PROTEIN REQUIREMENT OF GROWING THAI-INDIGENOUS BEEF CATTLE FED WITH RICE STRAW AS ROUGHAGE

Shengchang Chen

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

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ความต้องการโปรตีนสำหรับโคเนื้อพันธุ์พื้นเมืองระยะกำลังเจริญเติบโตที่ได้รับฟางข้าวเป็นอาหารหยาบ

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ปีการศึกษา 2553

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

Thesis Examining Committee

_____

(Asst. Prof. Dr. Banchorn Likitdecharote, DVM)

Chairperson

_____

(Asst. Prof. Dr. Pramote Paengkoum)

Member (Thesis Advisor)

_____

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Member

_____

(Dr. Amonrat Molee)

Member

_____

(Asst. Prof. Dr. Songsak Chumpawadee)

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_____	_____
(Prof. Dr. Sukit Limpijumnong)	(Asst. Prof. Dr. Suwayd Ningsanond)
Vice Rector for Academic Affairs	Dean of Institute of Agricultural Technology

เชิงชาง เชิน : ความต้องการโปรตีนสำหรับโคเนื้อพันธุ์พื้นเมืองระยะกำลังเจริญเติบโตที่ได้รับฟางข้าวเป็นอาหารหยาบ (PROTEIN REQUIREMENT OF GROWING THAI-INDIGENOUS BEEF CATTLE FED WITH RICE STRAW AS ROUGHAGE) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 159 หน้า.

การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความต้องการโปรตีนในโคเนื้อพันธุ์พื้นเมืองระยะกำลังเจริญเติบโต และศึกษาผลของสัดส่วนโปรตีนและโปรตีนที่ไม่ถูกย่อยในรูเมนต่อการย่อยได้ของโภชนะ กระบวนการหมักในรูเมน จุลินทรีย์ในรูเมน สมดุลไนโตรเจน และการสังเคราะห์จุลินทรีย์โปรตีนในรูเมน การศึกษาในครั้งนี้ประกอบไปด้วย 2 การทดลอง

การทดลองที่ 1 ใช้โคเนื้อเพศผู้พันธุ์พื้นเมืองในระยะกำลังเจริญเติบโต น้ำหนักตัวเฉลี่ย 154±13.2 กิโลกรัม ใช้แผนการทดลองแบบ 3x3 ละตินสแควร์แบบมีซ้ำ การศึกษาใช้อาหารผสมสำเร็จที่ประกอบด้วยโปรตีน 4.3, 7.3 และ 10.3 เปอร์เซ็นต์โดยน้ำหนักแห้ง ปริมาณการกินได้ของวัตถุแห้ง แอมโมเนีย-ไนโตรเจนในรูเมน และยูเรีย-ไนโตรเจนในกระแสเลือดเพิ่มขึ้นแบบเป็นเส้นตรง ($P<0.01$) ตามระดับของโปรตีนที่เพิ่มขึ้น ส่วนการย่อยได้ของวัตถุแห้ง อินทรียวัตถุ ผนังเซลล์ (neutral detergent fiber, NDF) และลิกนินเซลลูโลส (acid detergent fiber, ADF) ไม่มีความแตกต่างกันทางสถิติ เช่นเดียวกับความเป็นกรด-ด่างในรูเมน กรดไขมันระเหยได้ทั้งหมด จุลินทรีย์ที่ตรวจนับ และแบคทีเรียในรูเมน ไม่มีผลเนื่องมาจากระดับโปรตีนที่แตกต่างกัน อย่างไรก็ตาม การกินได้ของไนโตรเจน การขับออกของไนโตรเจนทางปัสสาวะ การดูดซึมไนโตรเจน การเก็บกักไนโตรเจน อนุพันธ์ของพิวรีนที่ขับออกมาทางปัสสาวะทั้งหมด และการสังเคราะห์จุลินทรีย์ไนโตรเจนเพิ่มขึ้นแบบเป็นเส้นตรง ($P<0.05$) ส่วนความต้องการโปรตีนสำหรับดำรงชีพในโคเนื้อพันธุ์พื้นเมืองระยะกำลังเจริญเติบโตคือ 3.54 กรัมต่อกิโลกรัมน้ำหนักเมแทบอลิก (g/kg $BW^{0.75}$)

การทดลองที่ 2 ใช้โคเนื้อพันธุ์พื้นเมืองระยะกำลังเจริญเติบโต จัดกลุ่มการทดลองแบบ 2x3 แฟกทอเรียลในแผนการทดลองแบบสุ่มในบล็อกสมบูรณ์ ประกอบด้วยอาหารที่ศึกษา 6 ทรีทเมนท์ ประกอบด้วยโปรตีน 2 ระดับ (10 และ 12 เปอร์เซ็นต์) และโปรตีนที่ไม่ถูกย่อยในรูเมน 3 ระดับ (15, 25 และ 35 เปอร์เซ็นต์) ปริมาณการกินได้ของวัตถุแห้ง อัตราการเจริญเติบโต การย่อยได้ของวัตถุแห้ง อินทรียวัตถุ ผนังเซลล์ และลิกนินเซลลูโลส ความเป็นกรด-ด่างในรูเมน กรดไขมันระเหยได้ทั้งหมด และการสังเคราะห์จุลินทรีย์ไนโตรเจน ไม่มีผลเนื่องมาจากระดับของโปรตีน อย่างไรก็ตาม ปริมาณการกินได้ของไนโตรเจน และไนโตรเจนที่ขับออกในโคกลุ่มที่ได้รับโปรตีน 12 เปอร์เซ็นต์ สูงกว่ากลุ่มโคที่ได้รับโปรตีน 10 เปอร์เซ็นต์ อย่างมีนัยสำคัญทางสถิติ ($P<0.05$) นอกจากนี้ การย่อยได้ของวัตถุแห้ง อินทรียวัตถุ ผนังเซลล์ และลิกนินเซลลูโลส ความเป็นกรด-ด่างในรูเมน กรดไขมันระเหยได้ทั้งหมด จุลินทรีย์ที่นับได้ และการสังเคราะห์จุลินทรีย์ไนโตรเจน ไม่มี

ผลเนื่องมาจากระดับของโปรตีนที่ไม่ถูกย่อยในรูเมน อย่างไรก็ตาม ปริมาณการกินได้ของอาหาร อัตราการเจริญเติบโต ปริมาณการกินได้ของไนโตรเจน การขับออกไนโตรเจนในมูล และไนโตรเจนที่เก็บกักในร่างกาย เพิ่มขึ้นแบบเป็นเส้นตรง ($P<0.01$) ตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมนที่เพิ่มขึ้น ในขณะที่แอมโมเนีย-ไนโตรเจนในรูเมน ยูเรีย-ไนโตรเจนในกระแสเลือดลดลงแบบเป็นเส้นโค้ง (quadratically, $P<0.05$) ตามระดับของโปรตีนที่ไม่ถูกย่อยในรูเมน จากการศึกษาพบว่า ไม่มีปฏิสัมพันธ์ร่วมระหว่างโปรตีนและโปรตีนที่ไม่ถูกย่อยในรูเมน ยกเว้นระดับยูเรีย-ไนโตรเจน ในกระเพาะรูเมน ความต้องการโปรตีนสำหรับเมแทบอลิซึม สำหรับการเพิ่มน้ำหนักตัว 1 กรัมต่อกิโลกรัมของน้ำหนักตัวเมแทบอลิก คือ 0.34 กรัมต่อกิโลกรัมน้ำหนักตัวเมแทบอลิก

จากการทดลองทั้งสองงานนี้ สามารถนำข้อมูลมาประมวลหาความต้องการโปรตีน พบว่าโปรตีนในอาหาร 10 เปอร์เซ็นต์ เพียงพอสำหรับความต้องการสำหรับการเจริญเติบโตของโคเนื้อพันธุ์พื้นเมือง ยิ่งกว่านั้นยังพบว่าโปรตีนที่ถูกย่อยในรูเมน 6.5 เปอร์เซ็นต์ เพียงพอสำหรับเป็นแหล่งไนโตรเจนสำหรับจุลินทรีย์ในรูเมน ในขณะที่สัดส่วนที่เหมาะสมของโปรตีนที่ไม่ถูกย่อยในรูเมนและโปรตีนที่ถูกย่อยในรูเมน คือ 65 ต่อ 35

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2553 ลายมือชื่ออาจารย์ที่ปรึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

SHENGCHANG CHEN : PROTEIN REQUIREMENT OF GROWING THAI-INDIGENOUS BEEF CATTLE FED WITH RICE STRAW AS ROUGHAGE. THESIS ADVISOR : ASST. PROF. PRAMOTE PAENGKOUM. Ph.D., 159 PP.

THAI-INDIGENOUS BEEF CATTLE/NITROGEN BALANCE/PROTEIN REQUIREMENT

This research aimed to study the protein requirement of growing Thai-indigenous beef cattle, effects of crude protein (CP) levels or varying ratios of undegradable intake protein (UIP) to degradable intake protein (DIP) on nutrients digestibility, ruminal fermentation, rumen microbes, nitrogen balance, and microbial nitrogen synthesis. The study was divided into 2 experiments.

In experiment 1 (metabolism trial), 6 male growing Thai-indigenous beef cattle with body weight (BW) of 154±13.2 kg were randomly assigned in replicated 3×3 Latin square design. Crude protein levels in total mixed ration (TMR) diets were 4.3%, 7.3%, and 10.3% based on dry matter (DM). Dry matter intake (DMI), ruminal ammonia nitrogen (NH_3-N), and blood urea nitrogen (BUN) concentrations increased linearly ($P<0.01$) with increasing CP levels. There were no significant differences in terms of ($P>0.05$) digestibility of DM, organic matter (OM), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Rumen pH, total volatile fatty acid (VFA), ruminal microbe counts, and bacterial populations were not affected by CP levels ($P>0.05$). However, nitrogen (N) intake, urinary N excretion, N absorption,

N retained (g/d, % of N intake), total purine derivative (PD, mmol/d and mmol/d/kg $BW^{0.75}$) and, microbial nitrogen synthesis (MNS; g/d and g/d/kg $BW^{0.75}$) increased linearly ($P<0.05$) with increasing dietary CP levels. The CP requirement for maintenance of growing Thai-indigenous beef cattle was 3.54 g/kg $BW^{0.75}$.

In experiment 2 (feeding trial), eighteen growing Thai-indigenous beef cattle were used in 2×3 factorial of randomized complete block design. There were 6 dietary treatments which contained 2 levels of CP (10% and 12% CP) and 3 levels of UIP (15%, 25% and 35% UIP). Dry matter intake, average daily gain (ADG), digestibility of DM, OM, ADF and NDF, ruminal pH, total VFA, microbe counts, and MNS were not different ($P>0.05$) between the cattle fed with 10% and 12% CP. However, N intake and urinary N excretion of 12% CP were greater ($P<0.05$) than 10% CP. On the other hand, the digestibility of DM, OM, ADF and NDF, ruminal pH, total VFA, ruminal microbes counts, and MNS were not affected ($P>0.05$) by UIP levels. Dry matter intake, ADG, N intake, fecal N excretion (g/d), and N retained (g/d, % of N intake) increased linearly ($P<0.01$) with increasing UIP levels. While, NH_3-N and BUN decreased quadratically ($P<0.05$) with increasing UIP levels. However, interaction of UIP × CP was not observed in this study except BUN at 0 hour. Metabolizable requirement protein of growth for 1 g/kg $BW^{0.75}$ gain is 0.34 g/kg $BW^{0.75}$.

Based on the two experiments conducted, it could be concluded that the 10% dietary CP was enough to meet the protein requirement for the growth of Thai-indigenous beef cattle. Moreover, 6.5 % DIP of dietary DM can provide

adequate N source for the requirement of ruminal microbes growth while the optimal ratio of UIP to DIP was 35: 65.

School of Animal Production Technology

Academic Year 2010

Student's Signature_____

Advisor's Signature_____

Co-advisor's Signature_____

Co-advisor's Signature_____

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Shengchang Chen

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CHAPTER I

INTRODUCTION

1.1 Rationale of this study

Livestock industry is an important and integral component of the agricultural sector in Thailand. There were 4,635,741 beef cattle in 1999, the major breed of beef cattle is still the indigenous breed, which comprised approximately 65% of total amount of beef cattle. Others breeds were purebred and crossbred which accounted for 1% and 33%, respectively (Tongthainan, 2001). Since then, the number of beef cattle was increasing gradually. According to the statistics of the Department of Livestock Development, the population of total beef cattle was 8,000,000 heads and approximately 5,600,000 heads (70%) was Thai-indigenous beef cattle (DLD, 2006). Due to the mad cow disease impacted on beef production in Europe, the greater demand for beef in the international market means a great potential opportunity for Thailand's beef cattle production. However, while beef cattle production has been accorded emphasis and priority by the Royal Thai government in its development plans, problems remain to be solved.

Nutritional recommendations provided to producers for growing and finishing beef cattle are generally the result of academic research ventures, economic considerations and personal experience. Today's beef cattle producers continually search for methods to optimize the economic performance of their enterprise.

The balance between protein and energy is first-line in beef cattle growth and production. If protein supplement is insufficient, the growth will be limited. On the other hand, excessive protein supplement means a waste of some protein. Protein is likely to be the most expensive component of any beef cattle diet on a basic unit. Therefore, it is important to know how the cattle use protein and to know the requirement of dietary protein.

Protein requirement may be different in term of animal breed, management, environment condition, protein quality, sex and daily gain (NRC, 1996). In addition, it has been recognized that whether animals are castrates or intact males or female influences growth of body tissues, carcass composition and efficiency of gain (Berg and Butterfield, 1976). Thus, how to estimate accurately the protein requirement for cattle is very important. Furthermore, Thailand is a tropical country with different climates and environment conditions from other countries. The nutrient requirements recommended by NRC and ARC are widely adopted to formulate diets around the world. Nevertheless, since the nutrient requirement equations were based on *Bos taurus* cattle, the nutrient requirements in growing Thai-indigenous bulls may not be the same as those recommended.

Good feeding management is efficient feeding management. If we want to obtain the maximal economic gains in Thai-indigenous beef cattle production, we should know the protein requirement of growing Thai-indigenous bulls. Moreover, the relative requirements of degradable intake protein (DIP) and undegradable intake protein (UIP) are poorly defined (NRC, 1996), and research is needed to gain a

greater understanding of microbial efficiency with various forage types and qualities (Lardy, Adams, Klopfenstein, and Patterson, 2004) and how it is affected by DIP and UIP supplementation. So we desire to get some precise basic data on the crude protein requirement for Thai-indigenous beef cattle through this experiment to erect the Thai-indigenous beef cattle protein requirement system.

1.2 Research objectives

1.2.1 To determine protein requirement for maintenance in growing Thai-indigenous beef cattle fed with rice straw as roughage.

1.2.2 To study the effects of crude protein concentration on nutrient digestibility, feed intake, ruminal fermentation, nitrogen balance, and performance of Thai-indigenous beef cattle.

1.2.3 To study effects of different ratio of UIP and DIP on performance, nutrient digestibility, ruminal fermentation, nitrogen balance and microbial nitrogen synthesis of Thai-indigenous beef cattle.

1.2.4 To estimate the protein requirement for body weight gain of growing Thai-indigenous beef cattle.

1.3 Research hypothesis

1.3.1 The crude protein requirement for Thai-indigenous beef cattle is lower than as recommended by NRC (1996).

1.3.2 The optimal proportion of UIP and DIP is different from recommendation by Haddad, Mahmoud and Talfaha (2005) and Sultan, Javaid, Nadeem, Akhtar, and Mustafa (2009).

1.4 Scope and limitation of the study

This study focuses on protein requirement for growing male Thai-indigenous beef cattle. Some cattle were bought from the market, and the others borrowed from a farm in Udon Thani province. The feedstuff of soybean meal, cassava meal, cassava chip, molasses, rice straw, urea, minerals and vitamins premix were bought from Suranaree University of Technology (SUT) feedstuff factory. The experiment was done in the SUT dairy farm.

1.5 Expected results

1.5.1 To obtain accurate data on Thai-indigenous cattle protein requirement through this study; to indicate the relationship of the project proposal to the main subject of the research program on “Establishment of feeding standard of beef cattle and feedstuff data in Indochina” (example: Nutritive values data base, cattle nutrients requirement database or other past/current research). Result from the experiment can be included in Table of Protein Requirement of Thai Feeding Standard.

1.5.2 The protein requirement of growing Thai-indigenous beef cattle is lower than as recommended by NRC (1996).

1.5.3 To indicate the optimal ratio of UIP and DIP in Thai-indigenous beef cattle.

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CHAPTER II

LITERATURE REVIEW

2.1 The beef cattle protein requirement

2.1.1 The definition of protein

According to Parish (2009) defining, protein in beef cattle diets is commonly expressed as crude protein (Figure 2.1). It is comprised both of true protein and non-protein nitrogen, equal nitrogen value in feed by 6.25 times, because proteins typically contain 16 percent nitrogen (1/0.16 = 6.25). True protein is sometimes called “natural protein”. It is either degradable or undegradable in the rumen. Ruminally degradable protein (RDP) is broken down in the rumen and is also referred to as degradable intake protein (DIP). Ruminally undegradable protein (RUP) is protein that is not broken down in the rumen but is potentially degradable in the small intestine. It is sometimes called undegradable intake protein (UIP) or rumen bypass protein. A minimum amount of DIP is needed in the diet to support microbial growth. Otherwise the intake and digestibility of the diet will be limited. Crude protein is the sum of UIP and DIP. Metabolizable protein (MP) is true protein absorbed by the intestine. It is made up of microbial protein and UIP.

2.1.2 The protein requirement of beef cattle

The beef cattle protein requirement includes maintenance requirement and growth requirement. NRC requirements (1985) for MP were base on the factorial method, the factorial included were metabolic fecal losses, urinary losses, growth,

fetal growth and milk. Metabolic fecal, urinary and scurf losses represent the requirement needed for maintenance.

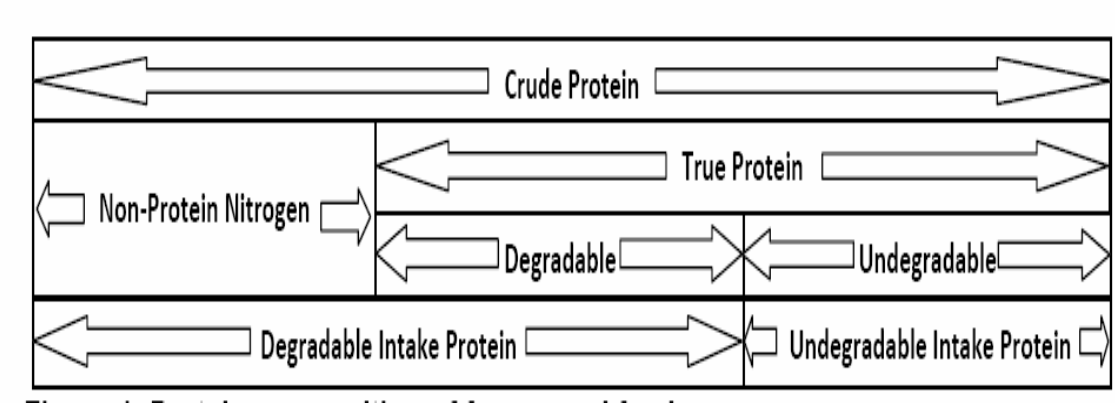

Figure 2.1 Protein composition of forage and feed (adopted from Parish, 2009)

There are variable protein requirements with different factors, such as breed, sex, body weight, daily gain, body condition, production functions, compensatory growth, environmental variation, forage availability and microbial yield (NRC, 1996). Three breed classes, early, medium and late maturing were recommended by ARC (1980), when defining the composition of the gains made by growing and finishing cattle, because of the large variation in energy and protein contents in the liveweight gain at different liveweight. They also recommended additional adjustments (±20%-30%) according to the sex of the animal, whether male, castrate or female. The nitrogen (N) requirements for feedlot cattle change during the finishing period, being greater during the initial part and diminishing during the later stages of finishing. The percentage of maintaining protein required in the diet is higher for young growing animals and declines gradually to maturity. Consequently, N is underfed early and overfed late in the feeding period (Klopfenstein and Erickson, 2002).

Nutrient Requirements of Beef Cattle (NRC, 1970), recommended CP levels were determined from practical experience with CP levels that provided suitable responses. Generally, crude protein is a gross measure of the N contained in a feedstuff. Nutrient Requirements of Beef Cattle (NRC, 1984) expressed protein requirements in terms of CP. However, that did not think about rumen degradation and re-synthesis of protein for use by the animal. In 1985, the Subcommittee on Nitrogen Usage in Ruminants (NRC, 1985) presented an excellent rationale for expressing protein requirements in terms of absorbed protein, a rationale adopted in 1989 by the Subcommittee on Dairy Cattle Nutrition (NRC, 1989). Since then absorbed protein (AP) has become synonymous with MP, a system that accounts for rumen degradation of protein and separates requirements into the needs of microorganisms and the needs of the animal.

A newer concept was described for meeting the MP requirement of beef cow (NRC, 1996). The concept of DIP is defined as that portion of consumed protein which digested in the rumen and utilized for microbial protein synthesis. UIP is that portion of protein which escapes this rumen degradation and passes on to the small intestine where it is absorbed and used to meet requirements for muscle growth and milk production. Metabolizable protein is supplied by microbial protein synthesized in the rumen and ruminally undegraded feed protein. Protein evaluation systems for beef (NRC, 1996) and dairy cattle (NRC, 2001) recognize that the intestinal digestibilities of proteins may differ by source. Before the 2001 revision of the dairy NRC, a constant digestibility of 80% was used for the UIP of all feedstuffs. However, the dairy NRC (2001) now used variable digestibility from 50 to 100%.

