controls whereas propionate and total VFA's were increased markedly in rations containing SBM (Davis et al., 1957; Hutjens and Schultz, 1971).

4.7 Conclusions

In conclusion, the results indicated that Caspurea used as a protein source replacement for total crude protein at 35% in ration did not affect feed intake, ADG and blood metabolites, rumen microbe populations, end-products of ruminal fermentation, digestibility. Therefore this study suggests that crude protein from Caspurea would replace for total crude protein in concentrate no more than 35%. Based on this experiment, Caspurea can be used as a protein source in Thai Native x Brahman beef cattle ration, especially when fed on urea-treated rice straw as a roughage.

4.8 References

AOAC.(1985). Official Methods of Analysis. **Association of official Chemists,** Washington, D.C.

Behnke, K. C., Deyoe, C. W., Bartley, E. E., and Griffel, G. W. (1973). Results from examing urea containing protein supplements by scanning electron microscropy. Page 25 in **Feedstuffs** Nov. 5.

Bloomfield, R. A., Kearley, E. D., Creach, D. O., and Muhrer, M. E. (1963). Ruminal pH and absorption of ammonia and VFA. **J. Anim. Sci.** 22: 833.

Briggs, P. K., Hogan, J. F., and Reid, R. L. (1957). The effect of volatile fatty acid, lactic acid, and ammonia on rumen pH in sheep. **Aust. J. Agric. Res.** 8 : 674-710.

Bromner, J. H., and Keeney, D. R. (1965). Steam distillation medthodes of determination of ammonium, nitrate and nitrite. **Annual. Chem. Acta.** 32 : 485 - 495.

Coombe, J. B., Tribe, D. E., and Morrison, J. W. C. (1960). Some experimental observations on the toxicity of urea to sheep. **Aust. J. Agr. Res.** 11: 247.

Davis, G. V., and Stallcup, O. T. (1967). Effect of soybean meal, raw soybeans, corn gluten feed, and urea on the concentration of rumen fluid components at intervals after feeding. **J. Dairy Sci.** 50 : 1638 – 1645.

Davis, G. V., and Stallcup, O. T. (1964). Influence of dietary nitrogen on nitrogen metabolism in the rumen. **J. Dairy Sci.** 47:1237.

Davis, R. F., Woodhouse, N. S., Keeny, M., and Beck, G. H. (1957). The effect of various levels of dietary protein upon the volatile fatty acids in the rumen of the dairy cow. **J. Dairy Sci.** 40: 75.

Freitag, R. R., Smith, W. H., and Beeson, W. M. (1968). Factors related to utilization of urea vs. protein nitrogen supplemented diets by the ruminant. **J. Anim. Sci.** 27 :478-485.

Goering. H. K., and Van Soet, P. J. (1970). **Forage Fiber Analyses** (apparatus, reagents, procedures, and some applications) Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Haskins, B. R., Wise, M. B., Craig, H. B., and Barrick, E. R. (1967). Effect of levels of protein, source of protein and antibiotic on performance, carcass characteristics, rumen environment and liver abscesses of steers fed all-concentrate rations. **J. Anim. Sci.** 26: 430.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25 – 32.

Helmer, L. G., Bartley, E. E., and Dyoe, C. W. (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Herrera-Saldana, R., Huber, J. T., and Poore, M. H. (1989). Influence of varying protein and starch degradabilities on performance of lactating cows. **J. Dairy Sci.** 72 : 1477-1483.

Holter, J. B., Colovos, N. F., Clark, R. M., Koes, R. M., Davis, H. A., and Urban, Jr. W. E. (1971). Urea for lactating cattle. V. Concentrate fiber and urea in a corn silage-high concentrate ration. **J. Dairy Sci.** 54: 1475-1482.

Huber, J. T., and Sandy, R. A. (1965). Response of dairy cows fed unlimited corn silage to three levels of urea and grain. (Abstr.) **J. Anim. Sci.** 24 : 887.

Huber, J. T., Sandy, R. A., Polan, C. E., Bryant, C. T., and Blaser, R. E. (1967). Varying levels of urea for dairy cows fed corn silage as the only forage. **J. Dairy Sci.** 50 : 1241-1246.

Hutjens, M. F., and Schultz, L. H. (1971). Addition of soybeans or methionine analog to high-concentrate rations for dairy cows. **J. Dairy Sci.** 54:1637-1646.

Jittakot, S. (1999). **Effect of cassarea as a protein replacement for soybean meal on feed intake, blood metabolites, ruminal fermentation, digestibility and microbial protein synthesis in dairy cows fed urea-treated rice straw as a roughage.** Khon Kaen, Thailand. Graduate School, Khon Kaen University. 171 pp. (M. Sc. Thesis).

Jones, G. M., Stephens, C., and Kensett, B. (1974). Utilization of starea, urea, or soybean meal in complete rations for lactating dairy cows. **J. Dairy Sci.** 58: 689-695.

Jouaney, J. P., and Ushida, K. (1999). The role of protozoa in feed digestion. **Asian-Aust. J. Anim. Sci.** 12: 113-126.

Kim, C. H. (2001). Effect of different protein sources given synchronously or asynchronously into the rumen of consuming a beef cattle diet high in concentrate on the synthesis of microbial protein. **J. Anim. Feed Sci. Technol.** 43 : 831-840.

Kolver, E., Muller, L. D., Vaga, G. A., and Cassidy, T. J. (1998). Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. **J. Dairy Sci.** 81:2017-2028.

Roffler, R. E., and Satter, L. D. (1973). Influence of ration composition on ruminal ammonia concentration. **J. Dairy Sci.** 56 : 663. (Abstr.)

Roman-Ponce, H., Van Horn, H. H., Mashall, S. P., Wilcox, C. J., and Randel, P. F. (1974). Complete rations for dairy cattle V. Interaction of sugarcane bagasse quantity and form with soybean meal, urea, and starea. **J. Dairy Sci.** 58: 1320-1327.

SAS. (1996). **SAS User's Guide: Statistics.** Version 6. 14th ed Cary, NC: SAS Inst

Schmidt, S. O., Jorgensen, N. A., Benevenga, N. F., and Burngart, V. H. (1973). Comparison of soybean meal, formaldehyde treated soybean meal, urea and starea for steers. **J. Anim. Sci.** 37 : 1233-1237.

Shabi, K., Arieli, A., Bruckental, L., Aharoni, Y., Zamwel, S., Bor, A., and Tagari, H. (1998). Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dietary cows. **J. Dairy Sci.** 81 : 1991-2000.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R., and Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. **J. Agric. Sci. Camb.** 120: 251-263.

Sommart, K., Parker, D. S., Rowlinson, P., and Wanapat, M. (2000). Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried Ruzi grass as substrates. **Asian-Aust. J. Anim. Sci.** 13: 1084-1093.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Susmel, P., Stefanon, B., Mills, C. R., and Candido, M. (1989). Change in amino acid composition of different protein sources after rumen incubation. **Anim. Prod.** 49:375-383.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Van Horn, H. H., Foreman, C. F., and Rodriguez, J. D. (1967). Effect of high-urea supplementation on feed intake and milk production of dairy cows. **J. Dairy Sci.** 50 : 709-716.

Whanger, P. D., and Matrone, G. (1966). Metabolism of lactic, succinic and acrylic acids by rumen microorganisms for sheep fed sulfur-adequate and sulfur efficient diets. **Biochimica et Biophysica Acta.** 136:27.

Witt, M. W., Sinclair, L. A., Wilkinson, R. G., and Buttery, P. J. (1999). The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food ad libitum. **Brit. Soc. Anim. Sci.** 69: 223-235.

CHAPTER V

EFFECT OF CASPUREA AS A PROTEIN SOURCE AND RUMEN UNDEGRADABLE PROTEIN LEVELS ON PERFORMANCE OF THAI NATIVE x BRAHMAN BEEF CATTLE

5.1 Abstract

This experiment aimed to study the effects of Caspurea (cassava pulp-urea) as a protein source and rumen undegradable protein levels on productive performance of Thai Native-Brahman beef cattle. Four Thai Native x Brahman beef cattle (approximately 365 days of age and average liveweight of 175.5±18.6 kg) were used in a 4 x 4 Latin square arrangement of treatments with 21-d periods. The treatments were levels of Caspurea as a protein source at 35% and rumen undegradable protein in concentrate at 30, 35, 40 and 45% . Concentrates were formulated to contain 14% CP and were fed at 2.0% BW. All animals were fed *ad libitum* with urea treated rice straw as roughage. The results showed that total dry matter (DM) intake (5.9, 5.8, 6.0, 6.0 kgDM/d) and DM digestibility (67.8, 68.9, 71.9, 69.9%) were highest ($P<0.05$) in 40% RUP. Dry matter intake increased linearly ($P<0.01$) as the level of RUP increased. Moreover, OM digestibility increased linearly ($P<0.05$) with increasing the level of RUP. Ruminal ammonia-N concentration (12.3, 11.0, 9.5, 11.0 mg%; $P<0.01$) was highest in 30% RUP. In addition, linearly decrease

in ruminal ammonia-N ($P<0.01$) reflected increases in level of RUP, however, at 45% RUP, NH_3-N was increased quadratically ($P<0.01$). Total volatile fatty acid (94.3, 113.25, 116.7, 112.1 mM/l; $P<0.01$) increased linearly ($P<0.01$) as the level of RUP increased, however, at 45% RUP TVFAs decreased quadratically ($P<0.01$). Bacteria population (2.2, 2.5, 2.6, 2.4 (x 10^{10} cell/ml); $P<0.05$) and protozoa population (1.9, 2.1, 2.2, 1.9 (x 10^5 cell/ml); $P<0.05$) were lowest in 30% RUP, but 35% and 40% RUP diets were not differ. Moreover, these data showed that increase in bacteria and protozoa populations reflected increased in level of RUP from 30 to 40%. However, bacteria and protozoa populations were decreased (quadratically, $P<0.05$) as the level of RUP increased from 40 to 45%. Nitrogen absorption (71.8, 70.4, 72.9, 69.6 g/d; $P>0.05$) was not significantly different among dietary treatments. Nitrogen retention (20.9, 19.7, 31.0, 21.5 g/d; $P<0.05$) was highest in 40% RUP and tended to increase as level of RUP increased, however, at N retention at 45% RUP tended to decrease (quadratically, $P=0.1$). Blood urea nitrogen (22.4, 21.6, 20.4, 21.2 mg%; $P>0.05$) was highest in 30% RUP. Body weight change (0.41, 0.49, 0.52, 0.42 kg/d; $P<0.01$) was highest in 40% RUP and lowest in 30% RUP. However, in 35% and 40% RUP were not differ, and these data showed that a tendency of increasing in body weight change reflected increases in level of RUP from 30 to 40%. Increases in level of RUP from 40 to 45% caused decrease in body weight change. The results also indicated that Thai Native x Brahman beef cattle performance were increased by rumen undegradable protein (RUP) levels at 40%. In conclusion, these results indicated that 35% CP from Capurea replacement for total crude protein and rumen undegradable protein levels at 40% in concentrate have positive effects on crossbred Native x Brahman beef cattle production.

5.2 Introduction

Studies with an extrusion-processed mixture of grain starch and urea (Starea) indicated that ammonia was released slower from this product both *in vivo* and *in vitro* than when a non-heated control mixture was used and, additionally, this product was superior to urea as a nitrogen supplement for lactating cows and for growing-fattenning cattle (Deyoe et al., 1968; Helmer et al., 1970). Utilization of urea rations for milk production has been improved by use of a mixture of gelatinized starch and urea (Starea), processed through an extruder cooker (Jittakhot, 1999). Rumen ammonia concentrations were reduced when this mixture was incubated with rumen microorganisms (Helmer et al., 1970). However, previous work with NPN supplementation showed that excessive rumen degradable nitrogen (RDN) from NPN sources such as Starea can result in increased rumen ammonia concentration and low efficient incorporation of N into microbial protein (Helmer et al., 1970). Moreover, if ruminally undegradable protein (RUP) is included in the diet in sufficient amounts, there is the potential to increase the amino acid flow from the abomasum and eventually modify the amino acid profile of protein reaching the duodenum, so that the efficiency of microbial growth and microbial protein production may be improved by balancing the overall daily ratio of RUP and RDP in the diet (Bach and Stern, 2000). The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia in the rumen and the subsequent observations that proteins or amino acids administered directly into the rumen (Schelling and Hatfield, 1968) has led to recent attempts to find ways of protecting soluble, high quality, dietary proteins from microbial degradation within the rumen. Decreasing the rumen solubility of casein (Faichney and Weston, 1971) and soybean meal protein (Peter et al., 1971) by treatment with formaldehyde would appear to be a potential method of decreasing

rumen degradation of high quality proteins and allowing more dietary protein by bypass the rumen to the abomasum and lower digestive tract. Reported here is work to determine the efficiency of N utilization from Caspurea by balancing daily levels of RUP in concentrate.

5.3 Objectives

The objectives of this research were to compare productive performance of Thai Native x Brahman beef cattle and utilization of dietary nitrogen when concentrate were formulated to contain 35% of Caspurea as a protein source with various levels of rumen undegradable protein in concentrate.

5.4 Materials and Methods

Four, Thai Native-Brahman beef cattle were used in the experiment. The animals were randomly assigned in a 4 x 4 Latin square designs with 21-d periods. The dietary treatments were formulated to contain 35% of Caspurea as a protein source with levels of rumen undegradable protein in concentrate, as follows:

T1= Ruminal undegradable protein at 30%

T2= Ruminal undegradable protein at 35%

T3= Ruminal undegradable protein at 40%

T4= Ruminal undegradable protein at 45%

All animals were fed *ad libitum* of urea-treated rice straw with fed concentrate (14% CP) at 2.0% BW, twice daily at 08.00 and 17.00. Each cow was housed in an individaul pen and free access to clean water all times. Daily collection of urine and faeces were made in the last 7 days of each period. Urine of individual animals was collected in 200 ml of 20% H_2SO_4 to keep the final pH of the urine lower than 3 all

times in a container. It is essential to acidify the urine to prevent bacterial activity. After recording the weight, urine was diluted 4 times to prevent precipitation of uric acid during storage. Duplicate urine samples of 50 ml were taken and stored at –20°C until analysis. Daily faeces collects in each period were bulked, mixed and a 5% sub sample taken. The sample of faeces were oven dried and ground (1 mm. Screen) for determination of DM, ash, OM, NDF, ADF and N content.

Rumen fluid and jugular blood were collected on the last day of each period. Ruminal pH was measured immediately after ruminal fluid sampling, 5 ml of 6 N HCL was added to 50 ml. Rumen fluid was collected 0, 3 and 6 h post feeding and jugular blood was collected at 0, 3 and 6 h post feeding and placed into heparinized vacationer tubes and centifuged at 2,500 x g for 15 minutes. Both rumen fluid and blood were stored at 5°C until analysis. Live weight of animals were measured at the beginning and at the end of each feeding period. Urea treated rice straw and concentrate were sampled every two weeks and the composited samples analyze for DM, NDF, ADF, ADL, CP and ash content. Neutral detergen fiber, acid detergent lignin of feeds and faeces were determined by the methods of Georing and Van Soest (1970) and DM, ash and crude protein were determined by the methods of AOAC (1985). Rumen fluid TVFA concentration was determined by titration technique of Briggs et al. (1957) and NH_3 –N were determined by the methods of Bromner and Keeney (1965).

5.5 Statistical analyses

All data obtained from the experiment were subjected to analysis of variance using Proc. GLM (SAS, 1996), treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torries, 1980) and all data obtained

from the experiment were subjected to the General Linear Models (GLM) procedure for orthogonal polynomial analysis of SAS (SAS, 1996).

5.6 Results and Discussion

The composition of the diets was shown in Table 5.1. Variation in CP within concentrates was small (approximately 14% CP) and was slightly higher than formulated at 14% CP. The high RUP (45% RUP) rations contained slightly higher NDF and ADF than other treatments. The effect of diet on feed intake is shown in Table 5.2. These results indicated that total dry matter intake (5.9, 5.8, 6.0, 6.0 kgDM/d; $P<0.05$) increased linearly ($P<0.01$) as the levels of Caspurea increased. Increase in DM intake is probably due to amino acid balance arising from increased RUP. Balance of essential amino acids enhanced feed intake.

Table 5.1 Feed formulation and chemical composition of dietary treatments.

	Caspurea(% of total CP) : RUP (%)				
Feed stuffs	**35:30**	**35:35**	**35:40**	**35:45**	
Caspurea	10.9	10.9	10.9	10.9	
Cassava pulp	55.6	41.8	37.8	15.4	
Rice bran	3.0	15.0	22.0	36.0	
Soybean meal	12.0	8.0	6.0	2.0	
Palm meal	3.0	13.0	20.0	34.0	
Molasses	13.0	9.0	1.2	0.0	
Urea	0.8	0.6	0.4	0.0	
Sulfur	0.2	0.2	0.2	0.2	
Lime stone	0.5	0.5	0.5	0.5	
Salt	0.5	0.5	0.5	0.5	
Mixed mineral	0.5	0.5	0.5	0.5	
Total	100.0	100.0	100.0	100.0	
Chemical composition (%)					**Urea treated rice straw**
DM	89.9	90.2	90.4	91.0	49.8
	..... % DM				
OM	91.9	91.7	91.6	89.7	89.1
NDF	18.8	19.4	19.9	20.2	68.0
ADF	9.3	9.7	10.1	11.4	47.1
ADL	4.2	4.3	4.3	4.5	8.7
Ash	8.1	8.3	8.4	10.3	10.9
AIA	1.4	1.5	1.5	1.6	1.5
CP	14.2	14.1	14.1	14.0	7.1

DM: dry matter, OM: organic matter, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, AIA: acid insoluble ash, CP: crude protein

Table 5.2 Effect of dietary treatments on feed intake and nutrient in take.

Items	Caspurea(% of total CP) : RUP (%)					Contrast*	
	35:30	35:35	35:40	35:45	SEM	L	Q
Feed intake(kgDM/d)							
Concentrate	2.4	2.4	2.4	2.5	0.01	0.078	0.082
Roughage	3.4[b]	3.4[b]	3.6[a]	3.6[a]	0.03	0.010	0.716
Total intake							
kgDM	5.87[b]	5.83[c]	5.99[ab]	6.04[a]	0.04	0.009	0.339
%BW	3.18[b]	3.17[b]	3.24[a]	3.24[a]	0.02	0.004	0.455
(g/kgBW$^{0.75}$)	116.9[b]	116.7[b]	119.6[a]	120.6[a]	0.72	0.004	0.862
Nutrient intake (kg/d)							
NDF	2.8[b]	2.8[b]	2.9[a]	2.9[a]	0.02	0.003	0.611
CP	0.59	0.58	0.60	0.59	0.01	0.253	0.506

[a,b] Means within a row with different superscripts differ ($P<0.05$), SE = standard error of the mean, NDF= Neutral detergent fiber, CP= Crude protein, *Orthogonal polynomial contrast L= linear, Q= quadratic

The effect of diet on feed digestibility is shown in Table 5.3. These results indicated that DM digestibility (67.8, 68.9, 71.9, 69.9%; $P<0.05$) were highest in 40% RUP. DM digestibility increased linearly ($P<0.01$) as the level of RUP increased. Moreover, OM digestibility increased linearly ($P<0.05$) with increasing the level of RUP. Crude protein digestibility of the diet containing RUP at 40% was higher ($P<0.05$) than other treatments. However, DM and NDF digestibility decreased (quadratically, $P<0.05$) as the levels of CP from Caspurea increased from 40 to 45%. These data indicated that matching supply of energy and N supply and balancing the overall daily ratio of RUP and RDP in the rumen may improve microbial growth and activity. The nitrogen requirement of rumen bacteria on a given diet can be estimated

from the amount and digestibility of organic matter digested by the animal, bearing in mind that there should be at least 30 g nitrogen, to be hydrolyzed to ammonia in the rumen, per kg of digestible organic matter in the diet (Ørskov, 1976). If the basal diet offered to the animals is deficient in nitrogen but contains large amounts of readily fermentable carbohydrates, it is advisable to supplement the diet with NPN so as to ensure the correct proportions of nitrogen and energy for microbial protein synthesis. The magnitude of the latter is rarely limited by nitrogen deficiency, as nitrogen can readily be added in form of NPN or, in the first instance by the intake of sufficiently large amounts of rumen-digestible organic matter, mainly carbohydrates.

Table 5.3 Effect of dietary treatments on digestibility and body weight change

Items	**Caspurea(% of total CP) : RUP (%)**					**Contrast***	
	35:30	**35:35**	**35:40**	**35:45**	**SEM**	**L**	**Q**
Digestibility (%)							
DM	67.8[b]	68.9[b]	71.9[a]	69.9[a]	0.65	0.020	0.059
NDF	56.2[b]	57.5[b]	61.9[a]	57.5[b]	0.75	0.048	0.009
CP	75.3[ab]	75.4[ab]	76.5[a]	73.2[b]	0.76	0.168	0.068
Body weight (kg)							
Initial weight	180.8	179.7	179.8	180.5	-		
Final weight	189.4	190.1	190.7	189.3	0.40	0.936	0.036
Body weight change (kg/d)	0.41[b]	0.49[a]	0.52[a]	0.42[b]	0.02	0.403	0.001

[a,b] Means within a row with different superscripts differ ($P<0.05$) SE = standard error of the mean, *Orthogonal polynomial contrast L= linear, Q= quadratic

Body weight change (0.41, 0.49, 0.52, 0.42 kg/d) (Table 5.3) was highest in 40% RUP and lowest ($P<0.01$) in 30% RUP. However, in 35% and 40% RUP were

not differ. Increase in body weight change reflected increases in level of RUP from 30 to 40%. Moreover, increases in level of RUP from 40 to 45% caused decreased in body weight change (quadratically, $P<0.01$). The animals fed the diet containing RUP at 40% had the highest body weight gains. The results indicated that the efficiency of microbial growth and microbial protein production may be improved by balancing the overall daily ratio of RUP and RDP in the diet. Bach and Stern (1999) reported that when the ruminal undegradable protein (RUP) is included in the diet in sufficient amounts, there is the potential to increase the AA flow from the abomasum and eventually modify the AA profile of protein reaching the duodenum, which enhanced body weight gains. The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia in the rumen and the subsequent observations that proteins or amino acids administered post-ruminally resulted in greater nitrogen retention than when these were administered directly into the rumen (Schelling and Hatfield, 1968) has led to recent attempts to find ways of protecting soluble, high quality, dietary proteins from microbial degradation within the rumen . Decreasing the rumen solubility of casein (Faichney and Weston, 1971) and soybean meal protein (Peter et al., 1971) by treatment with formaldehyde would appear to be a potential method of decreasing rumen degradation of high quality proteins and allowing more dietary protein by bypass the rumen to the abomasum and lower digestive tract.

Ruminal pH data are showed in Table 5.4 and Figure 5.1. Ruminal pH decreased linearly ($P<0.05$) as the levels of RUP increased and was lower ($P<0.05$) than other treatments when RUP level in concentrate at 45% was fed. Ruminal pH at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Low pH values appeared to be related to high VFA and high bacterial nitrogen

production. H-ion concentration appeared to be influenced more by VFA than by ammonia or lactate production (Stiles et al.,1970). Morever, the correlation between rumen pH and VFA was 0.8 (Roman-Ponce et al., 1974). Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns.

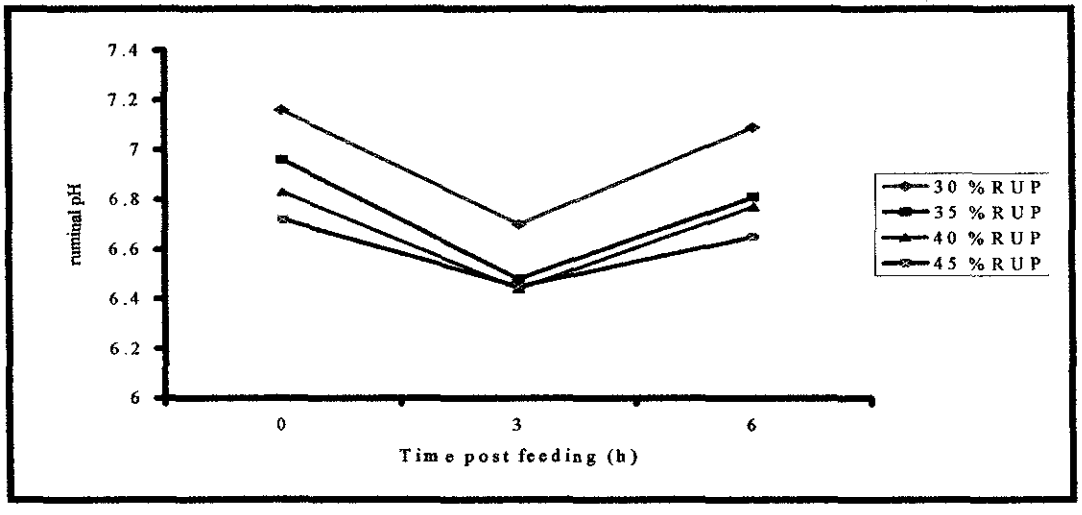

Figure 5.1 Hourly ruminal pH in cattle receiving a diet containing four levels of ruminal undegradable protein (RUP)

Ruminal ammonia-N concentration (12.3, 11.0, 9.5, 11.0 mg%) (Table 5.4) was highest ($P<0.01$) in 30% RUP. Moreover, the data showed that decrease in ruminal ammonia-N linearly ($P<0.01$) reflected increases in level of RUP. However, at 45% RUP rumen NH_3-N increased (quadratically, $P<0.01$). These results showed that the rumen NH_3-N concentration in animal fed the diet containing RUP at 40%

was lower ($P<0.01$) than other treatments. It is possible that more efficient capture of N for microbial protein synthesis. Reported by Kolver et al. (1998) that decreases ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis. Moreover, Helmer et al. (1970) have shown that slower hydrolysis of N results in more efficient incorporation of urea N into microbial protein. Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids, have but little effect on the yield of microbial matter (Ørskov et al., 1974). If, however, a certain microorganism causing a different type of fermentation predominated in the rumen, and if that microorganism were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist.

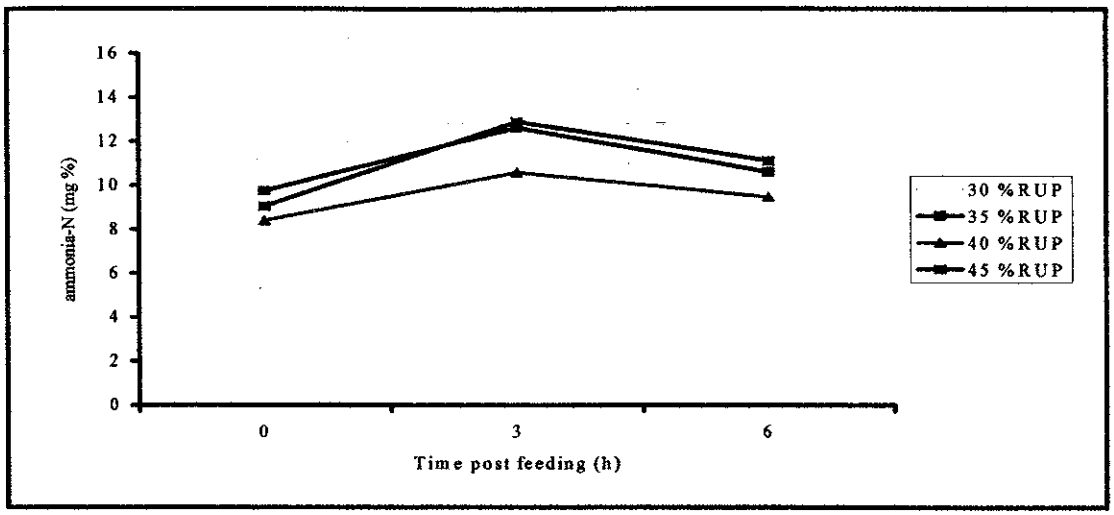

Figure 5.2 Hourly NH_3-N (mg%) in cattle receiving a diet containing four levels of ruminal undegradable protein (RUP)

Ruminal NH_3-N concentration at 0, 3 and 6 h post feeding are presented in Table 5.4 and Figure 5.2. Ruminal NH_3-N concentration at 3 h post feeding were increased similar in every dietary treatment. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Similar reported by Davis and Stallcup (1967) that peaks of amonia concentration in rumen contents 2 or 3 h after feeding. In contrast, Thompson et al. (1972) found that peak rumen ammonia for Starea rations 90 min after feeding. Higher NH_3-N concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feedings decreased indicating a more capture of N for increased microbial protein synthesis (Sinclair et al., 1993; Witt et al., 1999).