The Institute National de la Recherche Agronomique (INRA, 1988) suggested that the protein requirement for maintenance was 5.07 g CP/kg $BW^{0.75}$ or 3.25 g MP/kg $BW^{0.75}$. ARC (1980) recommended that the protein requirement for maintenance of British breed cattle was 4.42 g CP/kg $BW^{0.75}$. Wilkerson, Klopfenstein, Britton, Stock, and Miller et al. (1993) and NRC (1996) recommended that the protein requirement for maintenance of 253 kg growing calves was 5.94 g CP/kg $BW^{0.75}$ or 3.8 g MP/kg $BW^{0.75}$.

2.1.3 Evaluation of the Metabolizable Protein System (NRC, 1996)

Shrunk BW data (BWx0.96) were used to evaluate the MP system (NRC, 1996). Equations from the MP system (NRC, 1996) were used to determine animal and microbial requirements associated with actual performance. In addition, total digestible nutrients (TDN) intake, degraded intake protein (DIP), and undegraded intake protein (UIP) were determined from NRC (1996) ingredient profiles and actual DMI. Microbial CP synthesis (MCP) was calculated from dietary TDN concentration and was assumed to be 80% true protein and 80% digestible, therefore, bacterial MP (MPbac) derived from MCP was calculated as MCPx0.64 (NRC, 1996). Metabolizable protein arising from UIP (MP feed) is assumed to be 80% digestible; therefore, an adjustment factor of 0.80 was applied to UIP supplied by the diet. Total MP supplied to the animal is the sum of MPbac and MPfeed (NRC, 1996).

2.2 Effect of crude protein concentration on the performance

Crude protein (CP) supplementation increases performance variables that are commonly measured with cattle consuming high-concentrate diets (Huntington, Poore, Hopkins, and Spears, 2001), dairy cows consuming fermentable forage diets

(Klusmeyer, McCarthy, and Clark, 1990) and steers grazing low-quality forage (Bandyk, Cochran, Wickersham, Titgemeyer, Farmer, and Higgins, 2001). The optimal CP concentration means the point at which performance was maximized and protein wastage was minimized, the optimal CP concentration maybe correlate with beef cattle breed, protein sources and quality, basis of roughage and beef cattle growth periods. Although early data indicated the importance of protein in terms of maintenance and production status, as well as chronological age (NRC, 1924), optimizing CP levels is still a focus of modern research. Thomson, Preston, and Bartle (1995) suggested that the optimum CP level was between 12% and 13% of DM. Galyean and Gleghom (2001) reported that the mean CP level formulated by consulting nutritionists in the major cattle feeding areas of the U.S. was 13.3% of DM. The NRC (2001) suggests that the minimum requirement for DIP is 6.8% of dietary DM. Cole, Greene, McCollum, Montgomery, and McBride (2003) demonstrated steers fed the 14% CP diet tended ($P<0.1$) to have greater ADG and gain: feed (G:F) than steers fed the 12% CP diet. Gleghorn, Elam, Galyean, Duff, Cole, and Rivera (2004) and Cole, Clark, Todd, Richardson, Gueye, Greene, and McBride (2005) suggested that dietary CP concentration requirements of beef cattle for maximum rate of gain were approximately 11.5% of diet DM during the later stages of feeding. In contrast, Vasconcelos, Greene, Cole, Brown, and McCollum (2006) reported the performance was not affected significantly ($P>0.05$) in steer fed 10.0%, 11.5% and 13.0% CP of DM.

Changes in feed processing and application of implant practices have influenced the determination of CP levels in beef cattle diets. Carbohydrate digestion in the rumen is the most accurate predictor of microbial protein synthesis (Russell,

1992), and as feedstuffs are more thoroughly processed, an increased need of dietary CP is necessary (Cooper, Milton, Klopfenstein, and Jordan, 2002). In concert with increased grain processing, implants, by increasing protein accretion by the animal, have created a need for higher dietary CP. Moreover, by decreasing maintenance MP requirements, implants allow cattle to respond to increased dietary CP levels (DiCostanzo and Zehnder, 1999). Protein level and implant interactions were reviewed by Galyean (1996). Summaries of two trials found that implant effects were independent of dietary CP effects and included faster ADG with higher intakes, the ranking of implant strategy for ADG was high>medium>none (Galyean, 1996). Furthermore, increasing dietary CP level increased DMI and ADG. Similarly, another review of literature showed similar results to increased CP levels in cattle diets (DiCostanzo and Zehnder, 1999). Gleghorn et al. (2004) reported CP concentration above 13% seemed detrimental to ADG and HCW. With dry-rolled corn-based diets, Erickson, Klopfenstein, Milton, and Herold (1999), Cooper, Milton, and Klopfenstein (2000), and Trenkle (2002) reported no adverse effects on cattle performance when dietary CP concentrations were decreased during the later stages of the feeding period.

Modifying feedlot diets by decreasing CP while maintaining animal performance may be the most practical method to reduce N output (Todd, Cole, and Clark, 2006). Cole et al. (2005) suggest that modest changes in dietary CP concentration in the latter portion of the feeding period may have relatively small effects on overall beef cattle performance, but that decreasing dietary CP to 10% of DM would adversely affect performance of cattle fed high concentrate, steam-flaked, corn-based diets.

2.3 Protein Source in cattle diets

Supplemental protein sources vary widely in different countries and regions, and their usage depends largely on source availability and economic efficiency. Protein supplements are arbitrarily defined as having at least 20% crude protein, it includes animal protein sources, plant protein sources and unconventional protein sources. Animal proteins are from meat meal, fish meal and other animal protein. Plant protein sources include soybean meal, cottonseed meal and rapeseed meal, and so on. Most protein sources contribute other nutrients as well and many particularly plant proteins, contain deleterious or toxic factors. Soybean meal is the most important protein supplement for livestock in the worlds. Raw soybean is toxic to most animals, the toxins including protease inhibitors, lectins, phytoestrogens, saponins, goitrogens and etc. Cottonseed meal is the second most important plant protein supplement used in United States, the main toxin is gossypol. Rapeseed meal is an important protein source in China, India, Western Europe, and Canada, the toxins includes erucic acid and glucosinolates. The others protein sources are from linseed meal, peanut meal, sunflower meal, sesame meal, coconut meal and palm kernel meal. Non-protein nitrogen includes amino acids, peptides, amines, amides, nitrates, alkaloids and etc.

The ruminant has the unique ability to ferment feedstuffs before their entry in the lower gastrointestinal tract. Because of microbial fermentation in the rumen, ruminant can utilize non-protein nitrogen (NPN) source to satisfy part of their protein requirement. Oltjen (1969) reported that beef cattle can also survive and grow on all NPN diets through this of amino acid synthesis by ruminal microflora. Microbial protein formed from NPN compounds has a high nutritive value. Lambs and caves fed NPN and little or no protein grow well (Loosli, Williams, Thomas, Ferris, and Maynard,

1949). However, it is impossible to achieve maximum production with animals fed diets with only NPN as N sources because microbial synthesis of limiting amino acids is insufficient to meet the needs for production if muscle protein and milk in genetically superior animals.

The rumen microbes can utilize degraded protein and non-protein N sources. Ruminal microbes are unique in their ability to synthesis high quality proteins for use by the animal from relatively low quality feedstuffs, as long as they have an adequate supply of N, and a source of energy. Thus, ammonia, intact protein, and amino acids are sources of N available to the ruminal microflora (Russell et al., 1992). Microorganisms that ferment cellulose and hemicellulose grow slowly and utilize ammonia as an N source. However, starch-fermenting microorganisms grow more rapidly and can utilize ammonia or amino acids as their N source (Russell et al., 1992). Thus, it is evident that protein degradability must complement the fermentable carbohydrate source provided. Urea is commonly used as a source of DIP, and it is considered 100% ruminally degraded (NRC, 1996). The circumstances under dietary urea are not useful when the rumen contains an adequate quantity of fermentable nitrogen for maximum microbial growth. Urea is nontoxic but can cause ammonia toxicity, when the level of urea in the diet is excessive, the amount of ammonia absorbed can overwhelm the liver's capacity to detoxify it.

Milton, Brandt, and Titgemeyer (1997b) reported that supplementation with urea to achieve two CP levels (9% and 13%) did not improve ADG, but supplementation with soybean meal to achieve the same CP levels increased ADG as CP level increased. A shortage of MP has been indicated as the main cause for decreased performance during the early feeding stages (Sindt, Stock, Klopfenstein, and Shain, 1993; Milton et

al., 1997b) when ADG is more rapid than during later feeding stages, and the effects may be further accentuated when degraded true protein sources are fed rather than undegraded protein sources (Ludden, Jones, Cecava, and Hendrix, 1995). Microbial protein synthesis was maximized with urea in a high-concentrate diet (Devant, Ferret, Calsamiglia, Casals, and Gasa, 2001), followed in succession by the more ruminally degraded protein supplements. However, maximum concentrations of ruminal NH_3 may exceed microbial requirements, so that ruminal NH_3 is not limiting to microbial protein synthesis (Klusmeyer et al., 1990, Milton, Brandt, and Titgemeyer, 1997a). Nonetheless, optimum ruminal OM and starch digestion was achieved when ruminal NH_3 exceeded concentrations necessary to meet microbial needs. Furthermore, ruminal N digestion increased linearly with dietary urea, whereas digestion of non-urea feed N did no differ among supplemental CP sources containing varying levels of urea (Milton et al., 1997a). Thomson et al. (1995) reported daily gain, DMI, and gain: feed (G: F) increased linearly with increasing CP level, but CP source did not affect performance, source of CP also had minimal effects on carcass characteristics in finishing beef steers fed a 90% concentrate diet with 11%, 12%, or 13% CP and four sources of supplemental CP included a blood meal, corn gluten meal mix, cottonseed meal, soybean meal and urea. Gleghorn et al. (2004) used 100% urea, 50: 50 blend of urea and cottonseed meal and 100% cottonseed meal as three sources of supplemental CP (N basis) to experiment indicated crude protein concentration and source interactions were not observed difference for performance and carcass data, dry matter intake was not affected by source, increasing the proportion of supplemental urea increased carcass-adjusted ADG and G: F. Although providing supplemental CP increases performance by growing/finishing beef cattle, responses to CP concentration can vary with CP source (Huntington et al., 2001).

2.4 The requirement of DIP and UIP

Before MP system (NRC, 1996), CP requirements were based on the incorrect assumption that ruminal degradation of CP is constant across all feed sources. Natural feed sources vary in their level of ruminal degradability, whereas most non-protein N sources are essentially 100% degraded. Animal performance data suggest that the proper ratio of degraded: undegraded CP (DIP: UIP) should be got maximize performance (Stock, Merchen, Klopfenstein, and Poos, 1981; Milton et al., 1997a, b). Research has indicated that a balance of DIP and UIP is necessary for maximum productivity (Shain, Stock, Klopfenstein, and Herold, 1998). However, this balance depends on various factors, including body weight, implant status, feed source, and growth rate.

Providing adequate DIP is necessary for maximum microbial CP synthesis, which depends largely on carbohydrate digestion in the rumen (Russell et al., 1992). Thus, requirements for DIP should be greatest with high-grain diets that are based on extensively processed starch. Inclusion of DIP in such diets may alleviate ruminal ammonia shortages so that a loss of microbial yield does not occur.

Unique features of ruminant N digestion and metabolism require not only consideration of the tissue protein and amino acid needs of the ruminant animal, but also the N metabolism and requirements of the microbial population inhabiting the digestive tract, particularly the rumen (Galyean et al., 1996). Milton and Brandt (1994) compared urea and soybean meal as supplemental CP sources in 11.5% and 13.5% CP diets of steers implanted with Revalor S. A fifth dietary treatment of 13.5% CP with supplemental CP from cottonseed meal also was included in the experiment. Increasing urea to supply 13.5% CP tended to decrease DMI and decreased daily gain,

whereas increasing soybean meal to supply 11.5% Vs 13.5% CP increased gain. Cottonseed meal resulted in performance similar to that attained with soybean meal.

Koster, Cochran, Titgemeyer, Varizant, Abdelgadir, and Jean (1996) indicated that urea may be included as supplemental DIP at levels less than 75% of supplemental CP, without adversely affecting true ruminal and total tract OM digestion. The NRC (2000) suggests that the minimum requirement for DIP is 6.8% of dietary DM. According to NRC (2000), the 10% CP diet was calculated to be moderately deficient in CP and DIP, the 12% CP diet was calculated to be borderline deficient to adequate in CP and DIP, and the 14% CP diet was calculated to be adequate to slightly excessive in CP and DIP (Cole et al., 2003).

The relative requirements of grazing beef cows for DIP and MP are poorly defined, and research is needed to gain a greater understanding of microbial efficiency with various forage types and qualities and how it is affected by DIP and UIP supplementation (Reed, Lardy, Bauer, Gilbery, and Caton, 2007). Nutritional and physiological status can be characterized using blood metabolites and hormones and may be modified by protein supplementation (Cheema, Galyean, Caton, and Freeman, 1991; Wiley, Petersen, Ansotegui, and Bellows, 1991; Sletmoen-Olson, Caton, Olson, Redmer, Kirsch, and Reynolds, 2000). Owens, Zinn, and Kim (1986) reported ruminants fed high concentrate diets would probably have greater ruminal and postruminal fermentation than those fed low-quality roughage diets. Increasing ruminally available energy (starch) should increase the synthesis and efficiency of bacterial CP in the rumen and therefore increase the dietary degradable intake protein (DIP) requirement (NRC, 1996). But consumption of high-grain diets in large amounts and at rapid rates may increase the risk of subacute acidosis, intake variation,

or digestive disorders (Fulton, Klopfenstein, and Britton, 1979; Stock, Sindt, Parrott, and Goedeken, 1990).

Recent research indicated that the optimum level of supplemental DIP varies with degree of corn processing. Cooper et al. (2002) compared with three levels of processing, steam flaking, dry rolling, and high moisture harvesting and grinding, which were fed in conjunction with varying levels of urea as the source of supplemental CP, the results showed that optimum levels of DIP were 6.3%, 10.1%, and 8.3% (DM) for dryrolled, high moisture, and steam-flaked diets, respectively.

2.5 Effects of DIP and UIP supplementation on nutrient intake, digestion, rumen fermentation, nitrogen metabolism in ruminants fed low quality forages

Protein is one of the limiting nutrients in the diet of ruminant, ruminants growth require metabolizable protein for tissue synthesis. The metabolizable protein is supplied primarily by combinations of DIP and UIP. Degradable intake protein is required for ruminal microbial growth, this not only improves the ruminal fermentation but it also ensures an adequate supply of microbial protein to the host animal, however, microbial protein is unable to meet MP requirement of rapidly growing calves and supplementation of UIP should be added to ruminant diet to enhance the performance.

Because low-quality forages (<7% CP) are often limiting in protein, a positive relationship maybe exist between DIP supplementation and forage utilization. When dietary DIP is inadequate, the animal can sustain an adequate ruminal N supply through recycling of blood urea-N. The NRC (1985) suggests that if recycled N makes

up a large proportion of the total supply of DIP, the long-term protein needs of the animal may be underestimated, resulting in decreased production. To counterbalance this effect, we should provide the animal with additional UIP, it will not only provide additional MP for tissues deposition, but a portion of that UIP will serve as a source of N for endogenous recycling.

Imbalance of DIP and UIP in ruminant diet can compromise the microbial protein synthesis, ruminal digestion and protein availability to the animals (Santos, Theuber, and Huber, 1998; Reynal and Broderick, 2005). High level of dietary DIP causes excessive ammonia production in rumen which ultimately results in increased blood urea nitrogen (BUN) concentration (Butler, 1998; Dhali, Mishra, Mehla, and Sirohi, 2006; Javaid, Mahr un Nisa, and Shahzad, 2008). Therefore, the information on the optimal ratio of DIP and UIP in the diet for optimal growth in ruminant and how it is affected by DIP and UIP supplementation is very important. Different levels of DIP, UIP supplementation and different ratio of DIP to UIP maybe affect nutrient intake, digestion, N metabolism and performance in ruminants.

The effects of different level of DIP and UIP on nutrient intake, nutrient digestibility, ruminal pH, ruminal NH_3-N concentration, BUN, microbial count and N utilization were reviewed as following.

2.5.1 Dry matter in take (DMI)

Kalscheur, Baldwin, VI, Glenn, and Kohn (2006) observed the effect of different level of DIP and reported increased DMI in dairy cows with increased level of DIP. The DMI was 20.5, 21.0, 21.2 and 21.4 kg/d in cows fed 6.8%, 8.2%, 9.6% and 11% DIP of DM, respectively. Similary, Arroquy, Cochran, Villarreal, Wickersham, Llewellyn, and Titgemeyer (2004) reported that DMI increased with

increasing supplemental DIP ($P<0.05$). Ruminally degradable of dietary protein is vital significance as far as ruminal microbial activity and proliferation is concerned which can alter the nutrient digestion and nutrient intake. Provision of adequate amount of DIP ensures optimum microbial activity and proliferation which increases DM digestibility and intake (Westwood, Lean, Garvin, and Wynn, 2000). Similar results have been reported by Erdman and Vandersall (1983). However, DMI was not affected ($P>0.05$) with increasing DIP or UIP has been reported by some researchers (Sahlu Fernandez, Jia, Akinsoyinu, Hart, and The, 1993; Castillo et al., 2001; Bach, Huntingtun, Calsamiglia, and Stern, 2000; Reynal and Broderick, 2005).

Dry matter intake was decreased with increased level of DIP has been reported by other workers (Erb, Garverick, Challahan, Patton, and Monk, 1976, Scott and Hibberd, 1990; Westwood et al., 2000; Arroquy et al., 2004). Wilson, Martz, Campbell, and Becher (1975) reported a gradual decline in DMI with increasing DIP level (urea as DIP source) in cows. They attributed decreased DMI to increasing ruminal NH_3-N and blood urea concentrations due to high dietary DIP.

Kumar, Tiwar, and Kumar (2005) reported DMI increased from 12.89 to 13.20 kg/d when dietary UIP level was increased from 41% to 48% of dietary CP in crossbred cattle. Chaturvedi and Walli (2001) demonstrated 8.3% increase in DMI in early lactating crossbred cows when the UIP level increased from 29% to 43% of CP. Haddad, Mahmoud, and Talfaha (2005) and Kridli, Haddad, and Muwalla (2001) indicated that a linear increase in DMI with increasing the dietary UIP level (18, 27 and 34% of CP) in Awassi ewes. Similarly, Paengkoum, Liang, Jelan, and Basery (2004) examined the effects of different level of UIP (0, 2%, 4% and 6% of CP) in goat, they suggested that DMI increased linearly (729, 791, 818 and 829 g/d) with

increasing the level of UIP. Dry matter intake was improved with increasing the level of UIP in ruminants, because there is high demand of energy for coupling of increased supply of amino acids at cellular level. Increase in DMI with increasing UIP at the expense of DIP might be attributed to low level of ruminal NH_3-N and BUN concentrations due to decreased protein degradation.

In contrast, Henson, Schirigoethe, and Maiga (1997) indicated that feed intake was reduced (22.9 to 21.2 kg/d) when cows were fed high UIP (33.7% to 45.4% of CP), the plausible explanation of reduced DMI in their study might he attributed to animal protein sources which were used to increase the UIP levels which are usually unpalatable. Furthermore, DMI decreased with animal protein source might be due to low level of ruminal NH_3-N which might have reduced ruminal microbial proliferation, fermentation, digestibility and thus reduced nutrient intake (Faverdin, Bareille, and Verite, 1999). Moreover, reduced DMI due to reduced ruminal fermentation and microbial yield in ruminant cased by very low DIP level has been reported (Hume, Moir, and Somers, 1970; Orskov, 1992).

Sultan, Javaid, Nadeem, Akhtar, and Mustafa (2009) determined the effect of varying ratio of UIP to DIP (30: 70, 35: 65, 40: 60, 45: 55) on nutrient intake, they reported that a linear increase ($P<0.01$) in DMI, CP intake and NDF intake observed with increasing the UIP to DIP ratio in buffalo. However, Haddad et al. (2005) indicated no significant differences in DMI, CP intake, NDF intake DM and NDF digestibility with decreasing the DIP to UIP ration in lambs.

2.5.2 Digestibility of nutrition

Nutrient digestibility is affected by various factors. Sufficient availability of dietary DIP is of vital significance as far as ruminal microbial activity

and nutrient degradation is concerned. Providing DIP less than rumen microbial requirement affects adversely the ruminal fermentation by decreasing the microbial proliferation which not only reduces the VFA production but also decreases nutrient digestibility.

Fu, Felton, Lehmkuhlcr, and Kerley (2001) reported a linear increase in DM, OM and N digestibilities as the level of DIP increased (3.4%, 6.2%, 8.8% and 11.6% of DM) crossbred steers. Similar results were reported by Griswold, Apgar, Bouton, and Firkin (2003) that DM digestibility increased with increasing the level of dietary DIP in cows. They explained that increased nutrient digestibility with increasing level of DIP might be attributed to increased ruminal NH_3-N concentration which might lead to increase ruminal microbial activity. Increased ruminal microbial activity has been reported to increase nutrient digestibility in ruminants (Perdock, Leng, Bird, Habib, and Van Routed 1988). On the other hand, the nutrient digestibility was not affected by increasing level of DIP has been reported by other studies (Mishra and Rai, 1996, Castilo et al., 2001; Paengkoum et al., 2004). The plausible explanation of unaltered nutrient digestibility with increased DIP level might be attributed to various DIP sources and narrow range of DIP was used by them. Furthermore, Arroquy et al. (2004) reported that digestion of NDF and total tract OM increased linearly ($P<0.01$) with DIP, and tended (quadratic; $P = 0.08$) to plateau at the highest levels of supplemental DIP in beef cattle. This result is similar as Klevesahl et al. (2003) indicated that increasing the level of supplementation DIP from 0 to 0.123% of BW increased intakes of both forage OM and NDF and than decreased from 0.123% to 0.195%, resulting in an overall quadratic response ($P<0.01$) to DIP supplementation.