Table 5.4 Effect of dietary treatments on rumen fermentation

Items	Caspurea (% of total CP) : RUP (%)					Contrast*	
	35 : 30	35 : 35	35 : 40	35 : 45	SEM	L	Q
pH							
(hr-post-feeding)							
0	7.2 [a]	7.0 [b]	6.8 [c]	6.7 [c]	0.04	0.001	0.236
3	6.7 [a]	6.5 [b]	6.4 [b]	6.5 [b]	0.04	0.001	0.015
6	7.1 [a]	6.8 [b]	6.8 [b]	6.7 [c]	0.03	0.001	0.008
Mean	7.0 [a]	6.8 [b]	6.7 [b]	6.6 [b]	0.09	0.001	0.170
NH_3-N (mg%)							
0	11.2 [a]	9.7 [b]	8.4 [c]	9.1 [bc]	0.27	0.001	0.002
3	13.3 [a]	12.6 [b]	10.6 [c]	12.9 [ab]	0.20	0.003	0.001
6	12.4 [a]	10.6 [b]	9.5 [c]	11.1 [b]	0.18	0.001	0.001
Mean	12.3 [a]	11.0 [b]	9.5 [c]	11.0 [b]	0.65	0.003	0.001
TVFAs (mM/l)							
0	83.4 [d]	99.4 [c]	106.7 [a]	103.0 [b]	0.89	0.001	0.001
3	101.8 [c]	125.2 [ab]	125.9 [a]	122.6 [b]	0.98	0.001	0.001
6	94.8 [c]	114.9 [a]	117.4 [a]	110.6 [b]	0.85	0.001	0.001
Mean	94.3 [b]	113.2 [a]	116.7 [a]	112.1 [a]	4.57	0.001	0.001

[a,b,c,d] Means within a row with different superscripts differ ($P<0.05$)

*Orthogonal polynomial contrast L= linear, Q= quadratic

Total VFA concentrations in the rumen (Table 5.4 and Figure 5.3 (94.3, 113.25, 116.7, 112.1 mM/l; $P<0.01$) increased (linearly, $P<0.01$) as the level of RUP increased. However, at 45% RUP TVFAs decreased (quadratically, $P<0.01$). These

result showed that Total VFA concentration in animal fed the diet containing RUP at 40% was higher ($P<0.01$) than other treatments. The lowest ($P<0.01$) concentration recorded was on animals fed 30% RUP. The lowest concentration of VFA probably reflecting imbalances between RUP and RDP in the diet, resulting in decreased ruminal end product (Kim, 2001). Moreover, Witt et al. (1999) reported that higher VFA concentration might have been related to the microbial population in the same time as optimum pH. Moreover, Davis and Stallcup (1967) observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. Rumen microbe populations are presented in Table 5.4. The levels of RUP in concentrate had a significant influence upon both total bacteria and protozoa population was lowest ($P<0.05$) in 30% RUP. It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990).

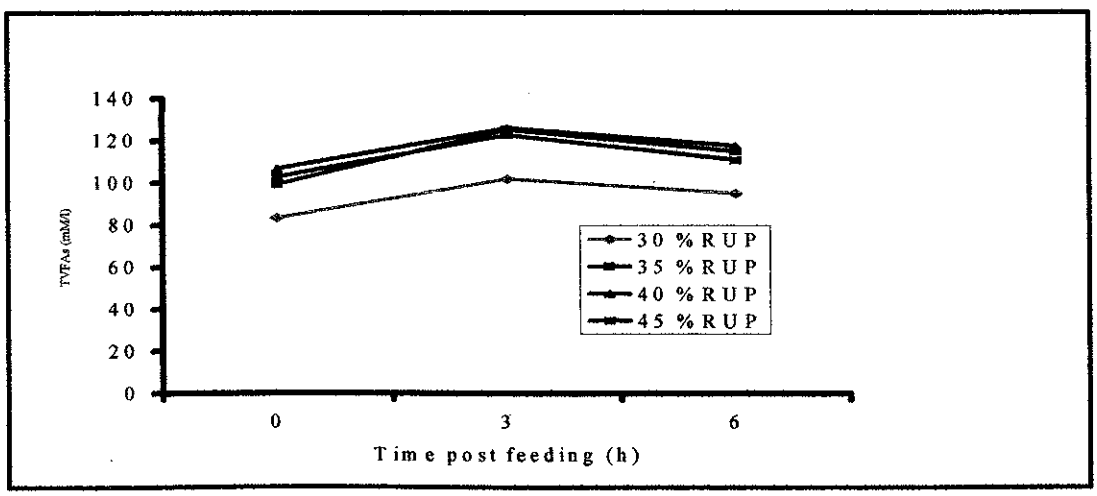

Figure 5.3 Hourly TVFAs in cattle receiving a diet containing four levels of ruminal undegradable protein (RUP)

TVFAs concentrations at 0, 3 and 6 h post feeding are presented in Table 5.4 and Figure 5.3. Ruminal TVFAs concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Higher VFA concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feedings decreased indicating a more capture of C-skeleton for increased microbial protein synthesis (Sinclair et al., 1993; Witt et al., 1999). The result agrees with Stiles et al. (1970) who reported that the rumen VFA concentration usually peaked 4 hours post-feeding. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microoganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids, have but little effect on the yield of microbial matter (Ørskov et al., 1974). If, however, a certain microorganisms causing a different type of fermentation predominated in the rumen, and if that microorganisms were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist. The result agrees with Stiles et al. (1970) who reported that the rumen VFA concentration usually peaked 4 hours post-feeding.

Table 5.5 Effect of dietary treatments on microbial population and blood urea nitrogen

Items	Caspurea (% of total CP) : RUP (%)					Contrast*	
	35 : 30	35 : 35	35 : 40	35 : 40	SEM	L	Q
Blood urea nitrogen(mg%)							
0	20.1 [a]	19.5 [a]	18.4 [b]	18.3 [b]	0.33	0.001	0.488
3	25.4 [a]	23.5 [b]	23.7 [b]	24.7 [ab]	0.77	0.397	0.015
6	21.8 [a]	21.8 [a]	19.1 [c]	20.6 [b]	0.31	0.001	0.028
Mean	22.4	21.6	20.4	21.2	1.22	0.125	0.245
bacteria (x 10^{10} cell/ml)	2.2	2.5	2.6	2.4	0.89	0.066	0.025
Protozoa (x 10^{5} cell/ml)	1.9 [c]	2.1 [ab]	2.2 [a]	1.9 [bc]	0.05	0.288	0.005

[a,b,c,d] Means within a row with different superscripts differ ($P<0.05$)

*Orthogonal polynomial contrast L= linear, Q= quadratic

Blood urea nitrogen concentrations are presented in Table 5.5 and Figure 5.4. Blood urea nitrogen concentration at 0 h after increased linearly ($P<0.05$) as the levels of RUP decreased. However, at 3 h post feeding BUN increased quadratically ($P<0.05$) as the levels of RUP decreased. Blood urea nitrogen concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding, rumen ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. however, at the 6 h post feedings decreased indicating a more excreted in the urine. (Sinclair et al., 1993; Witt et al., 1999). Ammonia liberated in the rumen is

utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971).

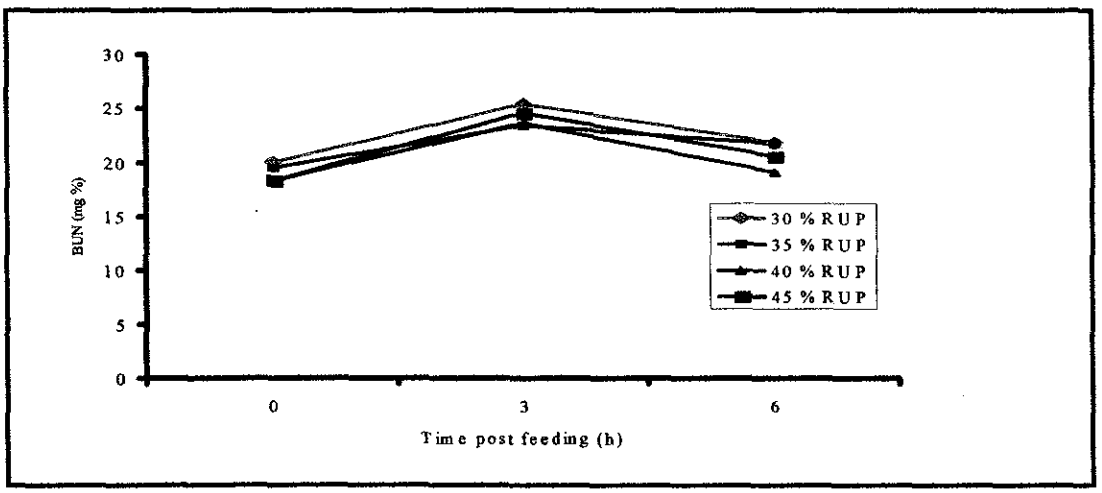

Figure 5.4 Hourly blood urea nitrogen (BUN) in cattle receiving a diet containing four levels of ruminal undegradable protein (RUP)

Rumen microbe populations are presented in Table 5.5. Bacteria population [2.2, 2.5, 2.6, 2.4 (x 10^{10} cell/ml)] and protozoa population [1.9, 2.1, 2.2, 1.9 (x 10^{5} cell/ml)] were lowest (P<0.05) in 30% RUP. However, in 35% and 40% microbial populations were not differ. Moreover, these data showed that increase in bacteria and

protozoa populations reflected increased in level of RUP from 30 to 40%. However, bacteria and protozoa populations were decreased quadratically ($P<0.05$) as the level of RUP increased from 40 to 45%. These result showed that microbe populations in animal fed the diet containing RUP at 40% was higher treatments ($P<0.05$) than other treatments. The lowest treatments ($P<0.05$) bacteria populations recorded was on animals fed 30% RUP. It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990). Sinclair et al. (1993) and Kim (2001) reported that higher synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased microbial protein synthesis. Moreover, Jouaney and Ushida (1999) reported that ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration. Therefore, high soluble N in diets and cassava starch possibly affected the rumen microbe population. Microbial biomass, net 15N incorporation into cells, volatile fatty acid production increased linearly with increasing levels of cassava inclusion in diets (Sommart et al., 2000).

Nitrogen balance study is shown in Table 5.6. Urinary N was similar across treatment. However, fecal N excretion increased quadratically ($P<0.05$) as the levels of RUP increased. Fecal N excretion was higher ($P<0.05$) than other treatments when RUP level in concentrate at 45% was fed, Fecal N excretion increase, indicating that excessive RUP can result in decrease protein digestibility. Nitrogen absorption (71.8, 70.4, 72.9, 69.6 g/d; $P>0.05$) was not significantly different among dietary treatments. Nitrogen retention (20.9, 19.7, 31.0, 21.5 g/d) was highest ($P<0.05$) in 40% RUP. Nitrogen retention tended to increased as level of RUP increased. In addition, at 45% RUP N retention tended to decreased (quadratically, $P=0.1$). This result agrees with

Cecava et al. (1991) who reported that excessive RUP can result in decreased microbial yield, and reduced ruminal OM and fiber digestion (Cecava and Parker, 1993). Therefore when protein digestibility decreased can result in increase fecal N excretion . Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). Supplementing diets of relatively low digestible organic matter content with NPN with is useless, as the bacteria are unable to utilize all the ammonia released in the rumen, the excess being absorbed into the bloodstream, converted to urea and excreted in the urine.

Table 5.6 Effect of dietary treatments on nitrogen balance

Items	Caspurea(% of total CP) : RUP (%)					Contrast*	
	35:30	35:35	35:40	35:45	SEM	L	Q
Urine N (g/d)	51.0	50.7	41.9	48.1	2.23	0.132	0.197
Urine N (g/kgBW $^{0.75}$)	1.04	1.04	0.86	0.98	0.05	0.143	0.244
Feces N (g/d)	23.0^{b}	22.8^{b}	22.3^{b}	25.6^{a}	0.68	0.052	0.044
N absorption (g/d)	71.8	70.4	72.9	69.6	1.12	0.438	0.437
N retention (g/d)	20.9^{b}	19.7^{b}	31.0^{a}	21.5^{b}	2.23	0.231	0.110
N absorption (%N intake)	75.3^{ab}	75.4^{ab}	76.5^{a}	73.2^{b}	0.76	0.168	0.068
N retention (%N intake)	21.6^{b}	21.1^{b}	32.5^{a}	22.7^{b}	2.46	0.230	0.107

^{a,b} Means within a row with different superscripts differ ($P<0.05$)

*Orthogonal polynomial contrast L= linear, Q= quadratic

5.7 Conclusions

The results indicated that Caspurea used as a protein at 35% in ration with 40% RUP can improve feed intake, body weight change, blood metabolites, rumen microbe populations, end-products of ruminal fermentation, digestibility and nitrogen balance. Therefore this study suggests that the diet containing of 35% Caspurea used as a protein in ration with 40% RUP has positive benefit on Thai Native x Brahman beef cattle productive performance.

5.8 References

AOAC. (1985). **Official Methods of Analysis.** Association of official Chemists, Washington, D.C.

Bach, A., and Stern, M. D. (2000). Measuring resistance to ruminal degradation and bioavailability of ruminally protected methionine. **Anim. Feed Sci. Tecnol.** 84: 23-32.

Bloomfield, R. A., Kearley, E. D., Creach, D. O., and Muhrer, M. E. (1963). Ruminal pH and absorption of ammonia and VFA. **J. Anim. Sci.** 22: 833.

Briggs, P. K., Hogan, J. F., and Reid, R. L. (1957). The effect of volatile fatty acid, lactic acid, and ammonia on rumen pH in sheep. **Aust. J. Agric. Res.** 8 : 674-710.

Bromner, J. H., and Keeney, D. R. (1965). Steam distillation methods of determination of ammonium, nitrate and nitrite. **Annual. Chem. Acta.** 32 : 485 - 495.