Kumar et al. (2005) demonstrated the digestibility of DM, CP and NDF increased from 57.3% to 59.1%, 57.3% to 58.2% and 42.8% to 41.6% as the UIP level increased from 41% to 48% of CP in crossbred cows, respectively. Paengkoum et al. (2004) examined the effect of increasing level of dietary UIP on digestibility in goats and reported digestibility of dry matter increased linearly from 53.1%, 53.5%, 53.8% and 54.1% when UIP level was increased from 0, 2%, 4% to 6% of CP, respectively. The CP digestibility also increased (from 50.6%, 54.8%, 54.9% to 54.9%) when the level of UIP was increased (from 0, 2%, 4 %to 6% of CP), respectively.

Sultan et al. (2009) determined the effect of varying ratio of DIP to UIP (70: 30, 65: 35, 60: 40, 55: 45) on nutrition intake, they reported that a linear decrease ($P<0.01$) in digestibility of DM and NDF was observed with increasing the DIP to UIP ratio in buffalo. Similarly, Wankhede and Kalbande (2001) determined the effect of different ratio of DIP and UIP on nutrient digestibility in Red Kandhari calves, they reported that the digestibility of DM, CP and NDF was increased from 48.2% to 58.8%, 57.3% to 63.5% and 55.7% to 62.2%, respectively. With decreasing the DIP from 65% to 55% of CP, similar result has been reported by Pattanaik, Sastry, Katiyar, and Murari Lal (2003). However, Haddad et al. (2005) indicated no significant differences in DM and NDF digestibility with decreasing the DIP to UIP ration in lambs.

Keery and Amos (1993) reported that digestibility of NDF was decreased from 52.8% to 43.6% when dietary UIP was increased from 35% to 44% of CP, respectively. Similarly, Chaturvedi and Walli (2000) reported a reduction in crude fiber digestibility due to increase in UIP level. Decreased fiber digestibility with increasing the UIP might be attributed to lower ruminal NH_3-N concentration which

might have decreased fiber degrading enzyme production by decreasing the ruminal cellutolytic bacterial activity in lactating crossbred cows. Ruminal bacteria require ammonia for growth. Low ruminal NH_3-N has the potential to inhibit microbial activity and decrease rate of fiber digestion.

2.5.3 Ruminal pH

Low ruminal pH is the result of fermentation of large amounts of available organic matter when the quantity of OM fermented increases, ruminal protein synthesis is increases (Hoover and Stokes, 1991). As a result, the negative relationship between pH and bacterial N flow is a consequence of the increased supply of energy with highly fermentable rations (Bach et al., 2005). Fu et al. (2001) determined the effect of different levels of DIP (3.4%, 6.2%, 8.8% and 11.6% of DM) on rumen pH in cannulated crossbred steers and indicated that it was not affected by DIP alteration. Lee, Hwang, and Chiou (2001) investigated the effect of varying DIP level on ruminal pH in goats and reported that rumen pH slightly decreased (6.21, 6.15 and 6,18) with increasing the level of DIP (62%, 64% and 68%) but statistically it remained nonsignificant. Similarly, Arroquy et al. (2004) and Klevesahl et al. (2003) indicated that there is no effects on ruminal pH with increasing DIP levels, but ruminal pH tended ($P<0.07$) to decline linearly in response to increasing level of DIP (Arroquy et al., 2004) in beef cattle fed low quality grass hay. Koster, Cochran, Titgemeyer, Varizant, Abdelgadir, and Jean (1996) found that ruminal pH decreased with increasing infusion of supplemental DIP in fistulated cows. Similarly, Bodine et al. (2000) reported a quadratic decrease in ruminal pH with increasing level of DIP addition to a low quality forage-based diet in either the presence or absence of supplemental corn. Baumann, Lardy, Caton, and Anderson (2004) determined the

digestion characteristics of steers and reported that pH was depressed with addition of DIP to corn base diets. The decline in ruminal pH with increasing DIP level reflected increased ruminal fermentation.

Reed et al. (2007) conducted the effect of pH with different UIP level of DM in steers, the observed When UIP levels from 0.8%, 19.6% to 40.6% of DM, the total pH was increased from 6.21, 6.28 to 6.32, respectively. They indicated that ruminal pH was increased linearly ($P<0.05$) with increasing UIP level of DM, and the pH was lower fed 0.8% UIP of DM diet than 19.6% and 40.6% UIP level of DM, but no different between 19.6% UIP and 40.6% UIP. However, Atkinson, Toone, and Ludden (2007) reported the total ruminal pH was not affected ($P>0.05$) by increasing UIP level in lambs.

2.5.4 Ruminal ammonia nitrogen (NH_3-N)

The DIP is degraded into peptides, amino acids, ammonia and branched chain fatty acids by rumen microorganisms. Recycled urea through saliva is another source of ruminal ammonia. Higher DIP level increases fermentation of diet (Davidson et al., 2003) while decreased ruminal NH_3-N concentration has been reported in cows fed high UIP level (Lee et al., 2001). Low level of ammonia in the rumen depresses bacterial growth that reduces rumen fermentation activities.

Kung, Huber, and Saner (1983) reported that increasing the DIP level in diets increased the NH_3-N concentration in the rumen. Lee et al. (2001) reported that increasing level of dietary DIP (62%, 64% and 68% of CP), NH_3-N concentrations (41.6, 49.8 and 53.1 mg/dL) were increased significantly ($P<0.01$) in lactating goats. Stokes, Hoover, Miller, and Blauweikel (1991) demonstrated that there was a linear increase in ruminal NH_3-N (8.15 and 21.2 mg/dL) with increasing dietary

DIP concentration (49.9%, 64.4% and 73.3% of CP). Increased ruminal NH_3-N with addition of DIP was reported by Baumann et al. (2004). Similarly, Fu et al. (2001) reported that a linear increase in ruminal NH_3-N concentration (2.53, 8.81, 22.96 and 25.68 mg/dL) was observed with increasing the level of DIP (3.4%, 6.2%, 8.8% and 11.6% of DM) in the diet of crossbred steers.

Sarwar, Mahr-un-Nisa, Bhatti, and Ali (1998) reported that ruminal NH_3-N concentration is higher (12.5 mg/dL) in buffaloes than cattle (10.1 mg/dL). Wanapat and Pimpa (1999) reported that ruminal NH_3-N concentration higher than 13.6 mg/dL in swamp buffaloes were considered optimum for DMI, microbial protein synthesis and digestibility. However, in dairy cow for optimum rumen fermentation and microbial yield, the ruminal NH_3-N should be between 10-25 mg/dL (Leng, 1990; Orskov, 1992). Moreover, Satter and Slyter (1974) reported that microbial protein production increased with increasing NH_3-N concentration and then reduced when ruminal NH_3-N reached 5 mg/dL in ruminal fluid. Maximum microbial protein flow from the rumen required ammonia concentration was 11 mg/dL (Balcells, Guada, Castrillo, and Gasa, 1993).

2.5.5 Volatile Fatty Acid (VFA)

Arroquy et al. (2004) determined the ruminal fermentation with different DIP supplementation concentration in beef cattle, they demonstrated when DIP supplementation from 0.15 g/kg, 0.87 g/kg to 1.59 g/kg of BW, the total VFA was increased from 50.2, 77.2 to 85 mM, concentration of ruminal VFA increased ($P<0.01$) linearly and quadraticly with increasing DIP supplementation. Similar results was observed by, Klevesahl et al. (2003). Wickersham, Titgemeyer, Cochran, Wickersham, and Gnad (2008) reported the total VFA was increased from 52.2, 62.2

to 69.8 when DIP supplementation was increased from 59, 118 to 177 mg/kg of BW, and it was increased linearly ($P<0.01$) with increasing DIP supplementation.

Reed et al. (2007) conducted the effect of VFA with different UIP level of DM in steers, the observed When UIP levels from 0.8%, 19.6% to 40.6% of DM, the total VFA was unaltered, it was 91.7, 93.9 and 92.1 mM, respectively. Similarly, Atkinson et al. (2007) reported the total VFA was not effect ($P>0.05$) by increasing UIP level in lambs.

2.5.6 Blood urea nitrogen (BUN)

Blood urea nitrogen (BUN) is an indicator of overall N status of ruminant. Increased BUN concentrations with increased dietary DIP probably can be explained by increased absorption of ruminal ammonia, resulting in higher ammonia being detoxified in the liver to form urea. A second source of urea produced by the liver is from the deamination and metabolism of amino acids. Circulating amino acids originate from UIP, microbial protein and body stores. Urea N circulating in the bloodstream are measured in either plasma urea N or serum urea N fractions and are often referred as BUN. The BUN due to DIP catabolism peaks about 4 to 6 h after meals where as the metabolism of UIP contributes to BUN continuously throughout the day (Elrod, Van Aniburgh, and Butler, 1993).

Increased BUN with increasing dietary DIP has been reported by Roseler, Ferguson, Sniffen, and Herrema (1993) and Higginbotham, Huber, and Walientine (1989). Increase in dietary DIP lead to increase BUN by increasing the concentration of ruminal NH_3-N (Dhiman and Satter, 1997). Wanapat and Pimpa (1999) found a linear increase in BUN (13.0, 17.8, 23.4, 29.3 and 39.3 mg/dL) with increased ruminal NH_3-N concentration (7.1, 8.8, 13.6, 17.6 and 34.4 rng/dL) in

swamp buffaloes. Vertanen (1966) reported higher BUN in cattle fed purified diets containing urea than those fed natural diets. The other researchers (Vongsamphan and Wanapat, 2004; Chumpawadee, Sommart, Vongpralub, and Pattarajinda, 2006) reported that an increase in ruminal NH_3-N concentrations increased BUN concentration.

2.5.7 Nitrogen utilization

Wickersham et al. (2008a, b) reported that N intake and retained increased ($P<0.01$) with increasing DIP supplementation. Davidson et al. (2003) investigated the effect of amounts and degradability of protein on N utilization and excretion in cows. They indicated that fecal N excretion was not affected ($P<0.05$) by DIP level, but urinary N was increased with increasing the DIP levels in cows. Kalscheur et al. (2006) reported there was a linear increase in urinary N and fecal N excretion with increasing the DIP level.

On the other hand, increased dietary UIP levels base on same DIP level in ruminant with consuming low quality forages, N intake and digestion linearly increased ($P<0.001$) with increasing levels of UIP in steer (Reed et al., 2007) and in lamb (Atkinson et al., 2007). Moreover, Paengkoum et al. (2004) indicated that N intake was increased from 17.85, 20.0, 20.54 to 20.96 g/d when goats were fed diets containing 0, 2%, 4% and 6% UIP of CP, respectively. A linear decrease in urinary N (4.9, 4.1, 3.7 and 3.5 g/d) was observed. The linear decrease of urine N was due to the ratio of DIP (100%, 98%, 96% and 94% of CP) was decreased and because of increased efficiency of ruminal microbes to capture maximum ruminal NH3-N. They reported that N retention was decreased linearly from 20.4%, 23.7%, 26.6% to 28.0% with increasing UIP from 0, 2%, 4% and 6% UIP, respectively. Similar results were

reported by Pattanaik et al. (2003) who observed that N retention was higher in calves fed high UIP (51% of CP) than those fed low UIP (45% of CP).

Sultan et al. (2009) indicated to decrease the ratio of DIP to UIP resulted increasing linearly ($P<0.01$) in N retention and diet containing DIP to UIP ratio 55:45 is considered optimum regarding N retention in buffalo calves.

2.5.8 Microbial growth and count

Stokes et al. (1991) reported that higher microbial protein production was noticed in cows fed diets containing 11.8% or 13.7% DIP than those fed 9% DIP of DM and explained that higher microbial protein synthesis in cows fed high DIP was due to high concentration of ruminal NH_3-N. Fu et al. (2001) conducted a study on crossbred steers and indicated that bacterial N production increased linearly with increasing dietary DIP level. Similarly, Hoover and Stokes (1991) who indicated that microbial growth increased linearly ($P<0.05$) with increasing ruminal NH_3-N due to increase in DIP content in the diet in an in vitro experiment. Moreover, total bacterial population increased by increasing the ruminal NH_3-N concentration has been reported by Pimpa et al. (1996), Suwanlee and Wanapat (1994) in buffalo. Wanapat and Pimpa (1999) who reported bacterial counts were 1.4×10^8, 1.7×10^8, 2.6×10^8, 3.7×10^8 and 1.5×10^8 cells/mL when ruminal NH_3-N concentrations were 7.1, 8.8, 13.6, 17.6 and 34.4 mg/dL in swamp buffalo, the result showed that the bacterial count increased with increasing NH_3-N concentration up to 17.6 mg/dL. However, it reduced when ruminal NH_3-N concentration was increased to 34.4 mg/dL. Furthermore, Sarwar et al. (1998) reported that the ruminal microbial population is greater in buffalo than cattle.

Meng, Xia, and Kerley (2000) determined the effect of replacing soybean DIP with urea base DIP on protozoal count. They reported that protozoal count was lower (0.4×10^3 cells/mL) when total DIP was supplied from urea compared with when urea base DIP was replaced with 30% or 70% soybean meal (3.0×10^3 or 4.0×10^3 cells/mL) in a continuous culture fermenters. Stokes et al. (1991) conducted a study on lactating Holstein cows to determine the effect of different level of DIP and non-structural carbohydrates on microbial protein production. They reported increased protozoa count (3.2×10^5, 7.4×10^5 and 8.1×10^5 cells/mL) when DIP was increased from 9%, 11.8% to 13.7% of DM, respectively. Wanapat and Pimpa (1999) reported that protozoal count responded quadratically (8.2×10^5, 8.4×10^5, 8.9×10^5, 10.7×10^5 and 6.4×10^5 cells/mL) with increasing NH_3-N concentration (7.l, 8.8, 13.6, 17.6 and 34.4 mg/dL, respectively).

Microbial count was decreased with decreasing ruminal NH_3-N has been reported by several researches (Maeng and Baldwin, 1976; Argyle and Baldwin, 1989). The decreasing microbial count with decreasing dietary DIP level might be attributed to decreased concentrations of ruminal NH_3-N, amino acids, peptides, or branched chain VFA required for microbial growth (Bryant and Robinson, 1962). Bach et al. (2005) reported increased bacterial count with increased ruminal amino acids and peptides concentrations. Dhiman and Satter (1997) reported that 9.3% DIP of DM supplied enough N precursors to support maximum microbial growth in cow. Similarly, 9.6% DIP of DM is considered sufficient to meet the N needs of microbial protein synthesis in the rumen (NRC, 1989). Thus, provision of adequate dietary DIP is essential to maximize microbial protein synthesis in the rumen before the supplementation of UIP.

2.6 The measurement of Acid-Insoluble Ash (AIA) to estimate nutrition digestibility

The traditional measurements of estimating dry matter digestibility was total collection of the feces from animals housed in metabolic pens or use external markers (eg. chromium oxide). The measurement of AIA was used natural markers in diet to determine the dry matter digestibility. Compared with AIA method, the measurement of external markers and total collection were often expensive and labour intensive, especially in grazing condition. Acid-Insoluble Ash has been used to estimate digestibility of diets fed to monogastrics and ruminants (Moughan Smith, Schrama, and Smits, 1991; pigs; Vogtmann, Pfirter, and Prabucki, 1975, poultry; Van Keulen and Young, 1977, sheep; Sunvold, Vanzant and Cochran, 1991, steers). Thonney, Palhof, DeCarlo, Ross, Firth, Quaas (1984) indicated that in all diets studied except those containing very small amounts of naturally occurring AIA there was no significant difference between mean digestibilities determined by total collection or AIA. High grain diets which contain only small amounts of naturally occurring AIA showed more variability between the two methods (Thonney et al. 1984).

2.7 The measurement of purine derivative excretion (PD) to estimate microbial protein synthesis

Ruminant can convert DIP into microbial protein by ruminally microbial to support the protein requirement for maintenace and growth. Therefore, how to predict the microbial protein synthesis accurately is very important. Six methods were often used in ruminant nutrition research as flowing (Orskov, 1992): (a) use of a protein free purified diet; (b) use of diamino pimelic acid; (c) duodenal nucleic acid as a marker

for microbial N; (d) adopt radioisotopes ^{35}S or ^{15}N or ^{32}P for determination of microbial N; (e) amino acid profile in postruminal digest; (f) use of urinary purine derivatives to estimate microbial N supply. The methods generally used for determining microbial protein production depend on the use of natural microbial markers such as RNA (ribonucleic acid) and DAPA (diamino pimelic acid) or of radioisótopos ^{35}S, ^{15}S, ^{32}P. However, those methods need to use rumen cannulated animal and complex procedures to operate. The purine excretion method is simple, non-invasive and does not require surgical preparation of the animal. It has been used widely in many laboratories. This is because ruminant feeds usually have a low purine content, most of which undergo extensive degradation in the rumen as the result of microbial fermentation. Absorbed nucleic acid purines are degraded and excreted in the urine as their derivatives, hypoxanthine, xanthine, uric acid and allantoin (Figure 2.2). The excretion of the PD is directly related to the purine absorption. With the knowledge of the purine-N: total-N ratio in microbial biomass, microbial N absorption can be calculated from the amount of purine absorbed which is estimated from urinary PD excretion. There was a positive relationship between microbial N flow and purine derivative in urine. The amount of microbial N can estimated from purine absorption which determined excretion of purine derivative in urine. However, purine derivative can not be use to predict microbial N flow across different physiological states of animal and species.

The equation of estimating intestinal absorbed purine :

$$\text{(a)}\ Y = 0.85x + 0.15w^{0.75}e - 0.25x \quad \text{(sheep)}$$

$$\text{(b)}\ Y = 0.85x + 0.385w^{0.75} \quad \text{(cattle)}$$

Where Y is purine derivative excretion (PD, mmol/d), x is purine absorption (mmol/d).

The equation of estimating microbial N synthesis:

Microbial $N(g/d) = 70x/0.83 \times 0.116 \times 1000 = 0.727x$ (Yu, Egan, Boon-ek, and Leury, 2002; De boever, lantcheva, Cottyn, De Canpeneere, Fiems, and Boucgque, 1998). The explain of equations:

a) The mean endogenous urinary PD excretion from the degradation of tissue nucleic acids, base on 14 observations with cattle, is 0.385 mmol/d/kg $BW^{0.75}$.

b) The recovery of absorbed purine as urinary PD is assumed to be 85%, with the other 15% being lost via nonrenal routes.

c) The digestibility of microbial purine in intestines a mean value of 0.83 is assumed.

d) The ratio of purine N to total N in mixed rumen microbes is taken as 0.116, assuming no effect of dietary treatment.

e) The N content of purines is 70 mg/mmol.

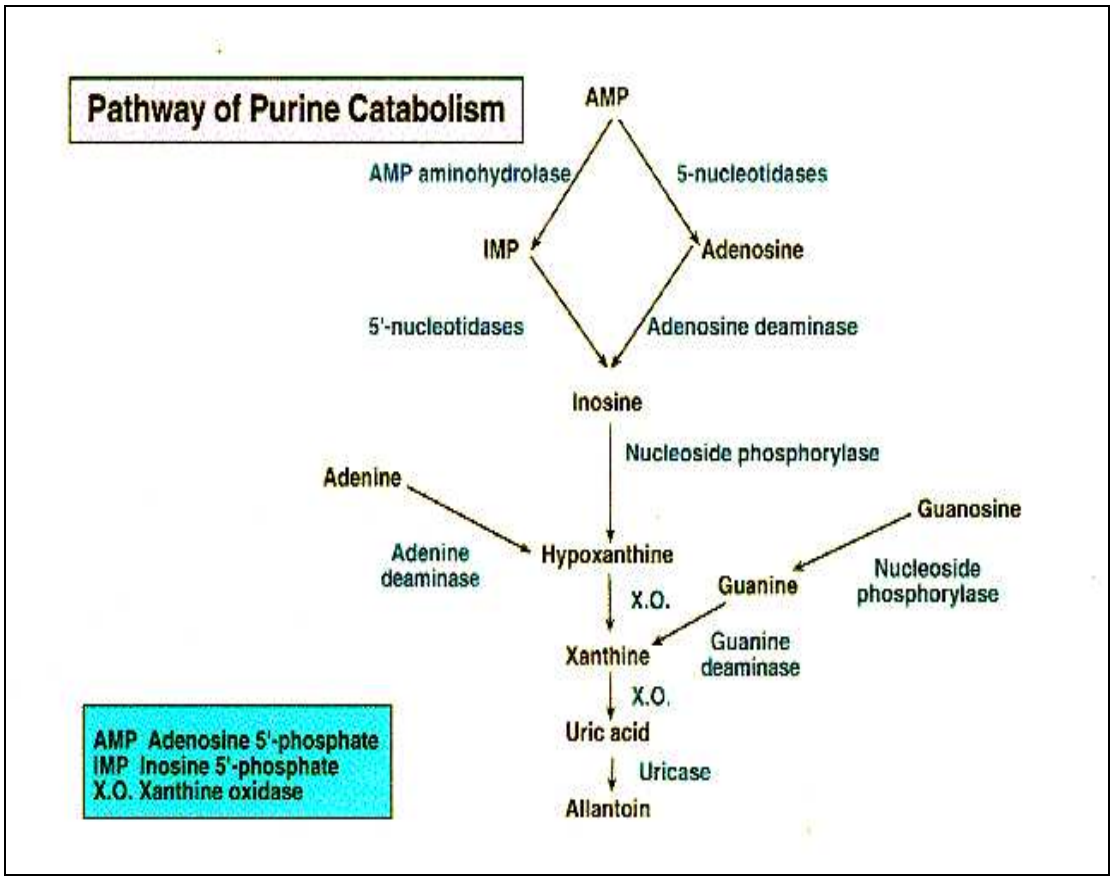

Figure 2.2 Degradation of purine nucleotides and formation of purine derivatives (Chen and Gomes, 1992）

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CHAPTER III

Effects of Dietary Protein on Feed Intake, Digestibility, Ruminal Fermentation, Ruminal Microbes and Nitrogen Utilization of Growing Thai-indigenous Beef Cattle

3.1 Abstract

Six male growing Thai-indigenous beef cattle with body weight (BW) of 154±13.2 kg were randomly assigned in replicated 3×3 Latin square design, and fed with different levels of crude protein (CP) in total mixed ration (TMR) diets. CP levels in diets were 4.3%, 7.3% and 10.3% base on dry matter (DM). Dry matter intake (DMI) increased linearly ($P<0.01$) with increasing CP concentrations. There were not significantly different ($P>0.05$) digestibility of DM, organic matter (OM), acid detergent fiber (ADF) and neutral detergent fiber (NDF). Ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) concentrations increased ($P<0.01$) with increasing CP levels. Moreover, there is a positive relationship between BUN and ruminal NH_3-N. Rumen pH, total volatile fatty acid (VFA), molar proportions of acetate, propionate and butyrate were not affected by CP levels ($P>0.05$). The counts of ruminal protozoa, ruminal fungi and ruminal bacterial, and the populations of cellulolytic bacteria, proteolytic bacteria and amylolytic bacteria were not affected ($P>0.05$) by dietary CP concentrations. Allantoin (mmol/d and mmol/d/kg $BW^{0.75}$), total purine derivative (PD) (mmol/d and mmol/d/kg $BW^{0.75}$), PD absorbed (mmol/d

and mmol/d/ kg $BW^{0.75}$) and microbial nitrogen synthesis (MNS) (g/d and g/d/ kg $BW^{0.75}$) increased linearly ($P<0.05$) with increasing dietary protein concentration. Uric acid (mmol/d and mmol/d/kg $BW^{0.75}$) and creatinine (mmol/d) of urinary excretion were not affected ($P>0.05$) by dietary CP levels. Nitrogen (N) intake, urinary N excretion, N digestibility, N retained (g/d, % of N intake) increased linearly ($P<0.05$) with increasing dietary CP levels. However, the fecal N excretion was not affected ($P>0.05$) by dietary CP levels. The CP requirement for maintenance of growing Thai-indigenous beef cattle was 3.54 g/kg $BW^{0.75}$. The regression equation was estimated by using linear regression of N retained and N intake. The value of N intake was the CP requirement for maintenance when nitrogen retained was zero.

Key Words: Thai-indigenous beef cattle, crude protein, maintenance, nitrogen balance

3.2 Introduction

Protein requirements for livestock are thought to be a function of many variables and not a specific figure for all conditions. There are varying protein requirements with different factors, such as breed, sex, body weight, daily gain, body condition, production functions, compensatory growth, environmental variation, forage availability and microbial yield effects (NRC, 1996). The Institute National de la Recherche Agronomique (INRA, 1988) suggested that protein requirement for maintenance of beef cattle was 5.07 g CP/kg $BW^{0.75}$ or 3.25 g MP/kg $BW^{0.75}$. Wilkerson, Klopfenstein, Britton, Stock, and Miller (1993) and NRC (1996) recommended that the protein requirement for maintenance of 253 kg growing calves was 5.94 g CP/kg $BW^{0.75}$ or 3.8 g MP/kg $BW^{0.75}$. Thai-indigenous beef cattle were

small in frame and low in growth rate. Furthermore, Thailand is a tropical country with different climates and environmental conditions from other countries. The nutrient requirements recommended by NRC and ARC are widely adopted to formulate diets around the world. Nevertheless, the nutrient requirement equations were based on *Bos taurus* cattle, but the nutrient requirements in growing Thai-indigenous bulls may not be the same as those recommended.

Rice straw was the most abundant and appropriate feed for cattle in tropical regions, but it contains poor protein, energy, minerals and vitamins contents. Protein supplementation with urea, cassava pulp and molasses can improve the utilization of low quality roughages through the supply of nitrogen (N) from DIP to rumen microbes. The objective of this study was to determine crude protein for maintenance requirement and effects of protein supplementation of rice straw-based diets on the ruminal fermentation and nitrogen utilization of Thai-indigenous bull calves.

3.3 Research objectives

3.3.1 To predict protein requirement for maintenance in growing Thai-indigenous beef cattle fed with rice straw as roughage.

3.3.2 To study the effects of crude protein concentration on nutrition digestion, feed intake, ruminal fermentation, nitrogen balance, and performance of Thai-indigenous beef cattle.

3.4 Materials and methods

3.4.1 Animals and diets

Six growing Thai-indigenous beef cattle, averaging 154±13.2 kg of body weight (BW) and approximately 18 months of age, were selected and housed in individual pens and then all of the cattle were treated against anthelmintics and intestinal parasites with Ivermectin. The cattle were assigned randomly in replicated 3×3 Latin square design (Table 3.1). Two weeks was the adjustment period before the experiment. Each experimental period consisted of 21 days, of which the last 7 days were the sample collection period, 4 days served as transition between each experimental period. The cattle were fed 7.3% CP diet during adjustment and each adjustment period. The cattle were fed with total mixed ratio (TMR) diets (T1, T2 and T3) which contained 4.3%, 7.3%, and 10.3% crude protein (CP) respectively, with similar amounts of 20% above maintenance metabolizable energy (ME). The ingredients and chemical composition are shown in Table 3.2. TMR diet was fed twice per day at 0830 h and 1530 h, the dry matter intake was estimated according to 2.5% of BW. Orts were weighed and recorded daily prior to the morning feeding to determine daily dry matter intake (DMI).

Table 3.1 The replicated square 3×3 Latin square design and the orders of treatment.

	1 square			**2 square**		
	B1	**B2**	**B3**	**B4**	**B5**	**B6**
P1	T1	T2	T3	T1	T3	T2
P2	T2	T3	T1	T3	T2	T1
P3	T3	T1	T2	T2	T1	T3

P=The period of treatment, B= The number of experiment beef cattle, T=The experiment treatment

Drinking water was not limited. The experimental cattle body weight was measured at both the beginning and the end of each period. At the last week of each experimental period, in order to determinate digestibility and nitrogen balance, samples of orts, fecal and urine were collected before new feed was given each morning.

Table 3.2 Ingredients and chemical composition of experimental diets (%, DM basis).

Ingredient (%, base dry matter of diet)	Dietary crude protein levels		
	4.3%	**7.3%**	**10.3%**
Rice straw	79.7	79.2	79.2
Cassava pulp	14.9	13.5	7.9
Soy bean meal	0.0	3.4	9.6
Molasses	3.0	1.0	0.4
Urea	0.4	0.9	0.9
Dicalcium phosphate	1.0	1.0	1.0
Vitamin–mineral premix[1]	1.0	1.0	1.0
Total	100	100	100
Chemical composition,			
DM	90.0	90.4	90.3
CP	4.3	7.3	10.3
Ash	15.9	15.9	15.6
NDF	59.5	59.1	58.0
ADF	40.3	40.1	39.3
ME (MJ/kg)	7.94	7.93	7.93

1The premix contained per kilogram of DM: 4×10^6IU Vitamin A, 0.4×10^6IU Vitamin D_3, 4000IU Vitamin E, 24 g Fe, 0.2 g Co, 2 g Cu, 10 g Zn, 0.5g I, 50 mg Se. DM = Dry Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, MP = Metabolizable energy.

3.4.2 Sampling

Five percent of daily urine and fecal were collected according to daily urine volume and daily fecal weight. A sample of urine of each animal was acidified with 20% H_2SO_4 to keep the final pH lower than 3. It is essential to acidify the urine to prevent bacterial activity. The sample of urine and fecal were pooled respectively when each sampling period was finished. After that, a 50 ml duplicate sample of urine and a 500 g duplicate sample of fecal were taken and stored at -20°C to determine

nitrogen balance and urinary purine derivatives (PD) excretion. Rumen fluid was sampled at 0 and 4 h after feeding on the last day of each period. The rumen fluid sample was filtered by layers of cheesecloth and measured with a pH meter immediately. Thereafter, 1 ml of the rumen fluid was transferred to a plastic bottle containing 9 ml 10% formalin (V:V = 1:9) as a preserving reagent by pipette for counting ruminal protozoa, fungi and bacteria. The rumen fluid (40 ml) was transferred into a 60 ml plastic bottle containing 10 ml 6 N of HCl for determination of ruminal ammonia-N (NH_3-N) and volatile fatty acids (VFA). At the same time, the rumen fluid was diluted 1 time (10^{-1}), 2 times (10^{-2}), 3 times (10^{-3}), 4 times (10^{-4}), 5 times (10^{-5}), 6 times (10^{-6}), 7 times (10^{-7}) and 8 times (10^{-8}), respectively. After that, 0.2 ml of the 4 times (10^{-4}) and 5 times (10^{-5}) diluted rumen fluid solution was inoculated in proteolytic and amylolytic culture medium, respectively. Zero point five ml of rumen fluid solution which has been diluted 7 times (10^{-7}) and 8 times (10^{-8}) was inoculated in cellulolytic culture medium, respectively. Then, the proteolytic and amylolytic culture medium was incubated at 39°C for 5 days, and the cellulolytic culture medium was incubated at 39°C for 21 days to determine cellulolytic, proteolytic and amylolytic bacteria population according to roll tube technique (Hobson, 1969). Jugular blood was sampled into heparinized vacationer tubes after rumen fluid was collected, and centrifuged at 5000 x g for 15 minutes to separate plasma, which was stored at -20°C for blood urea N (BUN) analysis (Crocker, 1967).

3.4.3 Chemical analysis procedures

The samples of feed, orts and fecal were ground through 1 mm screen and dried in a forced draught oven at 65°C for 48 hours. Dry matter, organic matter and ash, nitrogen balance were determined following standard procedure of AOAC

(1990) and fiber analysis was determined by methods supported by Van Soest, Robertson, and Lewis (1991). Volatile fatty acids (VFA) were determined by Gas Chromatography (GC, Hewlett-Packard GC system HP6890A; Hewlett-Packard Avondale, PA).

3.4.4 Procedures of counting of protozoa, fungi and bacteria

Rumen liquor (1 ml) was pipetted into a 50 ml plastic bottle containing 9 ml 10% formaldehyde for counting the number of protozoa, fungi and bacteria. Counting was done in 10X microscopic ocular, in a haemocytometer counting chamber (Figure 3.1), under an objective microscop of 40X, as described by Kamra et al. (1991).

The number of protozoa was calculated using Giri et al. (2005) formula:

number of protozoa $= \frac{NAD}{av}$, where N is the average number of protozoa/microscopic field, A is the area on slide on which the sample is spread (area of the cavity of haemocytometer), D is the dilution, a is the area of microscopic field and v is the volume of diluted SRL in the counting chamber.

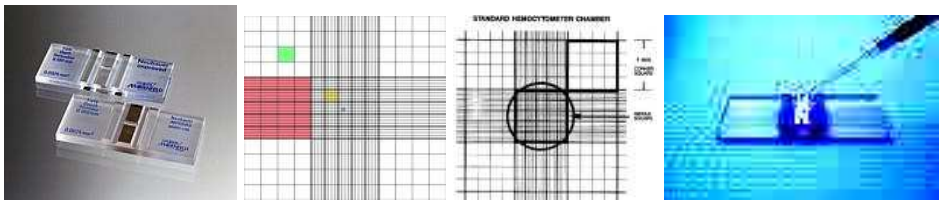

Figure 3.1 Haemocytometer counting chamber using for counting ruminal microbes.

In this experiment, there are 9 big squares in half of Haemocytometer counting chamber, 16 middle squares in 1 big square, and also 25 small squares in 1 middle square. Thus, 1 big square include 400 (16x25) small squares. Both the big square's width and length are 1mm and the depth is 0.1 mm. Therefore the volume of the big square is 0.1 mm^3. Usually, only the central big square was used to count ruminal microbes.

Calculating equation:

a) For protozoa, the number of protozoa was counted by 1 big square:

$$\text{number of protozoa/mL} = \frac{N}{400} \times 400 \times 10^4 \times D$$

where N is the average number of protozoa in 1 big square , D is the sample diluted times.

b) The numbers of fungi and bacteria were counted by small squares crossed by two diagonals in one big square:

$$\text{number of fungi (or bacteria) /mL} = \frac{N}{100} \times 400 \times 10^4 \times D$$

Where N is the average number of fungi or bacteria in middle squares crossed by two diagonals in one big square (100 small squares), and D is the sample diluted times.

3.4.5 Procedures of analysis of blood urea nitrogen (BUN)

1) The principles of BUN analysis

BUN or Plasma urea nitrogen (PUN) was determined by a Spectronic Genesys 5 spectrophotometer; the principle is shown in Figure 3.2

2) The brief procedures for determination of BUN are shown as following.

a) Stock forric chloride-phosphoric acid reagent:

Preparation of reagents: ($FeCl_3.6H_2O$ 15 g +DI water 30 ml) + H_3PO_4 (85%) 300 ml, mixed evenly, adjusted to 450 ml with DI water and kept in brown bottle.

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

96% H_2SO_4 150 ml + DI water 500 ml + Stock forric chloride + phosphoric acid regent 1 ml, mixed evenly and adjusted to 1000 ml with DI water.

c) Color reagent

Diacetyle monoxime 1.7 g + Thiosemicarbazide 0.3 g + DI water 100 ml mixed evenly and adjusted to 1000 ml with DI water, subsequently filtrated through waterman filter paper and kept in brown bottle.

d) Stock BUN standard (mg/dl)

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

e) Working BUN standard

Dilute stock BUN standard by 0.1 N HCl as following:

0.1 N HCl 90 ml + stock BUN standard 10 ml

0.2 N HCl 90 ml + stock BUN standard 10 ml

0.3 N HCl 90 ml + stock BUN standard 10 ml

0.4 N HCl 90 ml + stock BUN standard 10 ml

After that, the BUN standard solution containing urea 10, 20, 30 and 40 mg% respectively was obtained and then kept at 4°C.

f) Method

According to the Table 3.3, the reagents are transferred to screw cap tube with size 16x25 mm.

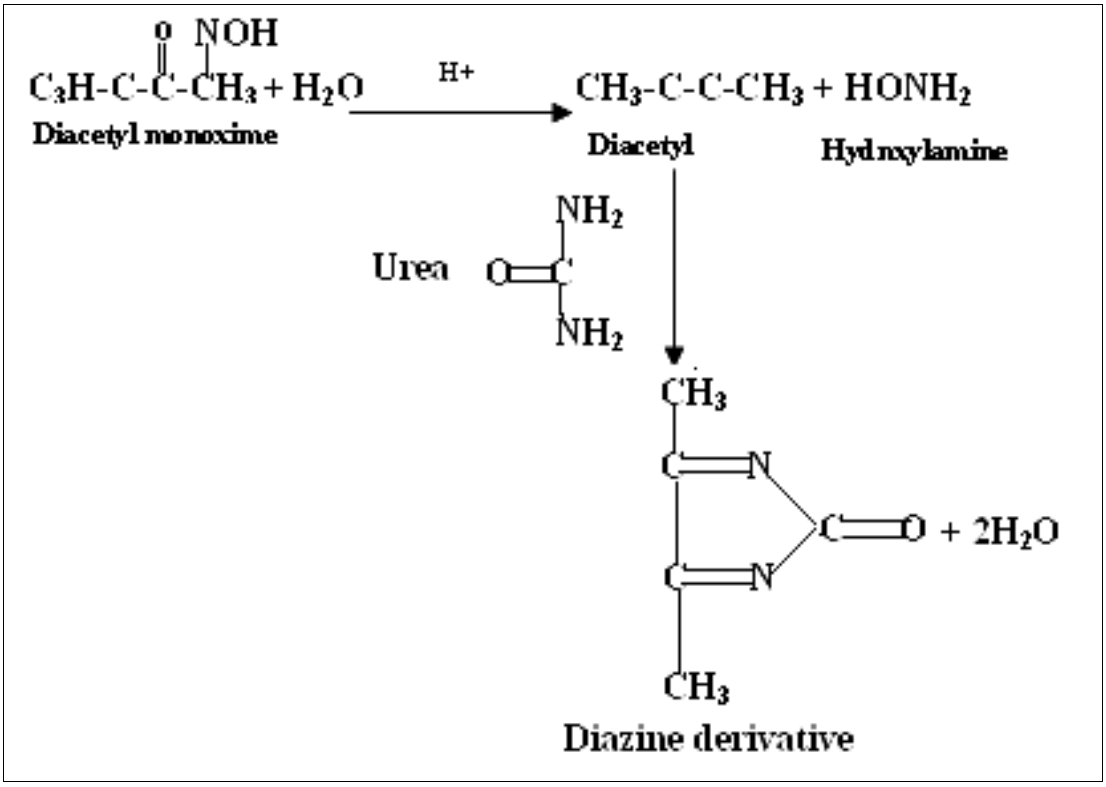

Figure 3.2 The principle of plasma urea nitrogen (PUN) determination

Source : Preston, Schnakenberg and Pfander (1964)

Table 3.3 The method of plasma urea nitrogen analysis.

Reagent	**Blank (no repetition)**	**Standard (3 repetition)**	**blood Sample**
DI water (µl)	20	-	-
Standard (µl)	-	20 (10, 20, 30, 40 mg%)	-
Sample (µl)	-	-	20
Color reagent (ml)	3.0	3.0	3.0
Acid reagent (ml)	2.0	2.0	2.0

g) Analyzing

The standard was run in triplicate and adopting none but the r^2 over 0.98. The samples and reagents were transferred into a 16x125 mm test tube by pipette, and the tubes were closed tightly with a screw cap lined with butyl rubber. Then the content was mixed evenly, supervening by boiling at 80°C until the color changed into pink and then it was cooled down to normal room temperature in cool water. Within 15 minutes after the preparation, the determination at 540-nanometer wavelength was done employing the blank to adjust the spectrophotometer to zero prior to it.

In this analysis, the standard curve and the regression equation was obtained by standard solution analysis as follows. $Y = 213.7x + 2.0802$, $R^2 = 0.9908$.

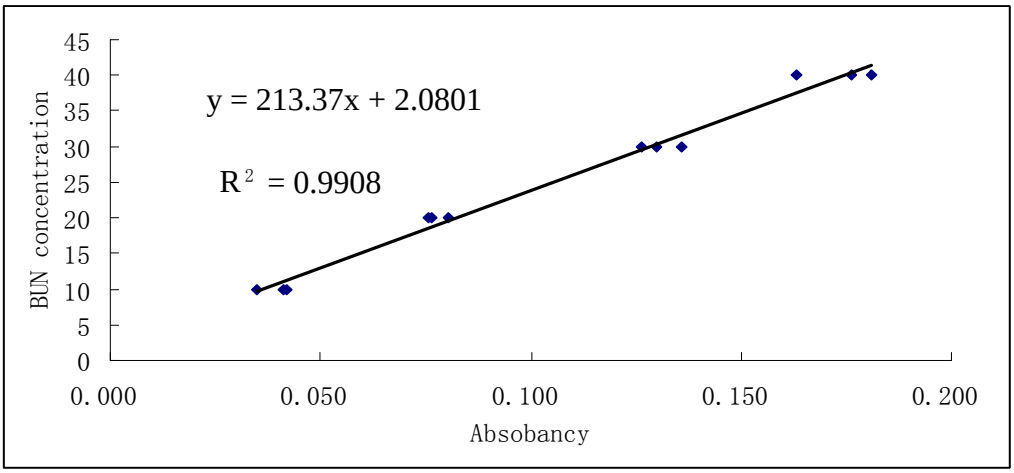

Figure 3.3 The relationship between standard of blood urea nitrogen (BUN) concentration and absorbancy.

3.4.6 The procedures of ruminal fluid VFA analysis determined by gas chromatography (GC).

1) Preparing sample of ruminal fluid for VFA analysis.

The samples of ruminal fluid collected from experiment cattle were centrifuged at 5000 x r for 15 min to get rid of food particles and ruminal microbe. Supernatant (1 ml) was transferred into a 2 ml vial to analysis total VFA and molar proportion of main VFA mix (acetate, propionate, and butyrate) by gas chromatography (GC).

2) Preparing VFA standard solution.

a) Preparing standard solution A contained 200 mM of acetic acid (C2), propionic acid (C3) and butyric acid (C4) mixed in DI water respectively.

C2 (0.574 ml) + C3 (0.761 ml) + C4 (0.924 ml) + 50 ml DI water

b) 5 mM mix standard solution 10 ml

0.25 ml of standard solution A was diluted to 10 ml by DI water

c) 10 mM mix standard solution 10 ml

0.5 ml of standard solution A was diluted to 10 ml by DI water

d) 20 mM mix standard solution 10 ml

1.0 ml of standard solution A was diluted to 10ml by DI water

e) 30 mM mix standard solution 10 ml

1.5 ml of standard solution A was diluted to 10 ml by DI water

f) 1 ml of 5mM mix standard solution, 10 mM mix standard solution, 20 mM mix standard solution, 30 mM mix standard solution were transferred into 2 ml vial, respectively.

The R^2 of the standard solution should be more than 0.98. For example, C2 in this standard solution analysis R^2 = 0.99866 (Figure 3.4). Otherwise, the standard solution have to be prepared again until R^2>0.98.