Coombe, J. B., Tribe, D. E., and Morrison, J. W. C. (1960). Some experimental observations on the toxicity of urea to sheep. Australian **J. Agr. Res.** 11: 247.

Davis, G. V., and Stallcup, O. T. (1967). Effect of soybean meal, raw soybeans, corn gluten feed, and urea on the concentration of rumen fluid components at intervals after feeding. **J. Dairy Sci.** 50 : 1638 – 1645.

Deyoe, C. W., Bartley, E. E., Pfost, H. B., Boren, F. W., Perry, H. B., Anstaett, F. R., Helmer, L., Stiles, D., Sung, A. C., and Meyer, R. (1968). An improved urea product for ruminants. (Abstr.). **J. Anim. Sci.** 27: 1163.

Faichney, G. J., and Weston, R. H. (1971). Digestion by ruminant lambs of a diet containing formaldehyde-treated casein. **Aust. J. Agr. Res.** 22:461.

Goering. H. K., and Van Soet, P. J. (1970). **Forage Fiber Analyses** (apparatus, reagents, procedures, and some applications) Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25 – 32.

Helmer, L. G., Bartley, E. E., and Deyoe, C. W. (1970). (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Herrera-Saldana, R., Huber, J. T., and Poore, M. H. (1989). Influence of varying protein and starch degradabilities on performance of lactating cows. **J. Dairy Sci.** 72 : 1477-1483.

Hobson, P. N. (1971). Rumen microorganisms. **Progr. Indust. Microbial.** 9: 41.

Hungate, R. E. (1966). The rumen and its microbes. Academic Press, London.

Jittakot, S. (1999). **Effect of cassarea as a protein replacement for soybean meal on feed intake, blood metabolites, ruminal fermentation, digestibility and microbial protein synthesis in dairy cows fed urea-treated rice straw as a roughage.** Khon Kaen, Thailand. Graduate School, Khon Kaen University. 171 pp. (M. Sc. Thesis).

Jouaney, J. P., and Ushida, K. (1999). The role of protozoa in feed digestion. **Asian-Aust. J. Anim. Sci.** 12: 113-126.

Kim, C. H. (2001). Effect of different protein sources given synchronously or asynchronously into the rumen of consuming a beef cattle diet high in concentrate on the synthesis of microbial protein. **J. Anim Feed Sci. Technol.** 43 : 831-840.

Kolver, E., Muller, L. D., Vaga, G. A., and Cassidy, T. J. (1998). Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. **J. Dairy Sci.** 81:2017-2028.

Ørskov, E. R. (1976). Nitrogen digestion and utilization by young and lactating ruminants. **Wld. Rev. Nutr. Diet.** 25, inpress.

Ørskov, E. R., Fraser, C., McDonald, I., and Smart, R. (1974). Digestion of concentrate in sheep. 5. The effect of adding fish meal and urea together to cereal diets on protein digestion and utilization by young sheep. **Brit. J. Nutr.** 31: 89-96.

Peter, A. P., Hatfield, E. E., Owens, F. N., and Garrigus, U. S. (1971). Effects of aldehyde treatments of soybean meal on *in vitro* ammonia release, solubility and lamb performance. **J. Nutr.** 101 : 605.

McDonald, I. W. (1948). The absorption of ammonia from the rumen of sheep. **Biochem. J.** 42: 584.

Roffler, R. E., and Satter, L. D. (1973). Influence of ration composition on ruminal ammonia concentration. **J. Dairy Sci.** 56 : 663. (Abstr.)

Roman-Ponce, H., Van Horn, H. H., Mashall, S. P., Wilcox, C. J., and Randel, P. F. (1974). Complete Rations for Dairy Cattle. V. Interaction of Sugarcane Bagasse Quantity and Form with Soybean Meal, Urea, and Starea. **J. Dairy Sci.** 58: 1320-1327.

SAS. (1996). SAS User's Guide: Statistics. Version 6. 14th ed Cary, NC: SAS Inst

Shabi, K., Arieli, A., Bruckental, L., Aharoni, Y., Zamwel, S., Bor, A., and Tagari, H. (1998). Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dietary cows. **J. Dairy Sci.** 81 : 1991-2000.

Schelling, G. T., and Hatfield, E. E. (1968). Effect of abomasally infused nitrogen sources on nitrogen retention of growing lambs. **J. Nutr.** 96:319.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R., and Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. **J. Agric. Sci. Camb.** 120: 251-263.

Sommart, K., Parker, D. S., Rowlinson, P., and Wanapat, M. (2000). Fermentation characteristics and microbial protein synthesis in an *in vitro* system using cassava, rice straw and dried ruzi grass as substrates. **Asian-Aust. J. Anim. Sci.** 13: 1084-1093.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Susmel, P., Stefanon, B., Mills, C. R., and Candido, M. (1989). Change in amino acid composition of different protien sources after rumen incubation. **Anim. Prod.** 49:375-383.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Witt, M. W., Sinclair, L. A., Wilkinson, R. G., and Buttery, P. J. (1999). The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food *ad libitum*. **Brit. Soc. Anim. Sci.** 69: 223-235.

CHAPTER VI

EFFECT OF AN EXTRUSION-PROCESSED MIXTURE OF CASSAVA PULP AND UREA WITH NATURAL PLANT PROTEIN (CASPUREA) AS A PROTEIN SOURCE ON PERFORMANCE OF THAI NATIVE x BRAHMAN BEEF CATTLE

6.1 Abstract

This experiment aimed to study the effects of an extrusion-processed mixture of cassava pulp and urea with natural plant protein (45% CP) and pelleting at high temperatures (Caspurea/Starea) as a protein source on productive performance in Thai Native x Brahman beef cattle. Eight Thai Native-Brahman beef cattle (approximatly 365 days of age and average liveweight of 195.5±24.4 kg) were used in double 4 x 4 Latin square arrangement of treatments with 21-d periods. The treatments were Caspurea with different natural plant protein (extrusion-processed mixture of cassava pulp and urea with natural plant protein and pelleting at high temperatures) as follows; 1) normal Caspurea (Control) 2) Caspurea+leucaena leaf 3) Caspurea+cassava leaf 4) Caspurea+soybean meal (SBM). Concentrates were formulated to contain 14% CP and 40% RUP and were fed at 2.0% BW. All animals were fed *ad libitum* with urea treated rice straw as roughage. The results showed that total dry matter intake (7.76, 7.76, 7.77 and 7.79 kgDM/d) and DM digestibility

(73.3, 74.1, 74.9 and 75.3%) were not significantly different ($P>0.05$) among dietary treatments. Ruminal ammonia-N (12.2, 11.5, 11.0 and 10.5 mg%) in animals fed the diet containing Caspurea+soybean meal was lower ($P<0.05$) than other treatments. Moreover, the results showed that total volatile fatty acid (98.6, 102.7, 105.5, 109.8 mM/l) was not differ ($P>0.05$) among dietary treatment. Bacteria population [1.9, 2.0, 2.2, 2.3 (x 10^{10} cell/ml)] and protozoa populations [2.4, 2.3, 2.5, 2.55 (x 10^{5} cell/ml)] were unaffected by the natural plant protein sources. N absorption (91.9, 92.6, 93.5, 93.5 g/d) and N retention (31.0, 32.2, 33.7, 34.3% N intake) were not significantly different ($P>0.05$) among dietary treatments. Blood urea nitrogen (21.6, 21.1, 20.0, 19.9 mg%) was affected by the natural plant protein sources and was lowest ($P<0.05$) in Caspurea+soybean meal group. Body weight change (0.48, 0.48, 0.51, 0.52 kg/d) in Caspurea+soybean meal group was higher ($P<0.05$) than normal Caspurea (Control) and Caspurea+leucaena groups. However, body weight change was not differ between Caspurea+cassava leaf and Caspurea+soybean meal groups. In conclusion, these results indicated that Caspurea+plant protein has positive effects on crossbred Native x Brahman beef cattle production.

6.2 Introduction

The use of urea by ruminants has been restricted by inefficient conversion of urea nitrogen to microbial protein by toxicity, by palatability and by urea segregation in mixed rations fed in meal form (Briggs, 1967; Chalupa, 1968; Reid, 1953). In a previous study (Helmer et al., 1970) an expansion-processed mixture of grain starch and urea (Starea) was approximately equal to soybean meal as a protein supplement for lactating cows. Cows fed grain rations supplemented with either soybean meal or Starea consumed more grain and produced more milk than cows fed the same grain

ration supplemented with urea. In another study (Helmer et al., 1970) Starea improved the utilization of urea *in vitro*. After 4 hours of fermentation *in vitro*, concentration of rumen ammonia was lower with Starea than with unprocessed corn and urea. The lower rumen ammonia was at least in part the result of more efficient utilization of ammonia by its conversion to microbial protein. Reduced consumption of high urea rations and loss of dietary nitrogen resulting from rapid hydrolysis in the rumen have prompted researchers to seek new means of improving NPN utilization in ruminant rations. Bartley et al. (1968) have attempted to reduce the solubility and to improve the acceptance of urea by cooking a grain-urea mixture at high temperatures. *In vitro* and that the product (Starea) equals soybean meal (SBM) as a nitrogen supplement for ruminants.

Other workers have attempted to stimulate microbial activity by supplying various nutrients that might be limiting in high urea diets Matrone et al. (1964) observed an invigorating influence of alfalfa meal on rumen microflora and Lowrey and McCormick (1969) state that feed consumption and gain were increased by the addition of 5% alfalfa meal to high urea diets. Thomas et al. (1951) reasoned from sulfur studies with lambs that sulfur deficiencies may limit NPN utilization. Gooddrich and Tillman (1966) have also demonstrated that the addition of sulfur improved urea utilization and nitrogen retention.

6.3 Objectives

The objectives of this research were to study the effect of an extrusion-processed mixture of cassava pulp and urea with natural plant protein and pelleting at high temperatures (Caspurea/Starea) as a protein source on performance in Thai Native x Brahman beef cattle.

6.4 Materials and Methods

Four, Thai Native x Brahman beef cattle were used in the experiment. The animals were randomly assigned in a double 4 x 4 Latin square designs with 21-d periods. The dietary treatments were formulated to contain 35% of CP from Caspurea (45% CP) and 40% RUP (extrusion-processed mixture of cassava pulp and urea with natural plant protein (cassava leaf, leucaena leaf and soybean meal) and pelleting at high temperatures; as follows:

T1= Caspurea (45% CP) (control)

T2= Caspurea + leucaena leaf (45% CP) (cassava pulp : urea : leucaena leaf = 70:14:16)

T3= Caspurea + cassava leaf (45% CP) (cassava pulp : urea : cassava leaf = 70:14:16)

T4= Caspurea + soybean meal (45% CP) (cassava pulp : urea : soybean meal = 76:14:10)

All animals were fed *ad libitum* of urea-treated rice straw with fed concentrate (14% CP) at 2.0% BW, twice daily at 08.00 and 17.00. Each cow was housed in an individaul pen and free access to clean water all times. Daily collection of urine and faeces were made in the last 7 days of each period. Urine of individual animals was collected in 200 ml of 20% H_2SO_4 to keep the final pH of the urine lower than 3 all times in a container. It is essential to acidify the urine to prevent bacterial activity. After recording the weight, urine was diluted 4 times to prevent precipitation of uric acid during storage. Duplicate urine samples of 50 ml were taken and stored at –20°C until analysis. Daily faeces collects in each period were bulked, mixed and a 5% sub sample taken. The sample of faeces were oven dried and ground (1 mm. Screen) for determination of DM, ash, OM, NDF, ADF and N content. Rumen fluid and jugular

blood were collected on the last day of each period. Ruminal pH was measured immediately after ruminal fluid sampling, 5 ml of 6 N HCl was added to 50 ml. Rumen fluid was collected 0, 3 and 6 h post feeding and jugular blood was collected at 0, 3 and 6 h post feeding and placed into heparinized vacationer tubes and centifuged at 2,500 x g for 15 minutes. Both of rumen fluid and blood were stored at 5°C until analysis. Liveweight of animals were measured at the beginning and at the end of each feeding period. Urea treated rice straw and concentrate were sampled every two weeks and the composited samples analyze for DM, NDF, ADF, ADL and ash and CP content. Neutral detergent fiber, acid detergent lignin of feeds and faeces were determined by the methods of Georing and Van Soest (1970) and DM, ash and crude protein were determined by the methods of AOAC (1985). Rumen fluid TVFA concentration was determined by titration technique of Briggs et al. (1957) and NH_3 – N were determined by the methods of Bromner and Keeney (1965).

6.5 Statistical analyses

All data obtained from the experiment were subjected to analysis of variance using Proc. GLM (SAS, 1996) and treatment means were statistically compared by Duncan's New Multiple Range Test (Steel and Torries, 1980) and orthogonal comparison using SAS (SAS, 1996).

6.6 Results and Discussion

The composition of the diets is shown in Table 6.1. Variation in CP within concentrates was small (approximately 14% CP) and was slightly higher than formulated at 14% CP. The effect of diet on feed intake is shown in Table 6.2. These results indicated that total DM intake (7.76, 7.76, 7.77 and 7.79 kgDM/d; $P>0.05$)

were not significantly different among dietary treatments. The result agrees with Stiles et al. (1970) who reported that Starea, an intimate mixture of gelatinized starch and urea, improved utilized of urea nitrogen and palatability of urea–containing rations. Therefore an extrusion-processed mixture of cassava pulp and urea with natural plant protein (cassava leaf, leucaena leaf and soybean meal) and pelleting at high temperatures did not affect feed intake. However, Caspurea, the unpleasant, bitter taste makes feed mixtures with a high proportion of urea unpalatable and adversely affects voluntary intake, especially by beef cattle. Reduced feed intake of high urea rations and loss of dietary nitrogen resulting from rapid hydrolysis in the rumen have prompted researchers to seek new means of improving NPN utilization in ruminant rations. Bartley et al. (1968) have attempted to reduce the solubility and to improve the acceptance of urea by cooking a grain-urea mixture at high temperatures. *In vitro* and that the product (Starea) equals soybean meal (SBM) as a nitrogen supplement for ruminants. Other workers have attempted to stimulate microbial activity by supplying various nutrients that might be limiting in high urea diets Matrone et al. (1964) observed an invigorating influence of alfalfa meal on rumen microflora. Lowrey and McCormick (1969) stated that feed intake and gain were increased by the addition of 5% alfalfa meal (natural plant protein) to high urea diets. Thomas et al. (1951) reasoned from sulfur studies with lambs that sulfur deficiencies may limit NPN utilization. Gooddrich and Tillman (1969) also demonstrated that the addition of sulfur improved urea utilization and nitrogen retention. Helmer et al. (1970) reported that cows receiving either soybean meal or Starea as the protein supplement in their grain ration consumed more grain and produced more milk than those receiving urea. Grain intake was lower for the urea supplemented group in all experimental periods. Differences between the soybean meal and Starea supplemented groups were not

significant. This finding was similar to those reported by Roman-Ponce et al. (1974) that urea (U) rations affected lower daily feed intake than soybean meal (SBM) and Starea rations. Jones et al. (1974) reported that cows fed SBM consumed more DM, which may explain the greater body weight gains, than those fed other rations. Intakes of DM were similar between soybean meal and Starea rations. Starea intake also surpassed that from the low protein ration. Losses in body weight with the Starea ration cannot be explained by milk production or feed consumption. Moreover, there were no differences between rations for apparent digestibility of DM, CP, ADF or cellulose.