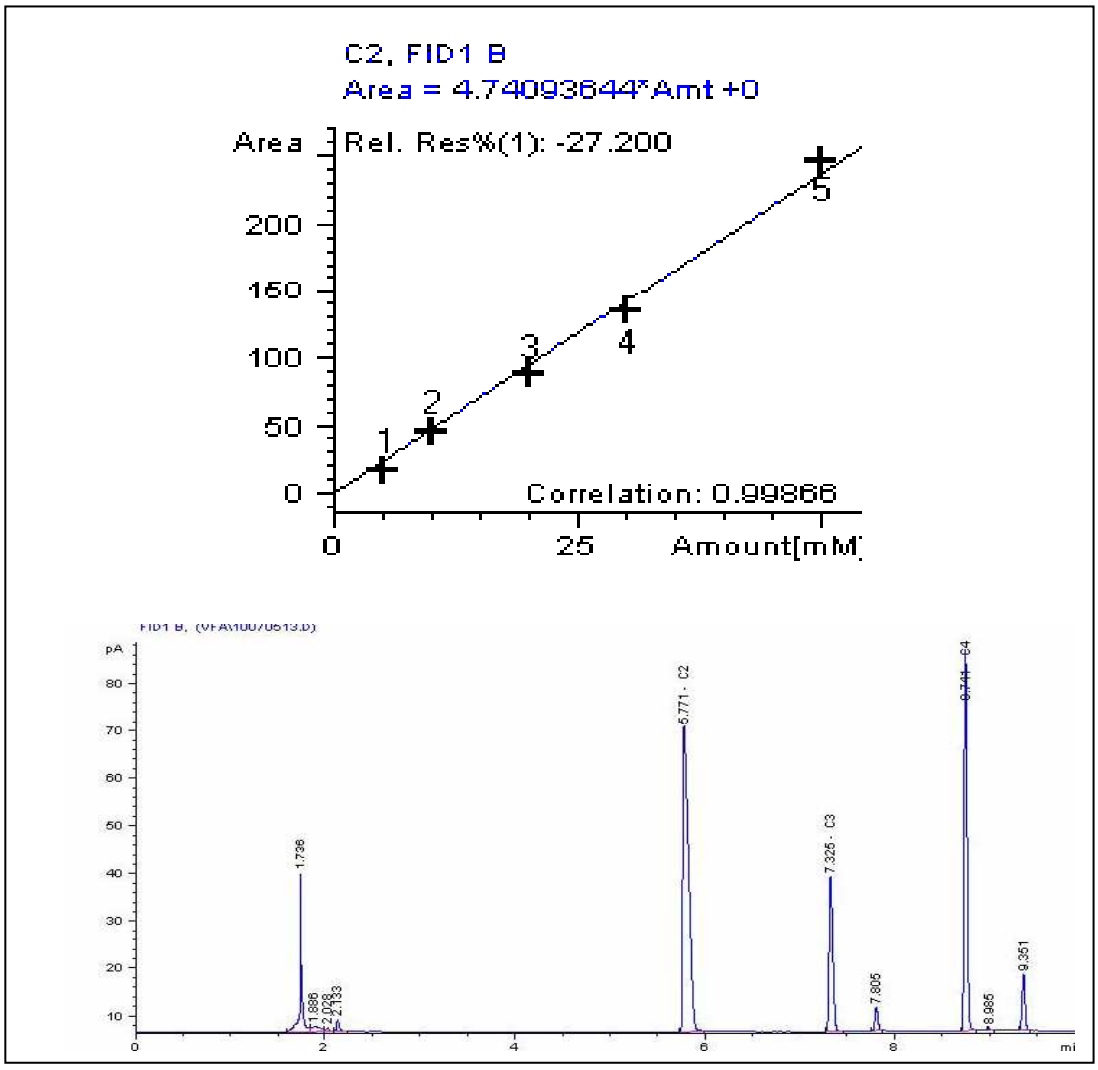

Figure 3.4 The relationship between C2 concentration and area in the C2 standard solution and the order of C2, C3, C4 arising.

3) Procedures of Analysis of VFA by Gas chromatography (GC)

Total VFA and molar proportion of acetic, propionic, and butyric acids in ruminal fluid were determined by HP6890 gas chromatography (GC) (made in USA) fitted with a Flame Ionization Detector (FID). In addition, a J&W 122~3232 column was applied for determination of VFA. The column temperature was fixed at

70°C for 4 min, then it increased at 13°C/min to 175°C which lasted for 27 min. Continually it increased at 4°C/min to 215°C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 ml/min flow rate and the oven temperature was 250°C. FID and injection temperature were fixed at 280°C, and a 1μL injection was done with a 10-μL injector.

3.4.7 The procedures of PD analysis (Chen and Gomes, 1992)

1) Limitation of the method

a) In the calculation, it is assumed that there is little dietary nucleic acid reaching the small intestine. This could be true with most diets, but may not be so when animals are fed with rations containing large amount of fishmeal.

b) The calculation of microbial N from purine content assumes that the ratio of purine: total N in mixed microbial population is constant.

c) Equations are species specific. The implication is that different models should be used for these species to relate PD excretion with intestinal flow of microbial protein.

d) At this stage, the values of microbial N flow calculated from PD excretion should not be taken as absolute values although results of limited number of experiments showed values obtained by the PD method were in good agreement with other methods. Nevertheless, this method is best used to compare differences in intestinal microbial N flow between dietary treatments.

2) Sampling principle

a) All the urine produced by animal was collected and separated from faces well.

b) To obtain a more realistic measurement of the daily PD excretion, urine collection should be made for more than 5 days. This helps to reduce the error due to the 'end-of-collection' variation in urine output from the animal.

c) The collection can be made as a bulk for the whole period. However, where analysis facility allows, it is better to make the urine collection daily. Daily collections will provide us with additional information on the variability of the daily measurement. From our experience, this day-to-day variation is usually about 10%.

d) Urine is collected into a container with approximately 100 ml of 10% H_2SO_4. The final pH of urine needs to be below 3. It is essential to acidify the urine to prevent bacterial destruction of PD in urine. It may be necessary to check the pH on the first day of collection and make necessary adjustment of the amount of acid used accordingly. Slight excess of acid will not matter.

e) PD concentration in straight urine is very high and precipitation (particularly of uric acid) can occur during storage. This will make it difficult for representative sampling for analysis. Dilution by 3-4 times will prevent the occurrence of precipitation.

3) Determination of purine derivative

a) Dilution of urine samples

The urine samples which have been previously diluted before storage still need further dilution. The next dilution should be to such an extent that the concentrations in the final samples will be within the range of the standards used in the assays (5-50 mg/L for both uric acid and allantoin). The dilution factor needed therefore depends on the feed intake, and thus microbial protein supply of the animal.

b) Analytical method

Urinary PD and creatinine were determined using HPLC analysis which consisted of a Multi-solvent delivery system (Waters model 600 E, USA), an injector (WISTTM model 712), a multi-wavelength detector (model 490E; set to 205 nm) and a double 4.6 mm × 250 mm C-18 reverse-phase column (Spherisorb®) following the procedure of Balcells et al. (1992). Purine derivatives were quantified by peak integration using Waters HPLC system controller software Maxima 820.

c) Estimation of microbial nitrogen (N) for cattle

$$PDe = 0.85\ PDa + 0.385\ w^{0.75} \quad \ldots\ldots(1)$$

The equation is for estimating intestinal PD absorption, Where PDe is purine derivative excretion (mmol/d), PDa is purine absorption, $w^{0.75}$ is the metabolic body weigh.

$$\text{Microbial N (g/d)} = 70 \times PDa / 0.83 \times 0.116 \times 1000 = 0.727\ PDa \ldots\ldots(2)$$

The following factors are used for the calculation of intestinal flow of microbial N (g /d) from the microbial purines absorbed (X mmol/d) (Equation 2):

i) Digestibility of microbial purines is assumed to be 0.83. This is taken as the mean digestibility value for microbial nucleic acids based on observations reported.

ii) The N content of purines is 70 mg N/mmol.

iii) The ratio of purine-N: total N in mixed rumen microbes is taken as 11.6:100.

3.4.8 The procedures of roll tube technique

1) Medium preparation for grouping bacteria by roll tube technique (Hobson, 1969).

a) Clarified rumen fluid

Fresh rumen fluid (300 ml) was taken from experiment cattle and filtered by nylon bag for preparing cellulolytic, proteolytic and amylolytic bacteria medium.

b) Mineral solution A and mineral solution B were prepared following Table 3.3.

Mineral solution A (2000 ml) and mineral solution B (2000 ml) were prepared according to Table 3.4. The chemicals were weighed by electronic analytical balance (0.0002 g), and then moved into a 2000 ml volumetric flask. Distilled water was replaced by RO-water. The RO-water was poured into a volumetric flask making the chemicals solve completely. Finally, the RO water was added to raise the volume to 2000 ml.

Table 3.4 The chemical composition in mineral solution A and mineral solution B.

Mineral solution A		
Mineral solution A(ml)	1000	2000
K_2HPO_4 (g)	3	6
Distilled water(ml)	1000	2000
Mineral solution B		
Mineral solution B(ml)	1000	2000
KH_2PO_4 (g)	3	6
$(NH_4)_2SO_4$(g)	6	12
NaCl (g)	6	12
$MgSO_4$(g).$7H_2O$(g)	0.6	1.2
$CaCl_2.2H_2O$ (g)	0.2	0.4
Distilled water(ml)	1000	2000

Source : Hobson (1969)

c) Anaerobic dilution solution

According to the chemical and ingredient requirement of anaerobic dilution solution (Table 3.5), the chemical ingredients were weighed or measured by electronic analytical balance (0.0002 g) or graduated flask. After that, they were moved into a 1000 ml filter flask, heated and mixed with a hotplate stirrer. At the same time, CO_2 was charged into the filter flask to render the dilution solution into an anaerobic condition until the solution preparation was completed. The solution was transferred into small glass bottles (about 10 ml), 5 ml dilution solution per glass bottle, by a thin plastic pipe when the solution color change from pink into clear in approximately 50 minutes. The bottles were covered with plastic covers and warped up immediately with aluminum foil. Finally, the bottles with dilution solution were sterilized (121°C, 15 minutes) in autoclave.

Table 3.5 The chemical composition of anaerobic dilution solution.

Anaerobic dilution solution(ml)	**300**	**400**
Mineral solution A(ml)	108	144
Mineral solution B(ml)	90	120
Cysteine hydrochloride(g)	0.10	0.13
Na_2CO_3(g)	0.6	0.8
Resazurin (g)	0.0003	0.0004
Distilled water (ml)	Until solution to 300ml	until solution to 400ml

Source : Hobson (1969)

d) Cellulose medium

Firstly, agar was put into glass bottles (40 ml), about 0.2 g per bottle. The other medium ingredients were weighed and measured accurately according to Table 3.6, and moved into a 1000 ml filter flask. Secondly, the flask with the solution was heated and mixed with a hotplate stirrer. At the same time, CO_2 was

pumped into filter flask to make the solution under an anaerobic condition until the medium preparation was completed. Thirdly, about being heated for 1 hour, the solution was moved into small glass bottles (about 40 ml) by a thin plastic pipe, 5 ml dilution solution per glass bottle. The bottles were covered with plastic covers and warped up with aluminum foil immediately. Finally, the bottle with dilution solution was sterilized (121°C, 15 minutes) in autoclave.

Table 3.6 Chemical composition of Cellulose medium.

Cellulose medium (ml)	**400**	**500**
Mineral solution A (ml)	60	75
Mineral solution B (ml)	75	90
Clarified rumen fluid (ml)	100	120
Agar (g)	8	10
Resazurin (g)	0.0004	0.0005
Bacto casitone (g)	4	5
Cellulose power (g)	4	5
$NaHCO_3$(g)	1.6	2
Cysteine hydrochloride (g)	0.2	0.25
Distilled water (ml)	Until the solution to 400	Until the solution to 500
pH	6.8-7.0	6.8-7.0

Source : Hobson (1969)

e) Proteolytic medium

According to Table 3.7 the chemical for proteolytic medium was prepared. Preparation of the proteolytic medium followed the same procedures for preparing cellulose medium.

Table 3.7 Chemical composition of proteolytic medium.

Proteolytic medium (ml)	**400**	**500**
Mineral solution A (ml)	60	75
Mineral solution B (ml)	60	75
Clarified rumen fluid (ml)	80	100
Agar (g)	10	12.5
Resazurin (g)	0.0004	0.0005
Tryptose (g)	1.2	1.5
Casein (g)	2.0	2.5
Cysteine hydrochloride (g)	0.2	0.25
$NaHCO_3$ (g)	2.0	2.5
Distilled water (ml)	Until the solution to 400	Until the solution to 500
pH	6.8-7.0	6.8-7.0

Source : Hobson (1969)

f) Amylolytic medium (starch medium)

The amylolytic medium was prepared according to the requirements of chemicals displayed in Table 3.8. The procedure of proteolytic medium preparation was the same as with cellulose and proteolytic mediums preparation.

2) Ruminal microbial inoculation

The glass bottle with mediums of cellulose, proteolytic and amylolytic were heated and changed into liquid by steam water. At the same time, the rumen fluid was diluted 1 time (10^{-1}), 2 times (10^{-2}), 3 times (10^{-3}), 4 times (10^{-4}), 5 times (10^{-5}), 6 times (10^{-6}), 7 times (10^{-7}) and 8 times (10^{-8}) respectively. When the hot bottle with medium was cold about 40°C, 0.2 ml of rumen fluid solution diluted by 4 times (10^{-4}) and 5 times (10^{-5}) was inoculated in proteolytic and amylolytic culture medium by injection respectively. Rumen fluid solution (0.5 ml) diluted by 7 times (10^{-7}) and 8 times (10^{-8}) was inoculated in cellulolytic culture medium, respectively. After inoculation, it was put into cool water and rolled by hand to make

the medium solidified equably around wall of bottle immediately. After this, the proteolytic and amylolytic culture mediums were incubated at 39°C for 5 days, cellulolytic culture medium was incubated at 39°C for 21 days to count the cellulolytic, proteolytic and amylolytic bacteria populations.

Table 3.8 Chemical composition of amylolytic medium.

Amylolytic medium (ml)	**400**	**500**
Mineral solution A (ml)	60	75
Mineral solution B (ml)	60	75
Clarified rumen fluid (ml)	80	100
Agar (g)	10	12.5
Resazurin (g)	0.0004	0.0005
Bacto casitone (g)	4	5
Soluble starch (g)	2.0	2.5
Cysteine hydrochloride (g)	0.2	0.25
$NaHCO_3$ (g)	2.0	2.5
Distilled water (ml)	Until the solution to 400	Until the solution to 500
pH	6.8-7.0	6.8-7.0

Source : Hobson (1969)

3.5 Statistics analysis

The analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedure (SAS, 1996) was used for statistical analysis of the data for the replicated 3×3 Latin square design. The model for Using Duncan's New Multiple Range Test and Orthogonal Analysis compares treatment means (SAS, 1996). The regression equation of nitrogen retention and nitrogen intake was determined by using simple linear regression (SAS, 1996).

3.6 Results and discussion

3.6.1 Effects of CP levels on dry matter intake (DMI) and digestibility

Effects of CP concentration on dry matter intake (DMI) and apparent digestibility of DM, OM, ADF and NDF are shown in Table 3.9. Dry matter intake in terms of kg per day (kg/d), g per kilogram per day (g/kg BW/d) and g per kilogram metabolic body weight (g/kg $BW^{0.75}$/d) increased linearly ($P<0.01$) with increasing crude protein concentration. Furthermore, DMI was lower ($P<0.05$) in cattle fed with 4.3% CP diet (3.15 kg/d) than 7.3% CP (3.64 kg/d) and 10.3% CP diets (3.77 kg/d). However, there were not significantly different ($P>0.05$) between 7.3% CP diet and 10.3% CP diet. Similarly, Paengkoum and Tatsapong (2009) used Thai native beef cattle to meet 5% CP, 7% CP, 9% CP and 11% CP diets demonstrated the DMI were not different ($P>0.05$) between 7% CP, 9% CP and 11% CP, but 5% CP was lower ($P<0.05$) than other protein levels. Also, this result agree with Perry, Shields, Dunn, and Mohler (1983) and Thomson, Prestonn, and Bartle (1995), who indicated DMI were improved ($P<0.01$) with increasing protein levels. In contrast, Devant, Ferret, Gasa, Calsamiglia, and Casals (2000), Basra, Nisa, Khan, Riaz, Tuqeer, and Saeed (2003), Yuangklang, (2009), Chantiratikul, Chantiratikul, Chumpawadee, and Kanchanmayoon (2009) and Tatsapong, Paengkoum, Pompa, and Hare (2010) suggested that DMI for calves did not affect by different protein levels. There were not significantly different ($P>0.05$) digestibility of DM, OM, ADF and NDF, but have a increased trendy with increasing CP levels. Similar result has been reported by

Table 3.9 Effects of dietary protein level on dry matter intake, average daily gain and nutrient digestibility.

	Dietary CP			SEM	Contrast P-value	
	4.3%	**7.3%**	**10.3%**		**Linear**	**Quadratic**
DMI (kg/d)	3.35^{B}	3.64^{A}	3.77^{A}	0.07	*	NS
DMI (% of BW)	2.28	2.37	2.42	0.06	NS	NS
DMI (g/kg $BW^{0.75}$/d)	76.54^{B}	83.39^{A}	85.62^{A}	0.53	*	NS
Digestibility (%)						
DM	54.66	58.84	59.27	2.64	NS	NS
OM	60.87	64.39	64.95	3.42	NS	NS
ADF	46.25	50.05	50.90	4.29	NS	NS
NDF	55.76	59.22	59.48	3.12	NS	NS
Initial weight (kg)	153.5	153.7	155.5	2.56	-	-
Final weight (kg)	153.5	153.8	156.7	2.62	-	-
BW change (g/d)	0	8	57	0.9	NS	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01) DMI = Dry Matter Intake, DM = Dry Matter, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, CP = crude protein , BW = body weight .

Atikinson, Toone, Harmon, and Ludden (2007). Devant et al. (2000) indicated digestibility about DM and OM were not affected by dietary protein concentration (14.3 % CP VS 17.3 % CP) in growing crossbred heifers. There was not different (P>0.05) body weight change among the treatment of 4.3%, 7.3% and 10.3 % CP. Although three levels CP diet were fed to beef cattle, but the dietary energy was low, just according to above 20 % of maintenance requirement, the growth maybe was limited, the low energy may be not enough to support the energy requirement for growing.

3.6.2 Effect of CP concentration on ruminal fermentation and blood urea nitrogen (BUN).

Effects of CP concentration on rumen fermentation are shown in Table 3.10. Ruminal pH was not different (P>0.05) at 0h, 4h and mean pH between 4.3%, 7.3% and 10.3% CP. The pH values at 0 hour were 7.03, 7.02 and 6.93, mean pH values were 7.07, 7.03 and 7.02, respectively. The values trend to be decreased with increasing CP level but statistics strictly. Reed, Lardy, Bauer, Gilbery, and Caton (2007) demonstrated that ruminal pH was not different between control and protein supplemented in steers. However, numerous researchers (Heldt et al., 1999; Mathis et al., 2000) have indicated lower pH with increasing levels of DIP supplementation. Because changes in pH are a result of changes in ruminal fermentation, wide range level protein may affect ruminal fermentation.

Ruminal ammonia-N (NH_3-N) concentration (0h, 4h and mean value) increased (P<0.01) with increasing levels of dietary protein from 4.3%, 7.3% to 10.3% CP respectively. Similarly, a few researchers (Devant et al., 2000; Arroquy et al., 2004; Klevesahl et al., 2003; Wickersham, Titgemeyer, Cochran, Wickersham, and Moore, 2008) suggested the concentration of ruminal NH_3-N increased with increasing protein levels.

The blood urea nitrogen (BUN) was 6.69, 22.53 and 35.61 mg/dl at 0 hour post feeding, and 12.04, 24.28 and 38.96 mg/dl at 4 hours post feeding when cattle was fed 4.3%, 7.3% and 10.3% of CP diet, respectively. Blood urea nitrogen increased (P<0.05) with increasing dietary CP at 0 hour and 4 hours post feeding, and it was significantly different (P<0.05) among treatments. Similar result has been reported in lambs (Bunting, Boling, Mackown, and Muntifering, 1987), growing

finishing Brahman cattle (Yungklang, 2009), Brahman crossbreed (Paengkoum and Yanee, 2009). Blood urea nitrogen is an indicator of overall N status of ruminant, it cased absorption of ruminal ammonia. Increased BUN with increasing dietary DIP (DIP) has been indicated by several researchers (Higginbotham, Huber, Wallentine, Johnson, and Andrus, 1989; Roseler, ferguson, Sniffen, and Herrema, 1993). A linear increase in BUN (6.69, 22.53 and 35.61 mg/dL at 0 hour post feeding, 12.04, 24.28 and 38.96 mg/dL at 4 hours post feeding) with increasing ruminal NH_3-N (13.75, 16.21 and 20.00 mg/dL at 0 hour post feeding, 12.45, 15.65 and 19.54 mg/dL at 4 hours post feeding), maybe a positive relationship existed between BUN concentration and ruminal NH_3-N concentration. This result supported findings by other researchers (Dhiman and Satter, 1997; Wanapat and Pimpa, 1999; Vertanen, 1996; Vongsamphan and Wandapat, 2004; Chumpawadee, Sommart, Vongralub, and Pattarajinda, 2006), who demonstrated that an increase of ruminal NH_3-N concentration increased BUN concentration.

Total volatile fatty acid (VFA) concentration, molar proportions of acetate, propionate and butyrate were not affected ($P>0.05$) by increased CP concentration at 0h, 4h and mean value. Similar results were reported by other studies (Reed et al., 2007; Atkinson et al., 2007). The dietary protein level from 4.3% CP, 7.3% CP to 10.3% CP, mean total VFA from 63.26 to 68.45 to 69.85, mean acetate from 61.52 to 65.72, to 63.95, mean propionate from 9.70 to 11.18, to 14.26 and butyrate from 8.88 to 12.62, to 13.20. Paengkoum and Tatsapong (2009) used yearling Brahman × Thai native beef cattle to meet 6%, 9%, 12% and 15% CP diets, which indicated no difference in total VFA concentration between 9%, 12% and 15% CP protein levels except that 6% CP was lower ($P<0.05$) than other protein levels.

Table 3.10 Effects of dietary crude protein levels on ruminal pH, ruminal ammonia nitrogen and volatile fatty acid.

	Dietary CP			SEM	Contrast P-value	
	4.3 %	**7.3 %**	**10.3 %**		**Linear**	**Quadratic**
Ruminal pH						
0 hr	7.03	7.02	6.93	0.49	NS	NS
4 hrs	7.10	7.05	7.11	0.61	NS	NS
Mean	7.07	7.03	7.02	0.58	NS	NS
Ruminal NH_3-N (mg %)						
0 hr	13.75^{B}	16.21^{B}	20.00^{A}	1.62	**	NS
4 hrs	12.45^{B}	15.65^{B}	19.54^{A}	1.47	**	NS
Mean	13.10^{B}	15.93^{B}	19.77^{A}	1.52	**	NS
BUN (mg/dL)						
0 hr	9.69^{C}	22.53^{B}	35.61^{A}	3.08	**	NS
4 hrs	12.04^{C}	24.28^{B}	38.96^{A}	2.85	**	NS
Total VFA (mM/L)						
0 hr	63.27	70.88	74.42	1.45	NS	NS
4 hrs	63.25	64.03	65.85	0.47	NS	NS
Mean	63.26	68.45	69.85	9.16	NS	NS
Acetate (mol/100 mol)						
0 hr	77.16	73.66	73.27	3.96	NS	NS
4 hrs	76.47	72.75	69.06	1.34	NS	NS
Mean	76.80	73.41	71.23	4.17	NS	NS
Propionate (mol/100 mol)						
0 hr	12.20	12.41	13.40	0.73	NS	NS
4 hrs	12.02	12.76	16.58	0.26	NS	NS
Mean	12.11	12.49	14.94	1.08	NS	NS
Butyrate (mol/100 mol)						
0 hr	10.64	13.94	13.33	0.32	NS	NS
4 hrs	11.52	14.49	14.36	0.41	NS	NS
Mean	11.09	14.10	13.83	0.41	NS	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01) CP = crude protein.

In addition, the present experiment confirmed the finding of Paengkoum and Tatsapong (2009) that molar proportions of acetate, propionate and butyrate are not affected by increased CP concentration.

3.6.3 Effects of dietary protein level on rumen microbe count and population.

The ruminal microbes populations are shown in Table 3.11. The counts of protozoa in ruminal fluid fed by 4.3%, 7.3% and 10.3% of dietary protein were 4.58, 3.75 and 1.83 (10^5 cell/ml) at 0 hour post feeding, 2.17, 1.67 and 1.42 (10^5 cell/ml) at 4 hours post feeding, respectively. The count of protozoa slightly decreased with increasing CP levels, but it was not significantly different (P>0.05) by statistics analysis. The counts of fungi (Zoospore) in ruminal fluid was not affected (P>0.05) by dietary protein concentration, the value of which was 1.96, 2.42 and 4.03 (10^6 cell/ml) at 0 hour post feeding, and 3.34, 3.48 and 5.61 (10^6 cell/ml) at 4 hours post feeding fed by 4.3%, 7.3% and 10.3% of dietary protein, respectively. The bacteria population was 8.77, 7.57 and 10.07 (10^9 cell/ml) at 0 hour post feeding, 8.43, 9.57 and 10.03 (10^9 cell/ml) at 4 hours post feeding. There was a slight increase with increasing dietary CP levels at 4 hours post feeding, but both of which were not affected (P>0.05) by CP levels at 0 hour and 4 hours post feeding.

The rumen microbial ecosystem comprises diverse symbiotic populations: obligatory anaerobic, ciliate protozoa and fungi, these microorganisms appear to account for most of the fermentative activity in the rumen. They can digest crude fiber and utilize non-protein nitrogen to synthesis microbial protein, providing the host ruminant nutrition requirement. In this study, the protozoa population was not

affected by dietary CP levels. This result was similar to Dayani, Ghorbani, Alikhani, Rahmani, and Mir (2007) finding who reported no effect (P>0.05) of dietary CP levels on protozoa population in sheep, because the main function of the protozoa is not in the hydrolysis of exogenous soluble protein, but in metabolizing bacteria and fungi protein (Morgavi, Sakurada, Tomita, and Onodera, 1994). However, there was a slight decrease (P>0.05) in the value of ciliate protozoa count with increasing dietary CP concentration. It might be due to presence of urea in the high CP level diet, because rumen protozoa are deficient in enzyme urease that is responsible for urea hydrolysis (Onodera, Nakagawa, and Kandatsu, 1977), and also they cannot use ammonia as nitrogen source for growth (Jouany, Ivan, papon, and Lassalas, 1992). Jouany, demeyer, and grain (1988), Takenaka and Itabashi (1995) and Ushida, Newbold, and Jouang (1997) indicated that total elimination of ruminal protozoa increased the bacterial population, maybe the protozoa inhibit the bacterial growth. Wallace and McPherson (1987) reported the predatory activity of protozoa against rumen bacteria is more significant. Leng and Nolan (1984) indicated defaunation decreased N recycling between bacteria, protozoa and ammonia pools resulting from engulfment and digestion of bacteria by protozoa and the result in increased rumen bacteria numbers caused by decreased protozoa population. In this research, the result agrees with the above point. There was a negative relationship between protozoa and bacterial population. Protozoa population decreased from 4.58 to 3.75 and to 1.83. The bacteria population increased from 7.57 to 8.77 and to 10.07 at 0 hour post feeding. Also at 4 hours post feeding, the protozoa population decreased from 2.17 to 1.67 and to 1.42, and the bacteria population increased from 8.43 to 9.57 and to 10.03.

The population of cellulolytic bacteria, proteolytic bacteria and amylolytic bacteria were not affected (P>0.05) by dietary CP levels, but there was an increasing tendency about the proteolytic bacterial population at 0 hour post feeding (2.46, 2.67 and 3.66 $\times 10^5$ cell/ml) and at 4 hours post feeding (3.04, 4.50 and 6.00$\times$ 10^5 cell/ml) fed 4.3%, 7.3% and 10.3% of CP diet, respectively. The cellulolytic bacteria had an increasing tendency with increasing dietary CP levels, but the fungi population tend to decrease with increasing dietary CP levels, and there seemed to a negative interaction between the ruminal cellulolytic bacteria and the fungi population. This finding was very similar to that was reported by Bernalier, Fonty, Bonnemoy, and Gouet (1992) and Bernalier, Fonty, Bonnemoy, and Gouet (1993). In their studies, ruminal cellulolytic bacteria were observed to inhibit the ability of fungi to hydrolyze cellulose and the inhibition of fungal activity was caused by an extra cellular protein released by cellulolytic bacteria. Santra (1995) suggested that increased concentrate in the diet increased the rumen amylase activity.

Table 3.11 Effects of dietary protein level on ruminal microbe.

	Dietary CP			SEM	Contrast P-value	
	4.3%	**7.3%**	**10.3%**		**Linear**	**Quadratic**
Protozoa （10^5 cell/ml）						
0 hr	4.58	3.75	1.83	1.84	NS	NS
4 hrs	2.17	1.67	1.42	0.76	NS	NS
Fungi (Zoospore, 10^6 cell/ml)						
0 hr	1.96	2.42	4.03	1.18	NS	NS
4 hrs	3.34	3.48	5.61	0.93	NS	NS
Bacterial (10^9 cell/ml)						
0 hr	7.57	8.77	10.07	1.30	NS	NS
4 hrs	8.43	9.57	10.03	2.33	NS	NS
Cellulolytic bacterial (10^7 cell/ml)						
0 hr	3.03	1.87	1.53	1.25	NS	NS
4 hrs	4.67	3.83	1.22	2.42	NS	NS
Proteolytic bacterial (10^5 cell/ml)						
0 hr	2.46	2.67	3.66	1.92	NS	NS
4 hrs	3.04	4.50	6.00	1.96	NS	NS
Amylolytic bacterial (10^5 cell/ml)						
0 hr	1.75	1.96	4.92	2.22	NS	NS
4 hrs	7.04	6.71	5.63	4.18	NS	NS

[A–C] Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.0); CP = crude protein

3.6.4 Effects of CP level on Nitrogen utilization:

Nitrogen utilization data are presented in Table 3.12. Nitrogen (N) intake, urinary N excretion, N absorption (%), N retained (g/d) and N retained (% of N intake) increased linearly with increasing ($P<0.01$) dietary CP concentration and were affected significantly ($P<0.01$) by dietary CP levels respectively. Similar results were reported by other studies (Devant et al., 2000; Castillo, Kebreab, Beever, barbi, Sutton, Kirby, and France, 2001; Cole, Greene, McCollum, Montgomery, and McBride, 2003; Archibeque, Freetly, Cole, and Ferrell, 2007; Reed et al., 2007). The fecal N excretion was not affected ($P>0.05$) by dietary CP concentration. This result agrees with Marini and Van Amburgh. (2003), Archibeque, Burns and Huntington (2001, 2002) and Reed et al. (2007) who observed no increase ($P>0.05$) in fecal N excretion as N intake increased. In this finding, the major effect of N excretion (g/d) was on urinary N output, the increase in dietary CP level increased ($P<0.05$) urinary N excretion by 4.17 g N/d and 7.31 g N/d, respectively. This result in agreement with Sutton, Cammell, Beever, Humphires, and Phipps (1998), Wright, Moscardini, Luimes, and McBride (1998), Castillo et al. (2001), Cole et al. (2003) and Archibeque et al. (2007) reported that the most significant effect of protein ration in the diet on N outputs was on urinary N excretion. In contrast, Hunter and Siebert (1980) observed increases in fecal N excretion with increasing protein supplementation. There was no significant difference of N retained (% of N intake, $P>0.05$) between 7.3% CP diet and 10.3% CP diet, but 4.3% CP diet was lower ($P<0.05$) than 7% and 10% CP. Castillo et al. (2001) indicated that N retained was not affected by CP concentration levels (210 g/kg DM and 290 g/kg DM). In this experiment, low N retained of 4.3% CP might be caused by inadequate UIP provision to help maintain the normal amount of ruminal microbial.

Table 3.12 Effects of dietary crude protein levels on nitrogen utilization (%, DM basis).

	Dietary CP			SEM	Contrast P-value	
	4.3%	**7.3%**	**10.3%**		**Linear**	**Quadratic**
N-intake (g/d)	22.21^{C}	43.86^{B}	63.06^{A}	1.96	**	NS
Fecal- N (g/d)	17.59	19.02	19.18	1.62	NS	NS
Urinary- N (g/d)	6.81^{C}	10.98^{B}	18.29^{A}	1.69	**	NS
N-absorption (%)	21.31^{C}	56.18^{B}	69.67^{A}	4.38	**	NS
N-retained (g/d)	-2.19^{C}	13.71^{B}	25.56^{A}	3.78	**	NS
N-retained (% of N-intake)	-9.65^{B}	31.04^{A}	34.60^{A}	9.59	**	NS

$^{A-C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); CP = crude protein.

3.6.5 Effect of dietary protein on microbe nitrogen (N) synthesis

The daily excretions of allantoin, uric acid, creatinine, total PD, PD absorbed and microbe production nitrogen are shown in Table3.13. Allantoin was the mian PD detect in the urine of the beef cattle, with values ranging from 16.12 to 42.11 mmol/d and 0.37 to 0.95 mmol/d/kg $BW^{0.75}$, respectively. Allantoin expressed both as mmol/d and mmol/d/kg $BW^{0.75}$ increased linearly (P<0.01) with increasing CP levels and dry organic matter intake (DOMI), and allantoin excretion in urine of cattle fed 10.3% dietary CP was higher (P<0.01) than fed 4.3% dietary CP. Similarly, total PD (mmol/d and mmol/d/kg $BW^{0.75}$), PD absorbed (mmol/d and mmol/d/ kg $BW^{0.75}$) and MNS (g/d and g/d/ kg $BW^{0.75}$) increased linearly (P<0.05) with increasing dietary protein concentration. The value of total PD (mmol/d and mmol/d/kg $BW^{0.75}$), PD

absorbed (mmol/d) and microbe nitrogen synthesis (MNS, g/d) in the urine of cattle which were fed 10.3% CP diet was significantly higher ($P<0.05$) than 4.3% CP diet, but compared with 4.3% CP diet and 10.3% CP, there was no difference when fed 7.3% CP diet. On the other hand, PD absorbed (mmol/d/kg $BW^{0.75}$) and MNS (g/d/kg $BW^{0.75}$) of the cattle fed 10.3% CP diet was higher ($P<0.05$) than 7.3% CP diet and 4.3% CP diet, but there was no difference ($P>0.05$) between 7.3% CP and 4.3% CP diet. The uric acid (mmol/d) and creatinine (mmol/d) were not affected ($P>0.05$) by dietary protein levels.

Several researchers indicated a significant ($P<0.05$) increase in allantoin and total PD but no significant ($P>0.05$) effect on uric acid in urine with increasing DMI and DOMI levels which had the same dietary CP concentration (liang, Mutsumoto, and Young, 1994; Chen, Samaraweera, Ørskor, and Abeygunawardene, 1996; Nolan, 1999). An increase of DMI and DOMI with same CP concentration diet implies an increase of CP intake. In this experiment, an increase of dietary CP levels led to increased CP intake. Therefore, the result of allantoin and uric acid in this experiment is in agreement with what they reported. Furthermore, similar result has been reported that increased PD excretion with increasing proportion of concentration in cows diet (Moorby, Evans, and Danelón, 2006). In contrast, Devant et al, (2000) indicated Urinary excretion of purine derivatives was from Table3.12 not affected ($P<0.05$) by protein concentration and degradability. They suggested that in high-concentrate diets NH_3-N concentration did not limit microbial growth. Creatinine (mmol/d) of urinary excretion was not affected ($P>0.05$) by dietary CP levels, a result similar to the finding in Malaysian Kedah Kelantan cattle (Pimpa, Liang, Jelan, and Abdullah, 2001), buffaloes (Chen et al., 1996), camel (Guerouali, Gass, Balcells,

Belenguer, and Nolan, 2004). On the other hand, Narayanan and Appleton (1980) demonstrated that total daily creatinine in urine is breed/species specific. The ruminal microbes depended on degradation of DIP to provide nitrogen sources to synthesis microbe protein. They derive their N ammonia, amino acids and peptides for their growth (Russell, Conner, Fox, Van Soest, and Sniffen, 1992). In this study, to improve the dietary CP levels result increase DIP levels, the MNS was increased ($P<0.05$) linearly with increasing dietary CP levels, the result similar as Stokes, Merchen, Klopfenstein, and Poos (1991) reported that higher microbial protein production was observed in cows fed diets containing 11.8% or 13.7% ruminal degradable protein (DIP) than 9% DIP. Also, Fu, Felton, Lehmkuhler, and Kerley (2001) conducted a study on crossbred steers and reported that bacteria N production increased linearly with increasing dietary DIP. They explained that higher microbial protein synthesis fed high DIP was due to high concentration of ruminal ammonia. Hoover and Stokes (1991) suggested that microbes growth increased linearly with increasing ruminal NH_3-N due to an increase in DIP content in the diet in an *in vitro* experiment.

Most ruminant feeds contain negligible amounts of nucleic acid. In the rumen, dietary nucleic acids are extensively broken down by micro-organisms. Therefore the nucleic acid arriving at the lower gut for digestion and absorption by the animal is essentially of microbial origin. In cattle, absorbed purines are almost completely converted into uric acid during passage across the intestinal mucosa before reaching the liver. Uric acid can then be converted into allantoin. Thus, Allantoin and uric acid are referred to as 'purine derivatives' (PD) to estimate the microbe nitrogen synthesis in cattle.

Table 3.13 Effects of dietary protein on microbe nitrogen synthesis.

Item	Dietary CP			SEM	Contrast P-value	
	4.3%	7.3%	10.3%		Linear	Quadratic
NH_3-N (g/L)	0.131^{B}	0.159^{B}	0.198^{A}	0.04	**	NS
DOMI (kg/d)	2.65^{B}	3.06^{B}	3.18^{A}	0.09	**	NS
DOMI (g/d/kg $BW^{0.75}$)	60.17^{B}	70.13^{A}	72.26^{A}	0.51	**	NS
Allantoin (mmol/d)	16.12^{B}	26.02^{AB}	42.11^{A}	8.70	**	NS
Allantoin (mmol/d/kg $BW^{0.75}$)	0.37^{B}	0.56^{B}	0.95^{A}	0.17	*	NS
Uric acid (mmol/d)	7.10	7.88	11.18	4.68	NS	NS
Uric acid (mmol/d/kg $BW^{0.75}$)	0.16	0.18	0.25	0.10	NS	NS
Creatinin (mmol/d)	2.45	3.75	2.43	1.02	NS	NS
Total PD (mmol/d)	23.22^{B}	33.90^{AB}	53.30^{A}	12.37	*	NS
Total PD (mmol/d/kg $BW^{0.75}$)	0.53^{B}	0.78^{AB}	1.21^{A}	0.32	*	NS
PD absorbed (mmol/d)	7.45^{B}	20.02^{AB}	44.58^{A}	14.38	*	NS
PD absorbed (mmol/d/kg $BW^{0.75}$)	0.13^{B}	0.49^{B}	1.02^{A}	0.28	**	NS
MNS (g/d)	5.41^{B}	14.56^{AB}	29.68^{A}	10.45	*	NS
MNS (g/d/kg $BW^{0.75}$)	0.12^{B}	0.33^{B}	0.67^{A}	0.22	*	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); CP = crude protein ; BW = body weight; DOMI = Dray organic matter intake; PD = Purine derivative; MNS = Microbial nitrogen synthesis.

3.6.6 The crude protein requirement for Thai-indigenous beef cattle.

Crude protein requirement for maintenance (CP_m) was determined by regression of crude protein retained on crude protein intake. A significant linear relationship between crude protein retained and crude protein intake, the equation

being y = 0.6895x-2.4434 (R^2 = 0.88, Figure 3.5). When crude protein retained was zero, the value of crude protein intake was crude protein maintenance. Thus, with the estimated equation, the value of maintenance of crude protein was computed to be 3.54 g CP/kg $BW^{0.75}$, which was lower than the crude protein requirement of *Bos Taurus* cattle suggested by NRC (1996) (5.94 g CP/kg $BW^{0.75}$) and ARC (1980) for British breed (4.42 g CP/kg $BW^{0.75}$). The dietary protein requirement for maintenance of Thai-indigenous beef cattle was low implied that not too much amounts of protein and concentrate required in Thai-indigenous beef cattle production. And also, the efficiency of protein intake utilization for growth was higher than the breed recommended by NRC (1996) and ARC (1980).

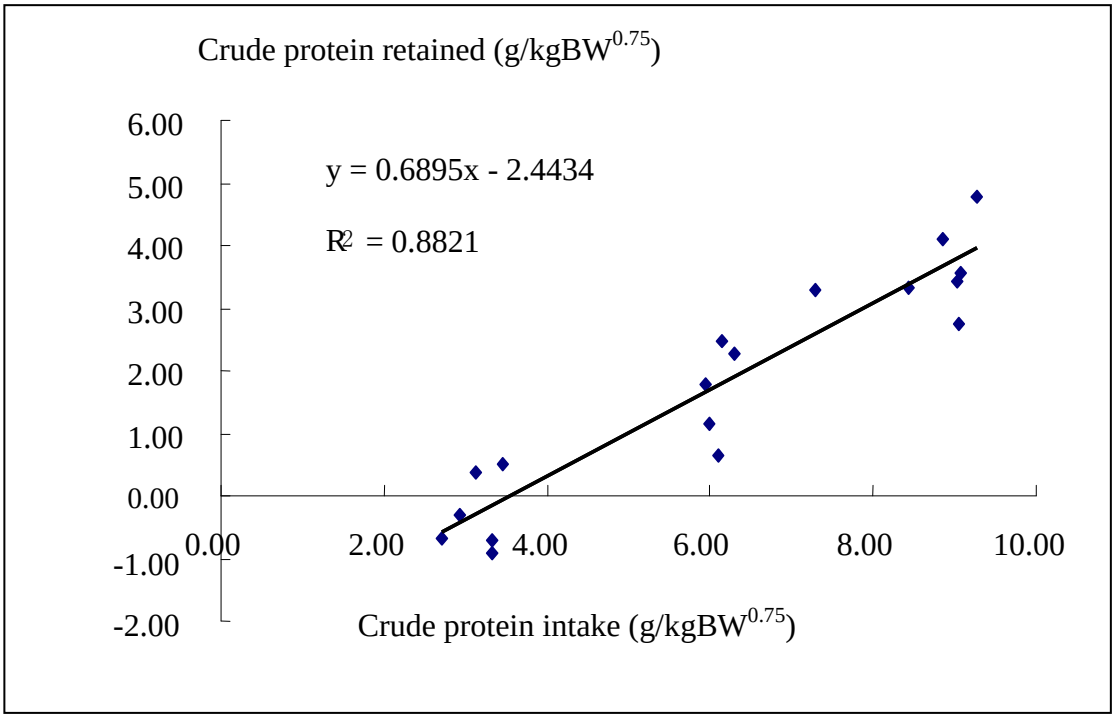

Figure 3.5 The linearly regression relationship between crude protein retained and crude protein intake**.**

3.7 Conclusions

Ruminal NH_3-N concentration increased significantly with increasing dietary CP level. BUN increased with increasing dietary CP level, and there is a positive relationship between BUN and ruminal NH_3-N. Allantoin (mmol/d and mmol/d/kg $BW^{0.75}$), total PD (mmol/d and mmol/d/kg $BW^{0.75}$), PD absorbed (mmol/d and mmol/d/kg $BW^{0.75}$) and MPN (g/d and g/d/kg $BW^{0.75}$) increased linearly ($P<0.05$) with increasing dietary protein concentration. Uric acid (mmol/d and mmol/d/kg $BW^{0.75}$) and Creatinine (mmol/d) of urinary excretion was not affected ($P>0.05$) by dietary CP levels. N utilization was improved by increasing dietary concentrate. The major effect of N excretion (g/d) was on urinary N output. The crude protein requirement for Thai-indigenous beef cattle is 3.54 g CP/kg $BW^{0.75}$, which is lower than that recommended by NRC (5.94 g CP/kg $BW^{0.75}$) and ARC (4.42 g CP/kg $BW^{0.75}$).