Table 6.1 Feed formulation and chemical composition of dietary treatments (%).

Feed stuffs	Caspurea+Natural Plant Protein				
	Control	Leucaena leaf	Cassava leaf	Soybean meal	
Caspurea	10.9	-	-	-	
Caspurea+leucaena leaf	-	10.9	-	-	
Caspurea+cassava leaf	-	-	10.9	-	
Caspurea+soybean meal	-	-	-	10.9	
Cassava pulp	37.8	37.8	37.8	37.8	
Rice bran	22.0	22.0	22.0	22.0	
Soybean meal	6.0	6.0	6.0	6.0	
Palm meal	20.0	20.0	20.0	20.0	
Molasses	1.2	1.2	1.2	1.2	
Urea	0.4	0.4	0.4	0.4	
Sulfur	0.2	0.2	0.2	0.2	
Lime stone	0.5	0.5	0.5	0.5	
Salt	0.5	0.5	0.5	0.5	
Mixed mineral	0.5	0.5	0.5	0.5	
Total	100.0	100.0	100.0	100.0	
Chemical composition (%)					**Urea treated rice straw**
DM	90.2	89.9	90.0	90.1	52.0
	..... %DM				
OM	91.1	89.9	90.0	90.1	87.8
NDF	18.3	18.2	18.4	18.1	69.1
ADF	9.3	9.2	9.4	9.0	48.0
ADL	4.1	4.2	4.3	4.1	8.3
Ash	8.9	10.1	10.0	9.9	12.2
CP	14.0	14.1	14.1	14.2	6.9

DM= dry matter, OM= organic matter, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, acid insoluble ash, CP= crud protein

Roman-Ponce et al. (1974) reported that lower intake for urea rations agrees with some results (Van Horn et al., 1967; Van Horn et al., 1975) although other investigators have reported no depression on feed intake by lactating cows using the same or higher urea (Colovos et al., 1967a; Colovos et al., 1967b; Hornter et al., 1971; Van Horn and Jacobson, 1971). Starea rations containing the same percentage of urea as urea rations (1.7%) and leading to slightly greater urea intakes than the former (275 and 256 g. per day) did not reduce feed intake as compared to SBM rations, which confirms results of Helmer et al. (1970) with lactating cows and of Schmidt et al. (1973) with steers. Equal responses in milk yield for Starea and SBM rations (higher than urea rations) agree with Helmer et al. (1970).

Table 6.2 Effect of dietary treatments on feed intake and nutrient intake.

Items	Capurea+Natural Plant Protein				SEM	Contrast*		
	Control	Leucaena leaf	Cassava leaf	Soybean meal		1 vs 2 & 3 & 4	3 vs 2 & 4	2 vs 4
Feed intake(kgDM/d)								
	2.8	2.8	2.7	2.7	0.04	0.928	0.971	0.912
Concentrate								
Roughage	5.0	5.0	5.0	5.1	0.12	0.930	0.995	0.930
Total intake								
kgDM	7.7	7.7	7.8	7.8	0.11	0.959	0.995	0.960
%BW	3.79	3.79	3.80	3.82	0.18	0.921	0.970	0.898
g/kgBW$^{0.75}$	143.2	143.4	143.7	144.3	6.08	0.915	0.987	0.894
Nutrient intake (kg/d)								
NDF	3.97	3.97	3.99	3.98	0.07	0.972	0.945	0.973
CP	0.75	0.75	0.74	0.74	0.01	0.815	0.984	0.813

[a,b] Means within a row with different superscripts differ ($P<0.05$) SE = standard error of the mean, *Orthogonal contrast

The effect of diet on feed digestibility is shown in Table 6.3. These results indicated that DM digestibility (73.3, 74.1, 74.9 and 75.3%; $P>0.05$) was not significantly different among dietary treatments. These data indicated that matching supply of energy and N supply and balancing the overall daily ratio of NPN and true protein from natural plant protein in the rumen may improve microbial growth and activity. However, Helmer and Bartley (1971) suggested that high level of urea in normal Starea may be utilized less efficiently than natural plant proteins such as soybean for ruminants. Jones et al. (1974) reported that cows fed SBM consumed more DM, which may explain the greater body weight gains, than those fed other rations. Intakes of were similar between soybean meal and Starea rations. Starea intake also surpassed that from the low protein ration ($P<0.05$). Losses in body weight with the Starea ration cannot be explained by milk production or feed consumption. Moreover, there were no differences between rations for apparent digestibility of DM, CP, ADF or cellulose. Addition of NPN to diets containing large amounts of readily digestible carbohydrates but small amounts of protein, or protein protected against deamination in the rumen, is particularly advisable, as in such diets there may be a shortage of nitrogen available to the bacteria for growth and may has affect on degradability. A correct synchronization of the rate of carbohydrate fermentation with the rate of ammonia release is of particular importance for the efficient utilization of NPN

Table 6.3 Effect of dietary treatments on digestibility and body weight change

Items	Capurea+Natural Plant Protein				SEM	Contrast*		
	Control	Leucaena leaf	Cassava leaf	Soybean meal		1 vs 2 & 3 & 4	3 vs 2 & 4	2 vs 4
Digestibility (%)								
DM	73.3	74.1	74.9	75.3	0.37	0.224	0.865	0.436
NDF	64.7	65.8	67.2	67.5	0.67	0.334	0.821	0.514
CP	76.5[b]	77.5[ab]	78.7[a]	79.2[a]	0.23	0.013	0.686	0.072
Body weight (kg)								
Initial weight	201.4	201.4	200.6	200.1	-	-	-	-
Final weight	211.50	211.5	211.3	211.0	7.87	0.979	1.00	0.965
Body weight change (kg/d)	0.48[b]	0.48[b]	0.51[a]	0.52[a]	0.01	0.064	0.206	0.016

[a,b] Means within a row with different superscripts differ ($P<0.05$) SE = standard error of the mean, *Orthogonal contrast

Body weight change (0.48, 0.48, 0.51, 0.52 kg/d; $P<0.05$) (Table 6.3) was similar in Caspurea+cassava leaf and Caspurea+soybean meal rations. Moreover, Caspurea+soybean meal ration was higher ($P<0.05$) than normal Caspurea (Control) and Caspurea+leucaena leaf. The result indicated that SBM has very high biological value for ruminant production, these results agree with the report of Susmel et al. (1989), who found that soybean meal had the high essential amino acid and biological value for ruminant. Moreover these data showed that the efficiency of microbial growth and microbial protein production may be improved by balancing the overall daily ratio of NPN and natural true protein in the diet. Bach and Stern (1999) reported that when the high biological protein is included in the diet in sufficient amounts, there is the potential to increase the AA flow from the abomasum and eventually modify the

AA profile of protein reaching the duodenum, which enhanced body weight gains. The result agrees with Helmer et al. (1970) who reported that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. The average change in body weight shows that dairy cows fed urea lost significantly more weight than those fed soybean meal or Starea. Differences in weight grains were not statistically significant between the soybean meal and Starea fed animals. Body weight change, like milk production, was directly related to grain intake. The unacceptability of the urea-containing ration had so reduced intake that normal production was not maintained even though body reserves were used to meet production and maintenance requirements. On the other hands, Starea and soybean meal rations were consumed in sufficient quantities to support high production and to maintain or increase body reserves.

Ruminal pH (6.4 and Figure 6.1) were affected by dietary treatments ($P<0.01$). The beef cattle fed the normal Caspurea had the highest ruminal pH. Moreover, the results showed that ruminal pH at 0, 3 and 6 after feeding in normal Caspurea rations were higher ($P<0.01$) than other treatments. However, ruminal pH was not significantly different between Caspurea+cassava leaf and Caspurea+soybean meal rations. Stiles et al. (1970) reported that no differences were observed in rumen pH between the rations. Although the mean pH for the rations was not different, several variables were negatively correlated with pH of the Starea-fed animals. Low pH values appeared to be related to high VFA and high bacterial nitrogen production. H-ion concentration appeared to be influenced more by VFA than by ammonia or lactate production. Moreover, Roman-Ponce et al. (1974) reported that lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with the

Urea opposite effect. The correlation between rumen pH and VFA was 0.8. Davis and Stallcup (1967) also observed more alkaline rumen pH when Urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with a more acid rumen pH. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns.

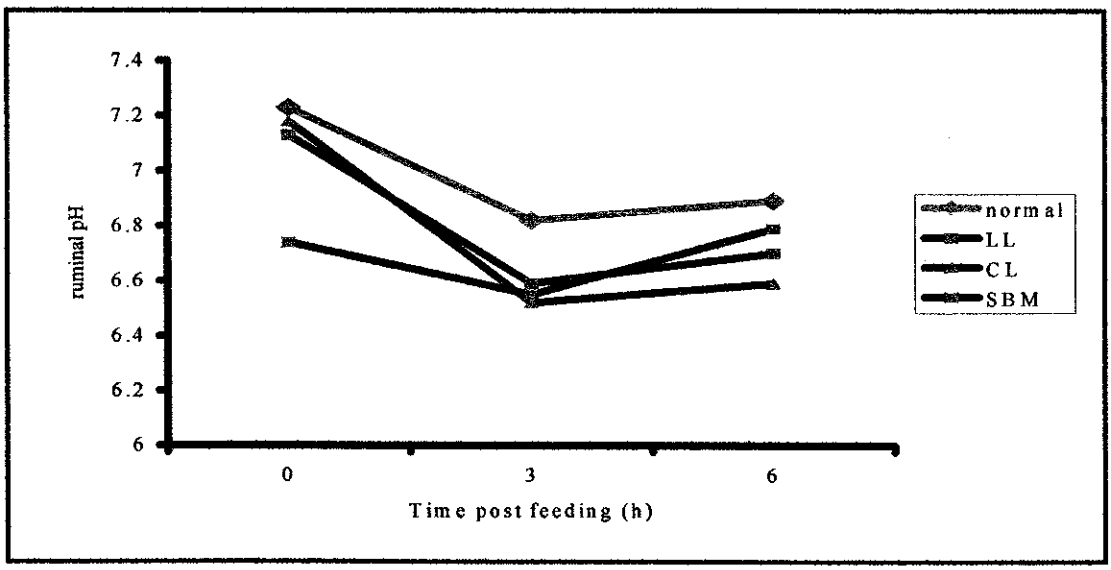

Figure 6.1 Hourly ruminal pH in cattle receiving a diet containing four type of Caspurea mixed with natural plant protein, Where Normal=normal Caspurea (Control), LL= leucaena leaf, CL= cassava leaf, SBM= soybean meal

Rumen NH_3-N concentration (6.4 and Figure 6.2) in animal fed the diet containing Caspurea+soybean meal was lower ($P<0.01$) than other treatments. Moreover, ruminal NH_3-N at 0, 3 and 6 after feeding in normal Caspurea rations were higher ($P<0.01$) than other treatments. However, ruminal NH_3-N was not differ between Caspurea+cassava leaf and Caspurea+soybean meal rations. It is possible that more efficient capture of N for microbial protein synthesis. Reported by Kolver et al. (1998) that decreases ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis. Moreover, Helmer et al. (1970) have shown that slower hydrolysis of N results in more efficient incorporation of urea N into microbial protein.

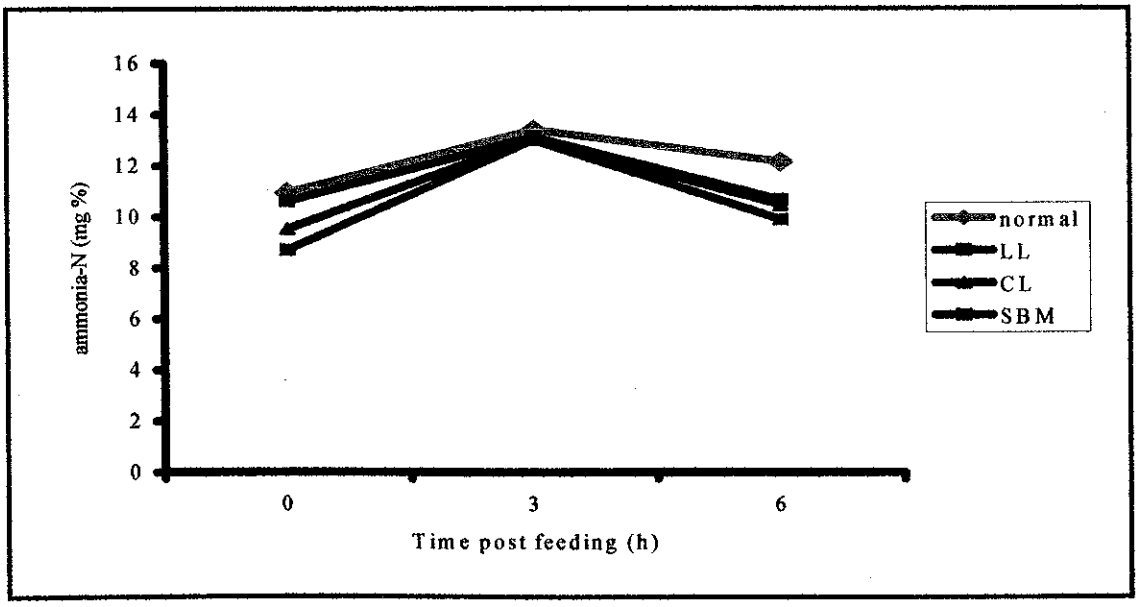

Figure 6.2 Hourly ruminal NH_3-N in cattle receiving a diet containing four type of Caspurea mixed with natural plant protein, Where normal= normal Caspurea (Control), LL= leucaena leaf, CL= cassava leaf, SBM= soybean meal

Ruminal NH_3-N concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Higher NH_3-N concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feedings decreased indicating a more capture of N for increased microbial protein synthesis (Sinclair et al., 1993; Witt et al., 1999). Roman-Ponce et al. (1974) reported that rumen ammonia N (NH_3-N) concentration was higher for all rations at 1 h after feeding than 2 h (28.2 and 19.0 mg%). Rumen ammonia was similar for urea and Starea rations but higher than SBM at 1 h ($P<0.05$) and 2 h. ($P<0.01$). In all treatments rumen NH_3-N concentration were higher at 1 h than 2 h (28.2 and 19.0). However, Davis and Stallcup (1967) reported that peaks of ammonia concentration in rumen contents 2 or 3 h after feeding either SBM, urea or SBM+urea rations. Thompson et al. (1972) found that peak rumen NH_3-N for Starea rations 90 min after feeding. Although rumen NH_3-N values for urea and Starea were not significantly different at either 1 or 2 h, the decline in NH_3-N from 1 to 2 h was 12.8 for urea and 6.4 mg% for Starea, suggesting a slower hydrolysis for Starea than Urea rations, in agreement with Stiles et al. (1970) and Schmidt et al. (1973). Many workers (as these data confirm) have obtained higher ammonia contents after feeding with urea than SBM rations (Davis and Stallcup,1964; Freitag et al.,1968; Schmidt et al., 1973; Thompson et al., 1972). Stiles et al. (1970) observed lower rumen ammonia content in the Starea-fed cows than urea-fed cows. Others (like these data) failed to find lowering of rumen ammonia with Starea as compared to urea (Schmidt et al., 1973) but did find a slow decline in rumen ammonia with Starea after feeding which agreed with these results. Coombe et al. (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. Bloomfield et

al. (1963) also indicated that increasing pH increased ammonia absorption and that low rumen pH was associated with high VFA absorption. These findings agree with many workers because rumen pH and VFA followed the above patterns, e.g. lower rumen pH observed for SBM and Starea rations resulted in higher VFA concentrations, with the urea rations having the opposite effect. The correlation between rumen pH and VFA was 0.8. Helmer et al. (1970) have shown that slower hydrolysis of urea results in more efficient incorporation of urea nitrogen into microbial protein. However, the daily gains of the steers fed urea and Starea were not different. Rumen ammonia levels in animal fed urea and Starea were higher than those for animals fed SBM and TSBM until the 3.5 h (urea) and 5.5 h (Starea) sampling times. There were no differences in the levels of rumen NH_3-N in animals fed the SBM or TSBM diets. Moreover, high rumen ammonia has been associated with higher content of SBM or crude protein in rations (Freitag et al., 1968). Davis and Stallcup (1967) also observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with more acid rumen pH. Schmidt et al. (1973) reported that at the 1.5 h sampling time, ruminal ammonia levels in animals fed urea or Starea were not different ($P<0.05$), perhaps indicating that Starea was hydrolyzed slower than urea (Stiles et al., 1970). If this were the case, then one would expect ruminal ammonia of the urea fed group to be higher than that of the Starea fed group prior to the initial post-feeding sample.

Table 6.4 Effect of dietary treatments on rumen fermentation

Items	Caspurea+Natural Plant Protein					Contrast*		
	Control	Leucaena leaf	Cassava leaf	Soybean meal	SEM	1 vs 2 & 3 & 4	3 vs 2 & 4	2 vs 4
pH (h-post-feeding)								
0	7.2[a]	7.1[a]	7.2[a]	6.7[b]	0.04	0.001	0.001	0.001
3	6.8[a]	6.6[b]	6.5[b]	6.6[b]	0.07	0.004	0.527	0.637
6	6.9[a]	6.7[b]	6.6[b]	6.8[a]	0.06	0.016	0.054	0.305
Mean	7.0[a]	6.8[ab]	6.7[b]	6.7[b]	0.13	0.008	0.886	0.257
NH_3-N (mg%)								
0	10.9[a]	10.6[a]	9.5[b]	8.7[c]	0.26	0.008	0.693	0.001
3	13.4	13.1	13.0	13.0	0.36	0.419	0.947	0.802
6	12.1[a]	10.6[b]	10.5[bc]	9.9[c]	0.21	0.001	0.471	0.021
Mean	12.2[a]	11.5[ab]	11.0[ab]	10.5[b]	0.77	0.029	0.988	0.147
TVFAs (mM/l)								
0	90.1[b]	92.4[b]	94.7[b]	103.9[a]	1.90	0.007	0.161	0.001
3	111.8	115.1	116.8	112.4	2.66	0.064	0.553	0.078
6	93.8[b]	100.5[a]	104.9[a]	103.1[a]	1.97	0.002	0.228	0.355
Mean	98.6	102.7	105.5	109.8	5.20	0.039	0.835	0.100

[a,b,c] Means within a row with different superscripts differ ($P<0.05$),Contrast* = Orthogonal contrast

Total VFA concentrations in the rumen are showed in Table 6.4. and Figure 6.3. The highest concentration (98.6, 102.7, 105.5, 109.8 mM/l) recorded was on animals fed Caspurea+soybean meal ($P<0.05$). However, TVFA concentrations were lowest in normal Caspurea ratoins. Moreover, TVFA were not significantly different ($P>0.05$) among Caspurea mixed with natural plant protein rations. These data indicated that matching supply of energy and N supply and balancing the overall daily ratio of NPN and true protein from natural plant protein such as soybean meal in the

rumen may improve microbial growth and activity (Kim, 2001). Moreover, Witt et al. (1999) reported that higher VFAs concentration might have been related to the microbial population in the same time as optimum pH. Rumen microbe populations are presented in Table 6.4. The natural plant protein sources in Caspurea had no effect on both total bacteria and protozoa populations (P>0.05). It is possible that higher synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990).

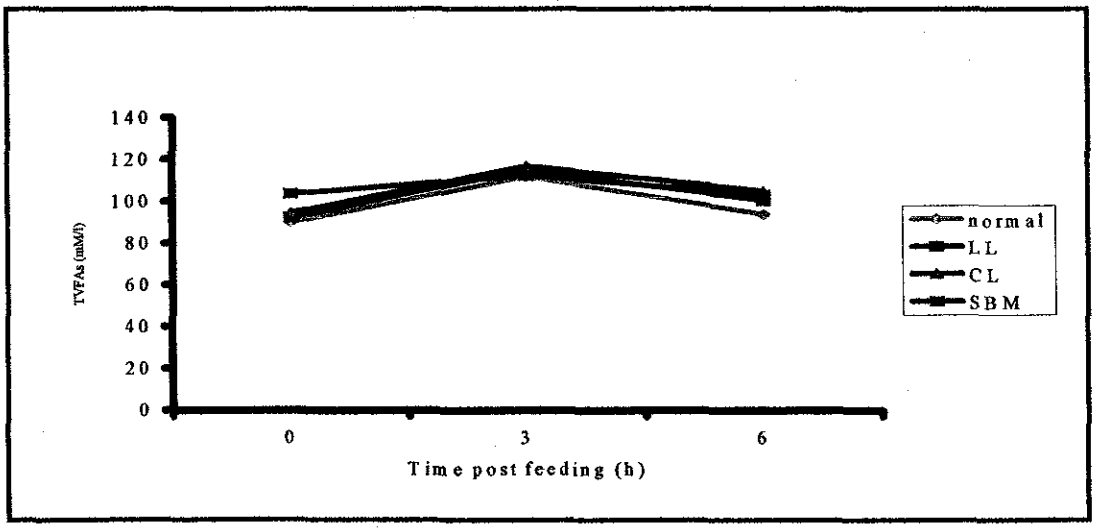

Figure 6.3 Hourly total volatile fatty acid (TVFA) in cattle receiving a diet containing four type of Caspurea mixed with natural plant protein, Where normal= normal Caspurea (Control), LL= leucaena leaf, CL= cassava leaf, SBM= soybean meal

Total volatile fatty acid (TVFA) concentrations at 0, 3 and 6 h post feeding are presented in Table 6.4 and Figure 6.3. TVFA concentration at 3 h post feeding was increased similar in all dietary treatment. The data in this experiment indicated that

ruminal fermentation was greatest at the 3 h post feeding. Higher VFA concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time; however, at the 6 h post feeding decreased indicating a more capture of C-skeleton for increased microbial protein synthesis (Sinclair et al., 1993; Witt et al., 1999).

Stiles et al. (1970) reported that the rumen VFA concentration usually peaked 4 hours post-feeding. While the total VFA concentration and acetic acid were highest for the Starea-fed animals. The molar proportions of propionic, iso-butyric and iso-valeric acids were significantly greater for those fed the control ration. This finding was similar to those reported by Roman-Ponce et al. (1974) that Starea and SBM rations result in higher ($P<0.01$) amount of total VFA than urea. Propionate concentration was similar in SBM and Starea rations and higher ($P<0.05$) in urea rations. Haskins et al. (1967) reported that no relationship of nitrogen source to molar percentages of VFA's but Davis and Stallcup (1967) found that more acetate and greater A/P ratios in rumen fluid from SBM than from urea-supplemented cattle. Moreover, the mechanism for the decreased A/P ratios in rumen fluid of steers receiving high sulfur diets may involve a more efficient, sulfur-dependent metabolic pathway. Whanger and Matrone (1966) offered evidence of propionate synthesis via the acrylate pathway from lactate, a compound utilized poorly by animal tissue. This system taps a supply of energy otherwise deficient in rations which are inadequate in sulfur and provides an additional source of propionate thereby improving the overall efficiency of the diet. However, Holter et al. (1971) also found that higher proportions of acetate and butyrate have been depressed compared to controls whereas propionate and total VFA's were increased markedly in rations containing SBM (Davis et al., 1957; Hutjens and Schultz, 1971).

Blood urea nitrogen concentrations (Table 6.4 and Figure 6.4) (21.6, 21.1, 20.0, 19.9 mg%) were affected by the natural plant protein sources and were lowest (P <0.05) in SBM group. However, the BUN were not significantly different (P>0.05) between Caspurea+cassava leaf and Caspurea+soybean meal rations. Moreover, when compaired between Caspurea+leucaena leaf and normal Caspurea rations were not differ (P>0.05). These data indicated that a slower rate of ammonia release and/more complete uptake of the NH_3-N for microbial protein synthesis when Caspurea mixed soybean meal was fed, but in the animals fed normal Caspurea was higher than those, these increases reflect the higher concentrations of ammonia in the rumen of these animals indicating that at a high NPN intake in the diet may cause imbalances between N and energy supply to rumen microorganisms and ammonia concentrations increase (Shabi et al., 1998), ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. Moreover, Sinclair et al. (1993) also reported that animals offered energy and nitrogen asynchronous diet had high blood urea.

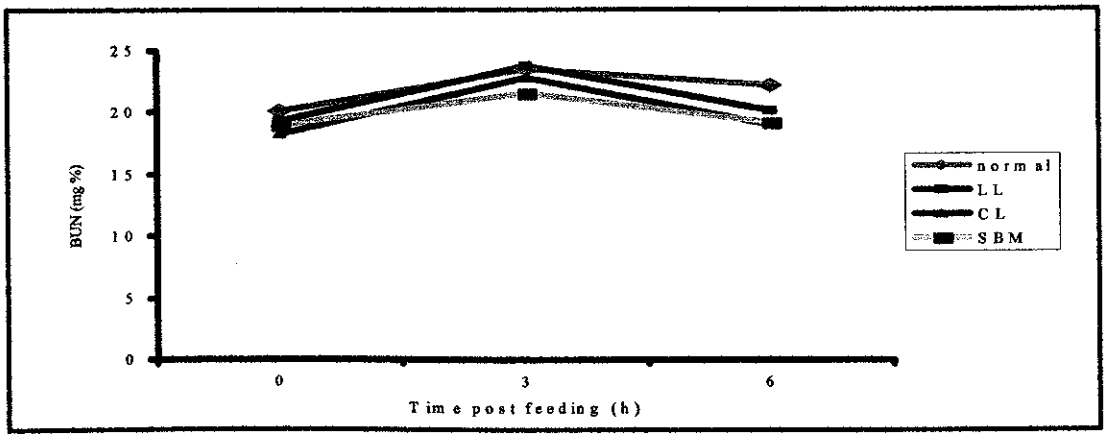

Figure 6.4 Hourly blood urea nitrogen (BUN) in cattle receiving a diet containing four type of Caspurea mixed with natural plant protein, Where normal= normal Caspurea (Control), LL= leucaena leaf, CL= cassava leaf, SBM= soybean meal

Blood urea nitrogen concentrations at 0, 3 and 6 h post feeding are presented in Table 6.5 and Figure 6.4. Blood urea nitrogen concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding, rumen ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. however, at the 6 h post feedings decreased indicating a more excreted in the urine. (Sinclair et al., 1993; Witt et al., 1999). Schmidt et al. (1973) reported that blood NH_3-N level were only slightly higher when urea was used as a nitrogen source than when SBM, TSBM or Starea were used as the nitrogen sources (1.2 and 1.1 μg NH_3-N/ml blood). An increase in blood NH_3-N levels would not be expected since the rumen NH_3-N concentrations at the 1 h sampling time were approximately one-half the 30 mM/liter rumen fluid necessary to result in a rise in peripheral blood ammonia. Blood urea nitrogen reached its peak concentration approximately 1 h after the rumen ammonia peak irrespective of supplemental nitrogen source. At 2.5 h, BUN was lower in the animals fed Starea than for those fed urea ($P<0.05$), and except for the 0 and 12 h samples, the levels for the animals fed Urea and Starea were higher than those for SBM and TSBM fed groups ($P<0.05$). These data indicate a slower rate of ammonia release and/or more complete uptake of the NH_3-N for microbial protein synthesis when SBM rather than urea was the nitrogen source. In contrast, reported by Jones et al. (1974) that the highest ($P<0.05$) blood urea nitrogen was with the SBM ration. Reducing dietary crude protein content resulted in lower BUN. Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein (Figure 2.1 and 2.2). In this way a part of dietary protein is converted into microbial protein of high nutritive value.

Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids, have but little effect on the yield of microbial matter (Ørskov et al., 1974). If, however, a certain microorganism causing a different type of fermentation predominated in the rumen, and if that microorganism were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist.

Table 6.5 Effect of dietary treatments on microbial population and blood urea nitrogen

Items	Caspurea+Natural Plant Protein					Contrast*		
	Control	Leucaena leaf	Cassava leaf	Soybean meal	SEM	1 vs 2 & 3 & 4	3 vs 2 & 4	2 vs 4
BUN (mg%)								
0	20.1[a]	19.4[ab]	18.3[c]	18.9[bc]	0.30	0.004	0.040	0.340
3	23.5[a]	23.8[a]	22.8[ab]	21.6[b]	0.42	0.129	0.813	0.003
6	22.2[a]	20.1[b]	19.0[b]	19.1[b]	0.46	0.001	0.308	0.167
Mean	21.6[a]	21.1[a]	20.0[b]	19.9[b]	0.07	0.014	0.507	0.123
Bacteria (x 10^{10} cell/ml)	1.9	2.0	2.2	2.3	0.08	0.218	0.991	0.365
Protozoa (x 10^{5} cell/ml)	2.4	2.3	2.5	2.6	0.08	0.898	0.907	0.499

[a,b,c] Means within a row with different superscripts differ ($P<0.05$), Contrast* = Orthogonal contrast

Rumen microbe populations are presented in Table 6.5. Bacteria population (1.9, 2.0, 2.2, 2.3 (x 10^{10} cell/ml); $P>0.05$) and protozoa population (2.4, 2.3, 2.5, 2.55 (x 10^{5} cell/ml); $P>0.05$) were affected by the natural plant protein sources. The result agrees with Helmer et al. (1970) who reported that Starea, an extrusion-processed mixture of grain or cassava and urea, has increased synthesis of microbial protein *in vitro* and *in vivo* (Stiles et al., 1970). It is possible that higher synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana et al., 1990). Sinclair et al. (1993) and Kim (2001) reported that higher synchonizing rate of degradation of dietary energy and N release in the rumen beneficially increased microbial protein synthesis. Moreover, Jouaney and Ushida (1999) reported that

ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration. Therefore, high soluble N in diets and cassava starch possibly affected the rumen microbe population. Microbial biomass, net 15^N incorporation into cells, volatile fatty acid production increased linearly with increasing levels of cassava inclusion in diets (Sommart et al., 2000). Moreover, carbohydrates are the main substrate for rumen bacteria and also the main constituent of the ruminant diet. They are the only substrate from which large amounts of energy can be derived under anaerobic conditions. In contrast to the bacteria for which carbohydrates are the energy source, the growth of those utilizing protein to cover their energy needs under anaerobic condition is very limited . Fat is not an energy source under the anaerobic conditions of the rumen, but the energy value of its hydrolytic product, glyceral, is similar to that of carbohydrate.

Table 6.6 Effect of dietary treatments on nitrogen balance

Items	Caspurea+Natural Plant Protein					Contrast*		
	Control	Leucaena leaf	Cassava leaf	Soybean meal	SEM	1 vs 2 & 3 & 4	3 vs 2 & 4	2 vs 4
Urine N (g/d)	54.8	54.2	53.5	52.9	0.77	0.626	0.975	0.696
Urine N (g/kgBW $^{0.75}$)	1.03	1.02	1.00	1.00	0.01	0.721	0.980	0.827
Feces N (g/d)	28.0^{a}	26.6^{ab}	25.3^{ab}	24.5^{b}	0.35	0.040	0.812	0.151
N absorption (g/d)	91.9	92.6	93.5	93.5	1.25	0.765	0.914	0.859
N retention (g/d)	20.9	19.7	21.0	21.5	2.23	0.216	0.791	0.384
N absorption (%N intake)	76.5^{b}	77.5^{a}	78.7^{a}	77.2^{ab}	0.23	0.013	0.686	0.072
N retention (%N intake)	31.0^{b}	32.2^{ab}	33.7^{a}	34.3^{a}	0.24	0.005	0.594	0.042

^{a,b}Means within a row with different superscripts differ (P<0.05),*Orthogonal contrast

Nitrogen balance study is shown in Table 6.6. Urinary N excretion was similar across treatment ($P>0.05$). However, The fecal N excretion in normal Caspurea ration higher than other treatments ($P<0.05$) Moreover, fecal excretion was lowest in Caspurea mixed soybean meal. Nitrogen absorption (91.9, 92.6, 93.5, 93.5 g/d) and N retention (31.0, 32.2, 33.7, 34.3% N intake) were not significantly different ($P>0.05$) among dietary treatments. Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity, is absorbed from the rumen into the blood and is converted in the liver to urea, of which a part is recirculated to the rumen with saliva or across the rumen wall, while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The type of rumen fermentation, and the kind of end-products, i.e. the molar proportion of volatile fatty acids, have but little effect on the yield of microbial matter (Ørskov et al., 1974). If, therefore, a certain microorganism causing a different type of fermentation predominated in the rumen, and if that microorganism were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist.

6.7 Conclusions

Inclusion, the results also indicated that Caspurea mixed soybean meal used as a protein can improve feed intake, body weight change, blood metabolites, rumen microbe populations, end-products of ruminal fermentation, digestibility and nitrogen balance.Therefore this study suggests that the diet containing of 35% Capurea mixed soybean meal used as a protein in ration with 40% RUP has positive benefit on Thai Native x Brahman beef cattle productive performance.

6.8 References

AOAC. (1985). **Oficial Methods of Analysis.** Association of Official Chemists, Washington, D.C.

Bach, A., and Stern, M. D. (1999). Measuring resistance to ruminal degradation and bioavailability of ruminally protected methionine. **Anim. Feed Sci. Technol.** 84: 23-32.

Bartley, E.E., Deyoe, C. W., Pfost, H. B., Anstaett, F. R., and Sung, A. C. (1968). An improved urea product for ruminants. **Anim. Nutri. And Health.** May: 10-13.

Bloomfield, R. A., Kearley, E. D., Creach, D. O., and Muhrer, M. E. (1963). Ruminal pH and absorption of ammonia and VFA. **J. Anim. Sci.** 22: 833.

Briggs, M. H. (1967). **Urea as a protein supplement.** 1st ed. Pergamon Press, Inc., Long Island City, New York.

Briggs, P. K., Hogan, J. F., and Reid, R. L. (1957). The effect of volatile fatty acid, lactic acid, and ammonia on rumen pH in sheep. **Aust. J. Agric. Res.** 8 : 674-710.

Bromner, J. H., and Keeney, D. R. (1965). Steam distillation medthodes of determination of ammonium, nitrate and nitrite. **Annual. Chem. Acta.** 32 : 485 - 495.

Chalupa, W. (1968). Problems in feeding urea to ruminants. **J. Anim. Sci.** 27: 207-215.

Colovos, N. F., Holter, J. B., Davis, H. A., and Urban, Jr. W. E. (1967a). Urea for lactating dairy cattle. I. Effect of concentrate fiber and urea levels on nutritive value of the ration. **J. Dairy Sci.** 50: 518-523.

Colovos, N. F., Holter, J. B., Davis, H. A., and Urban, Jr. W. E. (1967b). Urea for lactating dairy cattle. I. Effect of concentrate fiber and urea levels on nutritive value of the ration. **J. Dairy Sci.** 50: 523-528.

Coombe, J. B., Tribe, D. E., and Morrison, J. W. C. (1960). Some experimental observations on the toxicity of urea to sheep. **Aust. J. Agr. Res.** 11: 247.

Davis, G. V., and Stallcup, O. T. (1964). Influence of dietary nitrogen on nitrogen metabolism in the rumen. **J. Dairy Sci.** 47:1237.

Davis, G. V., and Stallcup, O. T. (1967). Effect of soybean meal, raw soybeans, corn gluten feed, and urea on the concentration of rumen fluid components at intervals after feeding. **J. Dairy Sci.** 50 : 1638 – 1645.

Davis, R. F., Woodhouse, N. S., Keeny, M., and Beck, G. H. (1957). The effect of various levels of dietary protein upon the volatile fatty acids in the rumen of the dairy cow. **J. Dairy Sci.** 40: 75.

Freitag, R. R., Smith, W. H., and Beeson, W. M. (1968). Factors related to utilization of urea vs. protein nitrogen supplemented diets by the ruminant. **J. Anim. Sci.** 27 :478-485.

Goering. H. K., and Van Soet, P. J. (1970). **Forage fiber analyses** (apparatus, reagents, procedures, and some applications) Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Goodrich, R. D., and Tillman. (1966). Effects of sulfur and nitrogen sources and copper levels on the metabolism of certain minerals by sheep. **J. Anim. Sci.** 25: 484.

Haskins, B. R., Wise, M. B., Craig, H. B., and Barrick, E. R. (1967). Effect of levels of protein, source of protein and antibiotic on performance, carcass characteristics, rumen environment and liver abscesses of steers fed all-concentrate rations. **J. Anim. Sci.** 26: 430.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25 – 32.

Helmer, L. G., Bartley, E. E., and Deyoe, C. W. (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Herrera-Saldana, R., Huber, J. T., and Poore, M. H. (1989). fluence of varying protein and starch degradabilities on performance of lactating cows. **J. Dairy Sci.** 72 : 1477-1483.

Holter, J. B., Colovos, N. F., Clark, R. M., Koes, R. M., Davis, H. A., and Urban, Jr. W. E. (1971). Urea for lactating cattle. V. Concentrate fiber and urea in a corn silage-high concentrate ration. **J. Dairy Sci.** 54: 1475-1482.

Hobson, P. N. (1971). Rumen microorganisms. **Progr. Indust. Microbial.** 9: 41.

Hungate, R. E. (1966). **The rumen and its microbes.** Academic Press, London.

Hutjens, M. F., and Schultz, L. H. (1971). Addition of soybeans or methionine analog to high-concentrate rations for dairy cows. **J. Dairy Sci.** 54:1637-1646.

Jittakot, S. (1999). **Effect of cassarea as a protein replacement for soybean meal on feed intake, blood metabolites, ruminal fermentation, digestibility and microbial protein synthesis in dairy cows fed urea-treated rice straw as a roughage.** Khon Kaen, Thailand. Graduate School, Khon Kaen University. 171 pp. (M. Sc. Thesis).

Jones, G. M., Stephens, C., and Kensett, B. (1974). Utilization of Starea, Urea, or Soybean Meal in Complete Rations for Lactating Dairy Cows. **J. Dairy Sci.** 58: 689-695.

Jouaney, J. P., and Ushida, K. (1999). The role of protozoa in feed digestion. **Asian-Aust. J. Anim. Sci.** 12: 113-126.

Kim, C. H. (2001). Effect of different protein sources given synchronously or asynchronously into the rumen of consuming a beef cattle diet high in concentrate on the synthesis of microbial protein. **J. Anim Sci. Technol.** 43 : 831-840.

Kolver, E., Muller, L. D., Vaga, G. A., and Cassidy, T. J. (1998). Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. **J. Dairy Sci.** 81:2017-2028.

Lowrey, R. S., and McCormick, W. C. (1969). Factors affecting the utilization of high urea diets by finishing steers. **J. Anim. Sci.** 28: 406-415.

Matrone, G., Bunn, C. R., and McNeill, J. J. (1964). Investigations of dietary factors in purified diets for ruminants. **J. Nutr.** 84: 215.

Ørskov, E. R., Fraser, C., McDonald, I., and Smart, R. (1974). Digestion of concentrate in sheep. 5. The effect of adding fish meal and urea together to cereal diets on protein digestion and utilization by young sheep. **Brit. J. Nutr.** 31: 89-96.

Reid, J. T. (1953). Urea as a protein replacement. A review. **J. Dairy Sci.** 36: 955.

Roffler, R. E., and Satter, L. D. (1973). Influence of ration composition on ruminal ammonia concentration. **J. Dairy Sci.** 56 : 663. (Abstr.)

Roman-Ponce, H., Van Horn, H. H., Mashall, S. P., Wilcox, C. J., and Randel, P. F. (1974). Complete rations for dairy cattle V. Interaction of sugarcane bagasse quantity and form with soybean meal, urea, and starea. **J. Dairy Sci.** 58: 1320-1327.

SAS. (1996). SAS User's Guide: Statistics. Version 6. 14th ed Cary, NC: **SAS Inst**

Schmidt, S. O., Jorgensen, N. A., Benevenga, N. F., and Burngart, V. H. (1973). Comparison of soybean meal, formaldehyde treated soybean meal, urea and Starea for steers. **J. Anim. Sci.** 37 : 1233-1237.

Shabi, K., Arieli, A., Bruckental, L., Aharoni, Y., Zamwel, S., Bor, A., and Tagari, H. (1998). Effect of the synchronization of the degradation of dietary crude protein and organic matter and feeding frequency on ruminal fermentation and flow of digesta in the abomasum of dietary cows. **J. Dairy Sci.** 81 : 1991-2000.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R., and Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. **J. Agric. Sci. Camb.** 120: 251-263.

Sommart, K., Parker, D. S., Rowlinson, P., and Wanapat, M. (2000). Fermentation characteristics and microbial protein synthesis in an in vitro system using cassava, rice straw and dried ruzi grass as substrates. **Asian-Aust. J. Anim. Sci.** 13: 1084-1093.

Stiles, D. A., Bartley, E. E., Meyer, R. M., Deyoe, C. W., and Pfost, H. B. (1970). Feed processing. VII. Effect of an expansion processed mixture of grain and urea (Starea) on rumen metabolism in cattle and on urea toxicity. **J. Dairy Sci.** 53 : 1436-1441.

Susmel, P., Stefanon, B., Mills, C. R., and Candido, M. (1989). Change in amino acid composition of different protein sources after rumen incubation. **Anim. Prod.** 49:375-383.

Thomas, W. E., Loosli, J. K., Williams, H. H., and Maynard, L. A. (1951). The utilization of inorganic sulfates and urea nitrogen by lambs. **J. Nutr.** 43:515.

Thompson, L. H., Wise, M. B., Harvey, R. W., and Barrick, E. R. (1972). Starea, urea and sulfur in beef cattle rations. **J. Anim. Sci.** 35 : 474-482.