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CHAPTER IV

Effects of Dietary Crude Protein and Undegradable Intake Protein on Nutrient Digestibility, Ruminal Fermentation, Nitrogen Utilization and Growth Performance of Growing Thai-indigenous Beef Cattle

4.1 Abstract

Eighteen heads of growing Thai-indigenous beef cattle were used in 2×3 factorial of randomized complete block design. The cattle were divided into 3 blocks according to their body weight. After that, all the cattle were divided into 6 groups, each group contained 3 cattle which were taken out from each block. There were 6 treatment diets which contained 2 levels of crude protein (CP) (10% and 12% of dry matter) and 3 levels of undegradable intake protein (UIP) (15%, 25% and 35% of CP). The digestibility of dry matter (DM), organic matter (OM), acid detergent fiber (ADF) and neutral detergent fiber (NDF), ruminal fermentation and microbes counts were not affected ($P>0.05$) by CP levels and UIP levels. Dry matter intake (DMI) and average daily gain (ADG) increased linearly ($P<0.01$) with increasing UIP levels. However, there were not different ($P>0.05$) between 10% and 12% CP. Ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) concentrations were not different ($P>0.05$) between 10% and 12% CP, and decreased quadratically ($P<0.05$)

with in creasing UIP levels. Microbial nitrogen synthesis (MNS) was not affected ($P>0.05$) by CP levels and the UIP levels. Nitrogen (N) intake and urinary N excretion of 12% CP were greater ($P<0.05$) than 10% CP. However, the N intake, fecal N excretion (g/d), N retained (g/d) and N retained (% of N intake) increased linearly ($P<0.01$) with increasing UIP levels. Urine N excretion (% of N intake) was decreased linearly ($P<0.01$) with increasing UIP levels. The interaction of UIP×CP was not observed except BUN at 0 hour post feeding in this study.

The estimation of the metabolizable protein (MP) requirement of 1 g/kg $BW^{0.75}$ gain was 0.34 g MP/kg $BW^{0.75}$ of growing Thai-indigenous cattle. The 10% dietary CP of DM was adequate to meet the protein requirement of growth and 6.5% DIP of DM diet can provide adequate N source for the requirement of ruminal microbes growth in growing Thai-indigenous beef cattle. The optimal diet contained 10% CP level of DM and the ration of UIP to DIP was 35: 65.

4.2 Introduction

Protein is an import and necessary nutrient for vital body function. In ruminants, the dietary crude protein is often divided into degradable intake protein (DIP) and undegradable intake protein (UIP). The DIP provides mixture of peptides, amino acids and ammonia to ruminal microbes to synthesis microbial protein. The UIP can escape the rumen digestion, and then is absorbed in small intestine to meet tissue synthesis requirement (NRC, 1996). Improving the dietary CP or DIP concentration may result not only in greater production performance, but also cause increasing the concentration of ruminal ammonia, blood urea nitrogen (BUN) and urinary N lost (Armentano, Bertics, and Riesterer, 1993; Christensen, Lynch, Clark,

and Yu, 1993; Butler, 1998; Castillo, Kebreab, Beever, Barbi, Sutton, Kirby, and France, 2001; Dhali, Mishra, Mehla, and Sirohi, 2006 and Javaid, Mahrum Nisa, and Shahzad, 2008). Ruminal ammonia absorbed through the ruminal wall, detoxified to urea in the liver (Lobley et al., 1995) and consequently harm host ruminant and leads to inefficient dietary N utilization. Animal performance data suggest that the proper ratio of degraded to undegraded intake protein (DIP: UIP) should be fed to maximize performance (Stock, Merchen, Klopfenstein, and Poos, 1981; Milton, Brandt, and Titgemeyer, 1997a, b). Imbalance of DIP and UIP in ruminant diet can compromise the microbial protein synthesis, ruminal digestion and protein availability to the animals (Santos, Santos, Theuber, and Huber, 1998; Reynal and Broderick, 2005). Thus, the information of the optimal ratio of DIP to UIP in the diet for optimal growth in ruminant and how it is affected by DIP and UIP supplementation is very important. Sultan, Javaid, Nadeem, Akhtar, and Mustafa (2009) reported diet containing 45% UIP in CP (UIP: DIP = 45: 55) is considered optimum regarding N retention in buffalo calves, but the optimum UIP concentration base on CP was 23% (DIP: UIP = 77: 23) in lambs (Hadded, Mahmoud, and Talfaha, 2005). For this reason, different breeds/ species ruminant maybe have different responses to varying ratio of DIP to UIP. The objectives of this experiment were to investigate the effects of two levels of dietary CP and three levels UIP on intake, ruminal fermentation, nutrient digestibility, microbial population, average daily gain (ADG), nitrogen utilization, microbial protein synthesis and get a “ideal” ratio of DIP to UIP in Thailand indigenous beef cattle diet. And also to determine the metabolizable protein requirement for body weight gain of growing Thai-indigenous cattle.

4.3 Research objectives

4.3.1 To study effects of different ratio of UIP and DIP on performance, nutrition digestion, ruminal fermentation, nitrogen balance and microbial nitrogen synthesis of Thai-indigenous beef cattle.

4.3.2 To predict the protein requirement for body weight gain in growing Than-indigenous beef cattle.

4.4 Materials and methods

4.4.1 Experimental animals

Eighteen heads of growing male Thai-indigenous cattle, with a range of body weight from 85 kg to 204 kg. The cattle were divided into three blocks (block 1: BW 85-125 kg, block 2 : 125-165 kg, block 3: 165- 205 kg) according to the body weight, and then all of the cattle were divided into 6 groups, each group containing 3 cattle, each head of cattle taken from each block respectively. The average body weight of groups were 143.7, 148.0, 145.7, 139.6, 149.0 and 150.3 kg respectively, and there were no difference ($P>0.05$) between the groups. All of the cattle were housed in individual pens and treated against anthelmintics and intestinal parasites with Ivermectin.

4.4.2 Treatments

There are 6 treatment diets (the ingredients and chemical composition are shown in Table 4.1) which contained 2 levels of CP (10% and 12% of DM) and 3 levels of UIP(15% UIP, UIP : DIP = 15: 85; 25% UIP, UIP: DIP = 25: 75; and 35% UIP, UIP: DIP = 35: 65), The treatments consisted of T1 to T6:

T1 = 10% CP + 15% UIP T4 = 12% CP + 15% UIP

T2 = 10% CP + 25% UIP T5 = 12% CP + 25% UIP

T3 = 10% CP + 35% UIP T6 = 12% CP + 35% UIP

Rice straw was fed as roughage only. The dry matter intake (DMI) was estimated according to 2.5% of body weight.

Table 4.1 Ingredients and chemical composition of experimental diets (%, DM basis).

	10% CP			**12% CP**		
	UIP levels (%)			**UIP levels (%)**		
	15	**25**	**35**	**15**	**25**	**35**
Rice straw	39.9	40.7	38.9	39.3	40.8	40.3
Cassava Chip	52.6	48.8	42.7	50.0	44.8	36.9
Soybean meal	2.2	7.3	16	4.6	10.6	20.8
Molasses	1.8	1.0	1.27	2.4	1.4	0.7
Urea	2.7	1.5	0.4	3.0	1.6	0.4
Dicalcium phosphate	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin-mineral premix1	0.4	0.4	0.4	0.4	0.4	0.4
Total	100.0	100	100	100	100	100
Forage: concentrate	40:60	40:60	41:59	39:61	41:59	40:60
Chemical composition (%)						
DM(%)	90.3	90.2	90.1	90.0	90.2	90.4
CP(% of DM)	10.1	10.2	10.0	12.1	12.1	12.3
UIP(% of CP)1	14.7	24.7	35.0	15.3	25.2	35.1
DIP(% of CP)2	85.3	75.3	65.0	84.7	74.8	64.9
Ash	6.24	6.58	6.80	6.26	6.72	7.15
NDF	53.91	53.31	50.22	52.54	51.89	49.05
ADF	21.1	21.8	21.5	21.0	22.0	22.2
ME (MJ/kg)	9.6	10.2	10.0	9.7	10.2	10.0

1The premix contained per kilogram of DM: 4×10^6IU Vitamin A, 0.4×10^6IU Vitamin D_3, 4000IU Vitamin E, 24 g ,Fe, 0.2 g Co, 2 g Cu, 10 g Zn, 0.5g I, 50 mg Se. DM=Dry Matter, NDF=Neutral Detergent Fiber, ADF= Acid Detergent Fiber. ME=Metabolizable energy. UIP1 = Undegradable intake protein (UIP = Soybean meal % × Soybean meal CP %× Soybean meal UIP % + Cassava chip %× Cassava chip CP %× Cassava chip UIP % +Rice straw %× Rice straw CP %×Rice straw UIP %) , UIP of feedstuff (% of CP): Soybean meal = 35% (NRC, 1996), Urea = 0 (NRC, 1996), Molasses = 0 (NRC, 1996), Cassava chip = 28.8 % (Chumpawadee, 2005), Rice straw = 72.1 % (Diao, and Tu, 2005). DIP2 = Degradable intake protein (DIP = 100 – UIP), ME = Metabolizable energy.

4.4.3 Experiment design and feeding management

Eighteen heads of growing Thai- indigenous beef cattle were used in a 2×3 factorial in randomized complete block design (RCBD). Each treatment has 3 heads of cattle. After 2 weeks adjustment period before the experiment began, the experiment period were 90 days. All of the beef cattle were weighed at initiation of the experiment. The diet required by the experiment was fed to the cattle twice per day at 0830 h and 1530 h, respectively. Concentrate and rice straw were fed separately. Orts were weighed daily prior to the morning feeding to determine daily dry matter intake (DMI). Access to drinking water was not limited. Body weight of each calf was measured one time per two weeks in the morning before the feeding.

4.4.4 Sampling

In the last 4 week of the experiment period, the cattle each were put into metabolic cages 7 days to collect sample of urine and fecal, and total collection of urine and feces were adopted to determine the digestibility, nitrogen balance and PD excretion. Samples of orts, feces and urine were collected before new feeds were given in each morning. Nitrogen balance analysis was followed the procedure of Schnieder and Flatt (1975). PD analysis procedure was accorded from Chen and Gomes (1992). The methods of fecal and urine collection and sample storage were the same as described in experiment 1. At the same time, the feces were sampled also, according to AIA procedure of Van Keulen and Young. (1977) to determine the nutrient digestibility.

Rumen fluid was sampled at 0 hour and 4 hours post-feeding on the last day of the experiment period. It was filtered with layers of cheesecloth. The ruminal pH was measured with a pH meter immediately and then divided into three

parts. The first part of rumen fluid sample was acidified with H_2SO_4 (20%) and stored -20ºC for analyzing ruminal ammonia N (NH_3-N) and VFA (Samuel, Sagathewan, Thomas, and Mathen, 1997). The second part will be fixed with 10% formalin solution in normal saline for determined total counts of protozoa, fungi and total bacteria population, and the last part was used to measure cellulolytic, proteolytic and amylolytic bacteria using the roll tube technique (Hungate, 1969; Hobson, 1969).

Jugular blood was sampled after rumen fluid was sampled. It was placed into heparinized vacationer tubes and centrifuged at 5000 x g for 15 minutes to separate plasma. The plasma was stored at -20ºC for blood urea N (BUN) analysis (Crocker, 1967). Feed samples were collected twice a week. All samples were stored in the laboratory to be analyzed later.

4.4.5 Procedure of determination of acid-insoluble ash (AIA) (Van Keulen and Yang, 1977)

1) Put the crucible into a muffle furnace (550-600°C) to heat 2 hour, and then let it cool in a desiccator at room temperature and weighed it.

2) Each duplicate 5 g sample of feed or feces (dried and ground 1 mm screen) was weighed, using a four figure balance, put into a 50 ml crucible, dried (2 hours) in a forced air oven (135°C), cooled in a desiccator to room temperature, re-weighed it and then it was ashed overnight at 475-500°C. Grab-sampling was used to collect feces at 08:00, 12:00 and 16: 00 daily for five days. These samples were composite within cows.

3) The ash was transferred to a round-bottom flask and 100 ml of 2 N HCl was added. The mixture was then boiled for 5 minutes on a muffle furnace (heat plate).

4) The hot hydrolysate was filtered (whatman No. 41) and washed free of acid with hot distilled water (85-100°C). The ash and filter paper were then transferred back into the crucible and ashed overnight at 475-500°C.

5) The crucible and content were cooled in a desiccator to room temperature and weighed.

6) Calculation

AIA was calculated as follows:

AIA (%) = (A-B)/C×100

Where: A = weight of crucible with ash

B = weight of crucible

C = weight of sample dry matter

Digestibility coefficient was calculated as follows:

$$\text{Digestibility coefficient of dry matter} = 100 - \frac{100 \times \%\text{AIA(feed)}}{\%\text{AIA(feces)}}$$

$$\text{Digestibility of nutrition} = 100 - \frac{100 \times \%\text{AIA(feed)} \times \%\text{Nutrient(feces)}}{\%\text{AIA(feces)} \times \%\text{Nutrient(feed)}}$$

4.4.6 Chemical analysis

Representative samples of feed, orts and feces were analyzed using AOAC. (1984) and fiber components (Van Soest, Robertson, and Lewis, 1991). The digestibility was measured by means of AIA, and diet and fecal samples were analyzed according to AIA procedure (Van Keulen and Young, 1977)

4.4.7 Parameters measure

The main parameters are DMI, ADG, rumen pH, nutrient digestibility, NH_3-N, Total VFA, BUN, Nitrogen balance, the counts of microbes, microbe nitrogen

synthesis and the metabolizable protein (MP) requirement for body weight gain of growing Thai-indigenous cattle.

4.4.8 Statistics analysis

The analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedure (SAS, 1996) was used for statistical analysis of the data for the 2×3 factorial of RCBD. Duncan's New Multiple Range Test and Orthogonal Analysis were performed to compare treatment means. The MP requirement for growth was predicted by simple linear regression between MP intake and ADG.

4.5 Results and discussion

4.5.1 Effects of dietary CP levels and UIP levels on growth performance

Effects of dietary CP levels and UIP levels on dry matter intake (DMI), organic matter intake (OMI) and average day gain (ADG) are shown in Table 4.2. The DMI and OMI were not affected (P>0.05) by 10% and 12% CP diets, and there was no interaction (P>0.05) between CP levels and UIP levels. The DMI and OMI were lower (P<0.05) in the cattle fed 15% UIP diet than in the cattle fed 25% and 35% UIP diet, but there was no difference (P>0.05) between 25% and 35% UIP. The result largely agreed with Sultan et al. (2009) examined the effect of UIP level in Nili Ravi buffalo and indicated DMI was increased from 3.81, 4.0 and 4.08 to 4.19 when UIP level was increased from 30%, 35% and 40% to 45% of CP, and that, DMI in UIP level of 30% and 35% were lower (P<0.05) in UIP levels of 40% and 45% of CP, but there was no significant difference (P>0.05) between 40% and 45% UIP levels.

Similar results have been reported by several researchers. Kumar, Tiwari, and Kumar (2005) indicated that DMI increased from 12.89 to 13.20 kg/d when UIP level was increased from 41% to 48% of dietary CP in crossbred cattle. Chaturvedi and Walli (2001) demonstrated when UIP level was raised from 29% to 43% of CP the DMI increased 8.3% in lactating cows. Furthermore, DMI increases with increasing UIP levels have been reported in dairy cows (Westwood, Lean, Garrin, and Wynn, 2000; Haddad et al., 2005) and in goats (PaengKoum, Liang, Jelan, and Basery, 2004). An increase in DMI with increasing UIP due to high concentration UIP led to a decrease of ruminal NH_3-N and blood urea nitrogen (BUN) concentration. Furthermore, urea was used as DIP sources, the diets are usually unpalatable with high concentration of urea, and consequently, DMI decreased. But in this study, the DMI was not affected when UIP level was increased from 25% to 35% of CP. Maybe 6.5% DIP ((100-35) ×10% = 6.5% of DM) of diet can meet the requirement for ruminal microbes growth. Similarly, the NRC (2000) suggests that the minimum requirement of DIP is 6.8% of dietary DM. On the contrary, Henson, Schirigoethe, and Maiga (1997) suggested feed intake decreased when the dietary UIP was increased from 33.7% to 45% of CP. It might have caused the high UIP level diet to provide low concentration of ruminal NH_3-N which led to reduce ruminal microbial proliferation, ruminal fermentation and thus reduced the nutrition intake (Faverdin, Bareille, and Veritc, 1999, Orskov 1992 and Hume, Moir, and Somers, 1970).

The OM intake increased linearly ($P<0.05$) with increasing UIP levels of CP, and 15% UIP was lower ($P<0.05$) than 25% and 35% UIP, but there was no difference ($P>0.05$) between 25% and 35% UIP. The result was in agreement with what has been reported in postpartum heifers (Strauch, Scholljegerdes, Patterson,

Smith, Lucy, Lamberson, 2001) and in steers (Guthrie and Wagner, 1988; McCollum and Horn, 1990; Koster, Cochran, Titgemeyer, Vanzant, Abdelgadir, and St-Jean, 1996). DIP levels may have been adequate, or the steers may have recycled adequate amounts of N to prevent reductions in OM intake. In contrast, Reed, Lardy, Bauer, Gilbery, and Caton (2007) indicated that the OM intake was not affected ($P<0.05$) by dietary UIP levels.

ADG increased linearly ($P<0.01$) with increasing UIP levels, and ADG fed 35% UIP level of CP diet was significantly higher ($P<0.01$) than when fed 25% and 15% UIP diet. Also, ADG was significantly much higher ($P<0.01$) in cattle fed 25% than when fed 15% dietary UIP level of CP. Similarly, MacDonald et al. (2007) reported that ADG was higher ($P<0.05$) in heifers fed high UIP concentration supplementation than when fed low UIP concentration. Haddad et al. (2005) demonstrated lamb fed middle concentration UIP (36.1 g/d) and high concentration of UIP (45.4 g/d) had greater ($P<0.05$) ADG than when fed low concentration UIP (25.5 g/d). However, Creighton, Wilson, Klopfenstein, and Adams (2003) reported a decline in ADG when UIP was provided in excess of the amount required, it could be explained by decreased forage intake due to excess UIP supplementation and the DIP was not enough to meet the ruminal microbial required. In this experiment, 35% UIP of CP was not excessive and 65% DIP of CP could to meet the needs of growing Thai-indigenous cattle.

4.5.2 Effects of CP levels and UIP levels of CP on nutrient digestibility

The digestibility of nutrient is shown in Table 4.3. The digestibility of DM, OM, CP, NDF and ADF were not affected ($P>0.05$) by CP levels and UIP levels, and there was no interaction between CP and UIP on digestibility.

The result of nutrient digestibility supported by other researchers (Swanson, Caton, Redmer, Burke, and Reynolds, 2000, Swanson, freetly, and Ferrell 2004; Bandyk, Cochran, Wickerham, titgemeyer, Famer, and Higgins, 2001; Salisbury, Krehbiel, Ross, Schultz, and Melton, 2004) who demonstrated no effect on apparent total tract digestion with increasing supplies of UIP. In contrast, Atkinson, Toone, Harmon, and Ludden (2007) indicated digestibility of OM, ADF increased ($P<0.05$) with UIP level, and digestibility of NDF increased quadratically ($P<0.05$). However, Sultan et al. (2009) examined the effect of varying DIP to UIP on nutrient digestibility, reported that the DM and NDF digestibility was greater ($P<0.05$) in Nili Ravi buffalo fed 30% and 35% UIP of CP diet than when fed 40% and 45%. An increase in dietary DIP led to an increase of ruminal ammonia N concentrations (Roffler and Satter, 1975; Baumann, Lardy, Caton, and Anderson, 2004) and than increased ruminal ammonia N concentration causes in increased ruminal microbial population and ruminal fermentation (Suwanlee and Wanapt, 1994; Pimpa, Wanapatt, Sommart, and Paker, 1996; Wanapat and Pimpa, 1999). Finally, higher microbial activity and growth with higher DIP (Fu, Felton, Lehmkuhlcr, and Kerley, 2001) results in higher DM, NDF and ADF digestibility (Griswold, Apgar, Bouton, and Firkin, 2003). According to this explanation, less than 35% UIP of CP (65% DIP) provided adequate ruminal NH_3-N for ruminal microbial growth requirement, and more than 40% UIP (60% DIP) of diet provided ruminal NH_3-N is inadequate in Nili Ravi buffalo examined by Sultan et al. (2009). That result supported this finding that 35% UIP (65% DIP) of CP diet could provide adequate ruminal NH_3-N for ruminal fermentation in growing Thai-indigenous beef cattle. Moreover, this finding about CP digestibility agreed with Sultan et al. (2009) who reported that the CP digestibility remained unaltered when buffalos were fed 30, 35, 40 and 45% UIP of CP diet, respectively.

4.5.3 Effects of CP levels and UIP levels on rumen fermentation

Effects of CP and UIP levels on ruminal pH, NH_3-N and BUN and VAF are shown in Table 4.4.