Van Horn, H. H., and Jacobson. (1971). Response of lactating cows to added increments of dietary protein and non protein nitrogen. **J. Dairy Sci.** 58:1101-1108.

Van Horn, H. H., Foreman, C. F., and Rodriguez, J. D. (1967). Effect of high-urea supplementation on feed intake and milk production of dairy cows. **J. Dairy Sci.** 50 : 709-716.

Van Horn, H. H., Marshall, S. P., Wilcox, C. J., Randel, P. F., and Wing, J. M. (1975). Complete rations for dairy cattle. III. Evaluation of protein percent and quality, and citrus pulp-corn substitutions. **J. Dairy Sci.** 58: 1101-1106.

Whanger, P. D., and Matrone, G. (1966). Metabolism of lactic, succinic and acrylic acids by rumen microorganisms for sheep fed sulfur-adequate and sulfur efficient diets. **Biochimica et Biophysica Acta.** 136:27.

Witt, M. W., Sinclair, L. A., Wilkinson, R. G., and Buttery, P. J. (1999). The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food ad libitum. **Brit. Soc. Anim. Sci.** 69: 223-235.

CHAPTER VII

OVERALL DISCUSSION AND IMPLICATION

The use of urea by ruminants has been restricted by inefficient conversion of urea nitrogen to microbial protein by toxicity, palatability and urea segregation in mixed rations fed in meal form (Briggs, 1967; Chalupa, 1968; Reid, 1953). In a previous study (Helmer et al., 1970) an extrusion-processed mixture of grain starch and urea (Starea) was approximately equal to soybean meal as a protein supplement for lactating cows. Cows fed grain rations supplemented with either soybean meal or Starea consumed more grain and produced more milk than cows fed the same grain ration supplemented with urea. In another study (Helmer et al., 1970) Starea improved the utilization of urea *in vitro*. After 4 hours of fermentation *in vitro*, concentration of rumen ammonia was lower with Starea than with unprocessed corn and urea. The lower rumen ammonia was at least in part the result of more efficient utilization of ammonia by its conversion to microbial protein. Current concepts of ruminant nutrition focus on maximizing rumen digestion and ruminal microbial protein production. Synchronization of the rate of dietary nitrogen and energy release has been suggested as a means of improving both microbial protein flow at the duodenum, and the microbial protein synthesis (Sinclair et al., 1993; Kolver et al., 1998; Kim, 2001).

This thesis presents four experiments that evaluated the effects of an extrusion – processed mixture of cassava pulp and urea (Caspurea) as crude protein sources on ruminal fermentation, microbe population, blood urea nitrogen, nutrient digestibility,

growth performance in Thai Native x Brahman crossbred beef cattle. The objectives of the first study was to compare performance of Thai Native-Brahman beef cattle and utilization of dietary nitrogen with levels of Caspurea replacement for soybean meal in concentrate. These results indicated that 50% replacement by Caspurea for soybean meal in concentrate unaffected crossbred Native x Brahman beef cattle production. This data was agreement with Helmer et al. (1970) who found that an expansion-processed mixture of grain starch and urea (Starea) was approximately equal to soybean meal as a protein supplement for lactating cows. However, NPN source is utilized less efficiently than natural plant proteins such as soybean meal for beef production (Helmer and Bartley, 1971). A study with high-producing cows receiving various levels of urea in a high concentrate ration showed that high-urea levels (20-40% of the total ration nitrogen) depressed both milk yield and efficiency (Huber and Sandy, 1965). As confirm by experiment 2 which suggests that crude protein from Caspurea would replacement for total crude protein in concentrate no more than 35%. Moreover, current concepts of ruminant nutrition focus on maximizing rumen digestion and ruminal microbial protein production. Balancing of the rate of dietary RDP and RUP degradation has been suggested as a means of improving both microbial protein flow at the duodenum, and the microbial protein synthesis (Sinclair et al., 1993; Kolver et al., 1998; Kim, 2001). Therefore the objectives of experiment 3 were to compare performance of Thai Native x Brahman beef cattle and utilization of dietary nitrogen when concentrate were formulated to contain 35% of CP from Caspurea replacement for total crude protein with varying levels of rumen undegradable protein in concentrate. These results indicated that crude protein of Caspurea replaced at 35% of total crude protein and rumen undegradable protein at 40% in concentrate has positive effects on crossbred Native x Brahman beef cattle

production, being similar to that suggested by NRC (2001). Finally, the use of Caspurea (NPN source) by ruminants has been restricted by inefficient conversion of N to microbial protein by toxicity and by palatability (Briggs, 1967; Chalupa, 1968), therefore the objectives of experiment 4 aimed to study the effects of an extrusion-processed mixture of cassava pulp and urea with natural plant protein (45% CP) (leucaena leaf, cassava leaf and soybean meal) and pelleting at high temperatures (Caspurea/Starea) as a protein source on performance of Thai Native x Brahman beef cattle. These results indicated that Caspurea+soybean meal has positive effects on crossbred Thai Native x Brahman beef cattle production. In conclusion, results from four experiments indicated that diets were formulated using Caspurea as a protein source replacement for SBM at 50% or 35% of total crude protein and contain 40% RUP in concentrate unaffected performance and has positive benefit effects in Thai Native x Brahman beef cattle. Moreover, extrusion-processed mixture of Caspurea with natural plant protein and pelleting at high temperatures has positive benefit effects and improve performance of Thai Native x Brahman beef cattle which tended to be higher than normal Caspurea.

References

Briggs, M. H. (1967). **Urea as a protein supplement.** 1st ed. Pergamon Press, Inc., Long Island City, New York.

Chalupa, W. (1968). Problems in feeding urea to ruminants. **J. Anim. Sci.** 27: 207-215.

Helmer, L. G., and Bartley, E. E. (1971). Progress in the utilization of urea as a protein replacer for ruminants. A review. **J. Dairy Sci.** 54 : 25 – 32.

Helmer, L. G., Bartley, E. E., and Deyoe, C. W. (1970). Feed processing. VI. Comparison of starea, urea, and soybean meal as protein source for lactating dairy cow. **J. Dairy Sci.** 53 : 883 – 887.

Huber, J. T., Sandy, R. A., Polan, C. E., Bryant, C. T., and Blaser, R. E. (1967). Varying levels of urea for dairy cows fed corn silage as the only forage. **J. Dairy Sci.** 50 : 1241-1246.

Kim, C. H. (2001). Effect of different protein sources given synchronously or asynchronously into the rumen of consuming a beef cattle diet high in concentrate on the synthesis of microbial protein. **J. Anim Feed Sci. Technol.** 43 : 831-840.

Kolver, E., Muller, L. D., Vaga, G. A., and Cassidy, T. J. (1998). Synchronization of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. **J. Dairy Sci.** 81:2017-2028.

National Research Council. (2001). **Nutrient requirements of dairy cattle.**7th ed. Acad. Sci. Washington, DC.

Reid, J. T. (1953). Urea as a protein replacement. A review. **J. Dairy Sci.** 36: 955.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R., and Buttery, P. J. (1993). Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. **J. Agric. Sci. Camb.** 120: 251-263.

APPENDICES

LAB PROCEDURE

Determination of Total Volatile Fatty Acid (TVFAs)

Materials

1. Distillation apparatus
2. Centrifuge
3. Four figure balance
4. Burette
5. Beaker
6. Erlenmeyer flask (250 ml)

Reagent and preparation

1. Sulfuric acid 1:1 Dilute 1 volume of concentrated sulfuric acid (H_2SO_4) slowly added to the same volume of water, stirring at intervals. Do not add water to sulfuric acid in order to avoid dangerous splashing.
2. Sodium hydroxide (NaOH) titrated 0.04 N solutions.
3. Phenolphthalein indicator solution. 0.5 g in 100 ml distilled water.
4. Methyl orange indicator solution. 0.05 g in 100 ml distilled water.
5. Acetic acid (CH_3COOH), 2000 mg/l. Dilute 1.9 ml of glacial acetic acid to 1000 ml with deiornize or distilled water. Tritrate with 0.04 N sodium hydroxide solution 10 ml of acetic acid solution (0.33 meq) require 8.3 ml of 0.04 N sodium hydroxide (Phenolpthalein indicator).
6. H_2O

Procedure

1. Sample: 150-200 ml ofrumen fluid is centrifuge for removing solids (2500 RPM, 15 minutes). The sample is given by suppernatant liquid.
2. Dilute 5 ml of rumen fluid to 100 ml wit distilled water in distillation test tube.
3. Acidified with sulfuric acid 1:1 added in 1 ml volumes up to the change of methyl orange indicator from red to yellow (pH 2.7).
4. Place into position in the stream distillng unit an Erlenmeyer flask and the test tube with the acidified sample.
5. Collect 100 ml of condensate.
6. Titration: after addition of 10 drops of phenolpthalein indicator solution, the samples are titrated using 0.04 N Sodium hydroxide up to a persisting pink color.

Calculations

1. The result is given by:

   mg/l TVFAs (as acetic acid) = (ml NaOH x normality x 60050)/(ml of Sample x F)

   TVFAs = Total volatile fatty acid (mg/l)

   F is a recovery factor of the used stream distillation unit obtained distilling known volumes (e.g. 10 m) of 2000 mg/l acetic acid solution and computing:

   F= (ml of used NaOH x 100)/(theoretical ml of NaOH).

Determination of Ammonia Nitrogen

Materials

1. Distillation apparatus
2. Centrifuge
3. Four figure balance
4. Burette
5. Beaker
6. Erlenmeyer flask (250 ml)

Reagent and preparation

1. Sulfuric acid (H_2SO_4) 0.01 N (dilute 0.5489 ml or 1.0099 g of concentrated sulfuric acid into 1000 ml of distilled water.
2. Sodium hydroxide (NaOH) 40%, (40 g of NaOH added to 100 ml of distilled water.
3. Boric acid 4% (4 g of boric acid added to 100 ml of distilled water)
4. Indicator (0.3125 g of methyl red and 0.2062 g of methylene blue dissolved in 95% ethanol 250 ml.
5. H_2O

Procedure

1. Sample: 150-200 ml ofrumen fluid is centrifuge for removing solids (2500 RPM, 15 minutes). The sample is given by suppernatant liquid.
2. Dilute 10 ml of rumen fluid to 100 ml wit distilled water in distillation test tube.

3. Added 50 ml of 40%NaOH
4. Place into position in the stream distilling unit an Erlenmeyer flask with 4% of boric acid and two drop of indicator.
5. Collect 200 ml of condensate.
6. The samples are titrated using 0.01 N H_2SO_4 up to a persisting purple colors.

Calculations

Ammonia-nitrogen (NH_3-N) mg% = ml H_2SO_4 x 0.28

In vitro gas production technique

Materials

1. Digital analytic balance
2. CO_2 gas
3. Suction pump
4. Stomach tube
5. Suction flask (2000 ml)
6. Thermos (2000 ml)
7. Cylinder (100, 500 and 1000 ml)
8. Pipet (0.5, 1.0 and 5.0 ml)
9. Thermometer (scale 50°C)
10. Hot plate stirrer
11. Syringe (60 ml)

12. Syringe glass (20 ml)
13. Needle (18 G x 1 and 25 G x 1)
14. Serum bottle (60 ml)
15. Hot air oven
16. Shaking water bath

Reagent and preparation

1. Distilled water 1,095 ml
2. Buffer solution 730 ml (dilute $NaHCO_3$ 35.0 g and 4.0 g NH_4HCO_3 to 1000 ml with distilled water)
3. Macro mineral solution 365 ml (dilute KH_2PO_4 6.2 g , Na_2HPO_4 5.7 g, NaCl 2.22 $MgSO_4.7H_2O$ 0.6 g to 1000 ml with distilled water)
4. Micro mineral solution 0.23 ml (dilute $MnCl_2.4H_2O$ 10.0 g, $CaCl_2.2H_2O$ 13.2 g, $CoCl_2.6H_2O$ 1.0 g, $FeCl_3.6H_2O$ 8.0 g to 100 ml with distilled water)
5. Resazurine solution indicator 1.0 ml (dilute resazurine 1.0 g to 100 ml with distilled water)
6. Oxigen reduction solution 60 ml (dilute $Na_2S.9H_2O$ 8.0 g with NaOH 1 M 60 ml)
7. Rumen fluid 660 ml

Procedure

1. The samples were ground through a 1 mm screen for the *in vitro* gas production technique.

2. Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inocculums was removed before the

morning feeding by vacuum pump and stomach tube technique into a 2 liters glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as descripbed by Sommart et al. (2000).

3. The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 60 ml serum bottle (Sommart et al., 2000). The bottles were pre-warmed in a water bath at 39°C for about 1 h prior to injection of 40 ml of rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were stoppered with rubbers stoppers, crimp sealed and incubated in a water bath setting at 39°C.

4. The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 ml glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 96 h (from 1-12 h, every 3 h from 13-24 h, every 6 h from 25-48h and every 12 h from 49-96 h) after incubation periods. Amount of cumulative gas volume from 1 to 96 h after incubations were fitted using the equation $y=a+b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979), where y, describes gas production at time t, *a*, the gas produced (ml) by instantaneous fermentation of the soluble and readily available fraction of feed, *b*, the gas produced (ml) by the fermentation of insoluble, but slowly fermentable fraction and *c*, the fractional rate (rate constant) at which gas is produced per hour (%/h).

BIOGRAPHY

Mr. Kanin Bunnakit was born on the 11th of June 1971 in Mahasarakham, Thailand. He finished high school from Kosum Wittayasun School, Mahasarakham. In 1994, he received Bachelors Degree in Bachelor of Science (Animal Science) from Khon Kaen University, Thailand. After graduation, he had experiences by working as the research assistant from 1995 to 1998 in Faculty of Agriculture, Department of Animal Science, Khon Kaen University. In 1999, he continued his Master Degree in Master of Science (Animal Science) and finished his Master Degree on the 14th of October 2003. Then he continued his Doctor Degree in School of Animal Production Technology at Institute of Agricultural Technology, Suranaree University of Technology. His research topic was effects of Caspurea as crude protein sources on performance of Thai Native x Brahman crossbred beef cattle. The result of this research has been presented as oral presentation at Chiang Mai University and Yunnan Agricultural University, Kunming, China. Moreover, while he was studying in Doctor Degree, he had experiences by working as the research and teaching assistant in School of Animal Production Technology.