Ruminal pH was not affected (P>0.05) by dietary CP and UIP levels and there was no CP×UIP interaction (P>0.05). But Reed et al. (2007) reported that ruminal pH was lower (P<0.05) in steers fed low UIP (0.8% of DM) supplementation than when fed medium UIP (19.6% of DM) and high UIP (40.6% of DM) supplementation. Furthermore, numerous researchers (Koster et al., 1996; Heldt et al., 1999; Mathis et al., 2000) have reported that lower pH with increasing levels of DIP supplementation because changes in pH are a result of changes in ruminal fermentation. However, there was no difference of pH in Thai-indigenous beef cattle in this experiment. Maybe 35% UIP diet can support adequate DIP for ruminal microbes and fermentation requirement and causes no change in ruminal pH.

The ruminal NH_3-N was not affected (P>0.05) by CP levels, there was no CP×UIP interaction (P>0.05) at both of 0 hour and 4 hours post feeding. But it was affected significantly (P<0.01) by UIP levels, and ruminal NH_3-N concentration decreased quadratically (P<0.05) with in creasing UIP levels. The ruminal NH_3-N concentration was higher (P<0.05) in Thai-indigenous beef cattle fed 15% UIP of CP diet than when fed 25% UIP of CP diet, but there was no difference (P>0.05) fed 35% UIP of CP diets with 15% UIP and 25% UIP. Similarly, it has been reported by several researchers that ruminal NH_3-N concentration increased with increasing DIP levels. Kung, Huber, and Saner (1983) indicated that increases in 55.0%, 58.8%, 63.3% and 66.0% DIP led to increases in ruminal NH_3-N 7.8, 10.9, 13.1 and 13.7 mg/dL at 4 hours post feeding in lactating cows. Lee, Hwang, and Chiou (2001) demonstrated

that increasing level of dietary DIP (62%, 64% and 68 % of CP) significantly increased NH_3-N concentrations (41.6, 49.8 and 53.1 mg/dL) in lactating goats. Furthermore, Stokes, Hoover, Miller, and Blauwcikel (1991), Fu et al. (2001) and Baumann et al. (2004) reported that there was a linear increase in ruminal NH_3-N with increasing dietary DIP concentration.

The blood urea nitrogen (BUN) was not affected ($P>0.05$) by CP levels, and there was no CP×UIP interaction ($P>0.05$) in BUN at 0 hr and 4 hrs post feeding. However, BUN was affected ($P<0.01$) by dietary UIP level of CP, and it decreased linearly ($P<0.01$) and decreased quadratically ($P<0.05$) with increasing dietary UIP levels of CP or decreasing dietary DIP levels of CP. Furthermore, the BUN was higher ($P<0.05$) when fed 15% UIP level of CP diet than when fed 25% and 35% UIP level of CP diet, but there was no difference ($P>0.05$) between 25% and 35% dietary UIP level of CP. Similar results have been reported by Sultan et al. (2009), who fed buffalo with 30%, 35%, 40% and 45% UIP of CP diets and suggested the BUN was higher ($P<0.05$) fed 30% UIP of CP diet than 35%, 40% and 45% UIP of CP diets, and there was no difference ($P>0.05$) between those fed 35%, 40% and 45% UIP levels of CP. On the other hand, BUN increases with increasing DIP levels have been reported by Higginbotham, Huber, and Walientine (1989) and Roseler, Ferguson, Sniffen, and Herrema (1993). The high BUN concentration was observed in high DIP concentration diet. This probably can be explained by increased absorption of ruminal ammonia N (Dhiman and Satter, 1997; Wanapat and Pimpa, 1999; Vongsamphan and Wanapat, 2004; Chumpawadee, Sommart, Vongpralub, and Pattarajinda, 2006).

The total VFA was affected ($P<0.05$) by CP level at 0 hour post feeding. Also, the total VFA in cattle fed 10% CP of DM diet (67.87 mg/dL) was

higher ($P<0.05$) than when fed 12% CP of DM diet (58.33 mg/dL), but there was no effect of UIP level and UIP×CP interaction on it. Because there was an interval of approximately 16 hours between the time the diet was fed to the experiment cattle and the time the ruminal fluid was sampled, a great part of the VFA was utilized for microbial protein synthesis. Therefore, the total VFA was lower in cattle fed 12% CP of DM diet than 10% CP of DM diet maybe due to the utilization efficiency of total VFA in cattle fed 12% dietary CP maybe is higher than when fed 10% dietary CP, because the VFA requirement increased linearly with increasing ruminal NH_3-N which was utilized by ruminal microbial.

The total VFA was not affected ($P>0.05$) by CP level and UIP×CP interaction at 4 hours post feeding, but it decreased linearly ($P<0.05$) with increasing UIP levels of CP and total VFA in cattle fed 15% UIP of CP diet was higher than when fed 25% and 35% UIP of CP diet. Reed et al. (2007) used a diet containing low UIP (0.8% of DM), medium UIP (19.6% of DM) and high UIP (40.6% of DM), and which contained different DIP level (24.8%, 22.1% and 19.2% DIP of DM) to examine the effect of UIP level on VFA. They reported the total VFA was not affected ($P>0.05$) by UIP level. Similarly, the total VFA was not affected by increasing UIP levels in lambs (Atkinson, Toone, and Ludden, 2007). Klevershan et al. (2003) indicated total VFA increased with increasing DIP levels and major shifts in total VFA concentration were consistent with the observed change in total DOMI in beef steers.

Molar proportion of acetate, propionate and butyrate, and the ratio of acetate to propionate were not influenced by UIP level of CP, CP level and UIP×CP interaction at 0 hour and 4 hours post feeding. But Reed et al. (2007) demonstrated that the molar proportion of acetate was lower ($P<0.05$) and propionate was higher

(P<0.05) when fed 1ow UIP level (0.8% of DM) diet than when fed medium UIP level (19.6% of DM) diet and high UIP level (20.6% of DM). Numerous researchers (Koster et al., 1996; Heldt et al., 1999; Mathis et al., 2000) suggested a decrease of molar proportions of acetate and an increase of molar proportions of propionate with increasing DIP supplementation in beef steers feed low-quality hay as roughage.

4.5.4 Effects of CP levels and UIP levels on microbial population and counts

The counts of protozoa, fungi and bacteria are shown in Table 4.5. The counts of protozoa neither at 0 hour nor 4 hours post feeding was not affected (P>0.05) by CP level, UIP level and UIP×CP interaction. But it tended to decrease (P>0.05) with increasing fed UIP level of CP in Thai-indigenous cattle, when the UIP level of CP increased from 15% to 25%, to 35%, the decrease of protozoa counts from 1.31×10^5 to 0.9×10^5, to 0.4×10^5 cells/mL at 0 hr and from 1.37×10^5 to 1.08×10^5, and to 0.96×10^5 cells/mL at 4 h post feeding. A similar result has been observed in dairy cow by Stokes et al. (1991), who reported increased protozoa count (3.2×10^5, 7.4×10^5 and 8.1×10^5 cells/mL) when DIP was increased from 9%, 11.8% to 13.7%, respectively. Furthermore, the protozoa count increased quadratically (P<0.05) with increasing ruminal NH_3-N concentration in buffalo (Wanapat and Pimpa 1999) and there was different responses in various DIP source (Meng, Xia, and Kerley, 2000).

The count of fungi at 0 hour and 4 hours post feeding were not affected (P>0.05) by CP level, UIP and UIP×CP interaction. No more information was provided about the response of count of fungi with DIP or UIP levels.

The counts of bacteria at 0 hour and 4 hours post feeding were not affected (P>0.05) by CP level, UIP level and UIP×CP interaction. When the UIP level

of CP increased from 15% to 25%, to 35%, the decrease of bacterial counts from 2.90 $\times 10^9$, 2.17×10^9 to 2.34×10^9 at 0 hr and from 4.23×10^9, 3.73×10^9, to 3.44×10^9 at 4 hrs post feeding. Although it tended to increase with increasing fed UIP level of CP in Thai-indigenous cattle, it was not affected ($P>0.05$) according to statistics analysis. Stokes et al. (1991) and Fu et al. (2001) reported that the population of bacteria increased linearly with increasing DIP concentration and they explained that higher microbial protein synthesis in cows fed high DIP was due to high concentration of ruminal NH_3-N. Furthermore, the count of bacteria increased with increasing ruminal NH_3-N has bee indicated in swamp buffalo (Pimpa et al., 1996; Suwanlee and Wanapat, 1994). Similarly, decreased microbial count with decreased ruminal NH_3-N has been reported by other studies (Argyle and Baldwin, 1989). However, Wanapat and Pimpa (1999) reported that the count of bacteria decreased when ruminal NH_3-N concentration increased to 34.4 mg/dL.

The population of proteolytic bacteria (0 hour and 4 hours post feeding) was not affected ($P>0.05$) by dietary CP levels and there was not a UIP×CP interaction on it. But it increased ($P<0.05$) linearly with increasing UIP levels of CP. The population of proteolytic bacteria was higher ($P<0.05$) in cattle fed 35% UIP of CP diet than those fed 15% and 25% UIP of CP diet, but there was no difference ($P>0.05$) between 15% and 25% UIP levels. Normally, the population of proteolytic bacteria should improve with increasing DIP concentration due to more degradable protein being degraded in rumen by proteolytic bacteria. Urea was used as main source DIP in 15% UIP of CP diet in this experiment. Thus, the true protein was a DIP source in 35% UIP of CP diet > 25% UIP of CP diet > 15% UIP of CP diet (Table 4.1). Therefore, there was a positive relationship between the population of proteolytic

bacteria and DIP from true protein source concentration, but only DIP concentration.

The population of amylolytic bacteria (0 hr and 4 hrs post feeding) was not affected (P>0.05) by dietary CP levels and there was not a UIP×CP interaction on it. But it increased (P<0.05) linearly with increasing UIP level of CP. The population of amylolytic bacterial was higher (P<0.05) in cattle fed 35% UIP of CP diet than those fed 15% UIP, but there was no difference (P>0.05) between 25% and 15% or 35% UIP level. Santra (1995) suggested that increased concentrate in the diet increased the rumen amylase activity.

Not only the population of cellulolytic bacteria at 0 hour but also at 4 hours post feeding were not affected (P>0.05) by dietary CP levels, and there was no UIP×CP interaction on it. But it decreased (P<0.05) linearly with increasing UIP levels of CP. The population of cullulolytic bacteria was higher (P<0.05) in cattle fed 35% UIP of CP diet than those fed 15% UIP and 25% UIP of CP diet, but there was no difference (P>0.05) between 15% and 25% UIP levels of CP diet.

4.5.5 Effects of CP level and UIP level on nitrogen metabolism

Effects of CP level and UIP level on nitrogen metabolism are shown in Table 4.6 The nitrogen (N) intake (g/d) was affected significantly (P<0.01) by CP level and UIP level, but there was no (P>0.05) CP×UIP interaction on it. The N intake when fed 12% CP of DM was greater (P<0.05) than when fed 10 CP % of DM diet in cattle. On the other hand, N intake was increased linearly (P<0.01) and qudratically (P<0.05) with increasing UIP levels of CP. N intake fed 25% and 35% UIP of CP diet were higher (P<0.05) than fed 15% UIP of CP diet. Similarly, the increase of N intake with increasing CP were reported by other studies (Devant et al., 2000; Castillo et al., 2001; Cole, Greene, McCollum, Montgomery, and McBride, 2003; Archibeque et al.,

2007; Reed et al., 2007). Furthermore, Paengkoum et al. (2004) indicated the N intake increased ($P<0.05$) when dietary UIP level of CP was raised from 0, 2%, 4% to 6 % in goat. Atkinson et al. (2007) reported that N intake linearly increased ($P<0.01$) with increasing levels of UIP supplementation in lambs. In contrast, Sultan et al. (2009) used the ratios 30 : 70, 35 : 65, 40 : 60 and 45 : 55 of UIP to DIP to determine the effect of UIP and DIP on N intake and demonstrated that the N intake decreased ($P<0.01$) with increasing ratios of UIP to DIP in buffalo. In this experiment, Urea was used as the main resource of DIP, the N intake increased with increasing UIP levels due to low UIP level diet containing high concentration of urea to decrease the DMI by urea bad palatability.

The fecal N excretion (g/d) was affected ($P<0.01$) significantly by UIP level and CP level, the fecal N excretion was higher when fed 12% CP diet than when fed 10% CP diet. At the same time, the fecal N excretion increased linearly ($P<0.01$) and quadratically ($P<0.05$) with increasing UIP levels. It was higher when fed 25% and 35% UIP level than when fed 15% UIP level, but there was no difference ($P>0.05$) between 25% and 35% UIP. But the fecal N excretion expressed as % of nitrogen intake was not affected ($P>0.05$) by CP levels. A same result has been reported that linear increase in fecal N (g/d) in Saanen goat when DIP to UIP ratio decreased (Paengkoum et al., 2004). This result agreed with Atkinson et al. (2007) who suggested that the fecal N excretion (g/d and % of N intake) increased ($P<0.01$) linearly as UIP supplementation increased. However, Salisbury et al. (2004) observed no difference in fecal N or urinary N output between supplemented with a low UIP vs. a high UIP when consuming a low quality grass hay mixture in wethers.

The urine N excretion (g/d) was affected (P<0.05) by CP level, the urine N excretion was higher (P<0.05) fed 12% CP diet than 10% CP diet, but it was not affected (P>0.05) by UIP level and UIP×CP interaction. When the urine N excretion expressed as % of N intake, it was not affected (P>0.05) by CP level and no UIP×CP interaction was observed on it. But it was affected (P<0.01) significantly by UIP levels, the urine N excretion (% of N intake) decreased (P<0.01) linearly with increasing UIP level. It was higher when fed 15% UIP level diet than when fed 25% and 35% UIP diet, and no difference (P>0.05) was found between 25% and 35% UIP. The difference of effect on urine N excretion (g/d) and urine N excretion (% of N intake) is perhaps due to low N intake in low UIP level diet. A similar result has been indicated that the urine N excretion decreased with increased ratios of UIP to DIP in buffalo (Sultan et al., 2009). Kalscheur, Baldwin, Glenn, and Kohn (2006) also demonstrated a linear increase in urinary N excretion with increasing dietary DIP levels in dairy cows. On the other hand, Swanson et al. (2000) and Atkinson et al. (2007) suggested that urinary N output increased as UIP increased in wether fed low-quality grass hay.

Neither of the N retained (g/d) no N retained (% of N intake) was affected by 10% and 12% CP of DM diet, but they increased (P<0.05) linearly with increasing dietary UIP levels of CP. The N retained was lower (P<0.05) when fed 15% UIP level of CP than when fed 25% and 35% UIP level of diet. There was no difference (P>0.05) between 25% and 35% UIP of CP. This result supported Sultan et al. (2009) who reported that N retained increased (P<0.05) linearly with increasing UIP level of CP which determined four UIP levels of CP (30%, 35%, 40% and 45%) in buffalo. Similar results have been indicated in lambs (Atkinson et al., 2007), in

crossbred calves (Pattanaik, Sastry, Katiyar, and Murari, 2003), and in wether (Swanson et al., 2000). In contrast, Salisbury et al. (2004) demonstrated no difference in N retention in wethers supplemented with low UIP vs. high UIP and consuming low-quality forage.

4.5.6 Effect of CP level and UIP level on purine derivative (PD) excretion and microbe nitrogen (N) synthesis

The data on allantion, uric acid, creatinine, total PD, PD absorbed and microbe nitrogen synthesis are shown in Table 4.7 The allantoin (mmol/d) and allantoin (mmol/100 g N, of Nitrogen intake) were not affected (P>0.05) by CP level, UIP level and there was not any CP×UIP interaction on them either. Similar results about uric acid, creatinine, total PD excretion, PD absorbed and microbes N synthesis were obtained in this study.

The PD of xanthine and Hypoxanthine was not observed in this experiment, possibly because the concentration was not high enough to be measured. This finding agreed with Chen and Gome (1992) who reported that allantoin, uric acid, xanthine and hypoxanthine are all present in sheep urine, but only allantoin and uric acid are found in cattle urine. This may be because in cattle the high activity of xanthine oxidase in the blood and tissues (also kidney) converts xanthine and hypoxanthine into uric acid prior to excretion in the urine.

Several researchers indicated a significant (P<0.05) increase in allantoin and total PD, but no significant (P>0.05) effect was observed on uric acid in urine with increasing DMI and DOMI levels which had the same dietary CP concentration (Liang, Matsumoto, and Young ,1994; Chen et al., 1996; Nolan, 1999; George, Dipu, Mehra, Verma, and Singh 2006).

On the other hand, Devant, Ferret, Gasa, Calsamiglia, and Casals (2000) indicated urinary excretion of purine derivatives was not affected ($P<0.05$) by protein concentration and degradability, than suggested that in high-concentrate diets NH_3-Nconcentration was not limiting microbial growth.

Creatinine (mmol/d) of urinary excretion was not affected ($P>0.05$) by dietary CP levels, this result was similar to the finding in Malaysian Kedah Kelantan cattle (Pimpa, Liang, Jelan, and Abdullah, 2001), buffaloes (Chen et al., 1996), camel (Guerouali, Gass, Balcells, Belenguer, and Nolan, 2004). On the other hand, Narayanan and Appleton (1980) demonstrated that total daily creatinine in urine is breed/species specific.

The microbe N synthesis expressed as g/d and % of N intake was not affected by dietary CP and UIP level. This result indicated that the diet contained 10% CP and 35% UIP of CP provided DIP can meet the requirement of ruminal fermentation and bacterial protein synthesis in growing Thai-indigenous beef cattle.

The ruminal microbes growth depended on degradation of DIP to provide nitrogen sources to synthesis microbe protein, and it derives their N from ammonia, amino acids and peptides for their growth (Russell, Conner, Fox, Van Soest, and Sniffen, 1992). Stokes et al. (1991) reported that higher microbial protein production was observed in cows fed diets containing 11.8% or 13.7% DIP than fed 9% DIP diet. Also, Fu et al. (2001) conducted a study on crossbred steers and reported that bacterial N production increased linearly with increasing dietary DIP. They explained that higher microbial protein synthesis fed high DIP was due to high concentration of ruminal ammonia. Hoover and Stokes (1991) suggested that microbes growth increased linearly with increasing ruminal NH_3-N due to an increase in DIP content in the diet in an *in vitro* experiment.

In this study, although to increase the dietary UIP levels resulted in a decrease in DIP level, it was still enough to meet the ruminal microbial growth requirement. Thus, the microbail nitrogen synthesis (MNS) was not affected (P>0.05) with increasing dietary UIP levels.

4.5.7 The metabolizable protein (MP) requirement for body weight gain

MP is defined as the true protein absorbed by the intestine, supplied by microbial protein and UIP, MP = (microbial synthesis protein + UIP)×0.8 (NRC, 1996). MP requirement for body weight gain were estimated from linearly regression average daily gain (g/kg $BW^{0.75}$) against MP absorbed (g/kg $BW^{0.75}$). A significant linear relationship between the MP absorbed and ADG was estimated, the equation being y = 0.34x+2.77 (R^2 = 0.69 Figure 4.1). The estimation of the MP requirement for g/kg $w^{0.75}$ gain was 0.34 g MP/kg $BW^{0.75}$ of growing Thai-indigenous cattle according to this equation.

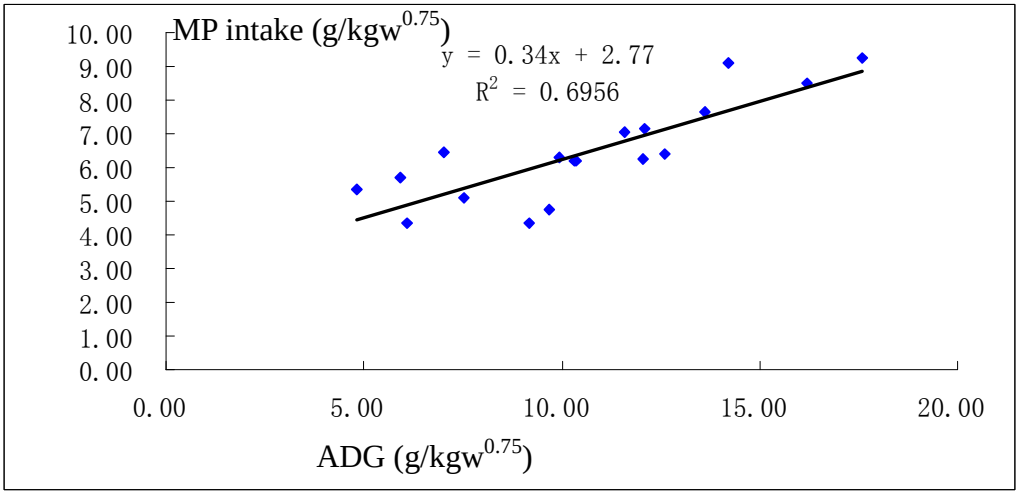

Figure 4.1 The linear regression relationship of metabolizable protein (MP) and average daily gain (ADG).

4.5.8 The relationship between BUN and ruminal NH_3-N

A significant linear relationship was found between the BUN and ruminal NH_3-N which was estimated by linear regression, the equation being y = 92.045x + 3.6661 (R^2 = 0.5498 Figure 4.2). Similarly, the concentration of ruminal ammonia N is closely related to the BUN concentrations (Ropstad, Vik-Mo, and Refsdal, 1989; DePeters and Ferguson, 1992; Broderick and Clayton, 1997; Rodriguez, Stallings, Herbein, and McGilliard, 1997). Excessive ruminal ammonia N is absorbed through the ruminal wall, and converted to urea N in the liver andenters the circulatory system. The amount of ruminal ammonia N absorption is mainly determined by the concentrations of ruminal ammonia N and the ruminal pH (Webbet, Bartly, and Meyer, 1972). Furthermore, Gustafsson and Palmquist (1993) reported that the peak BUN concentration occurred 1.5-2.0 hours after the ruminal ammonia N peak.

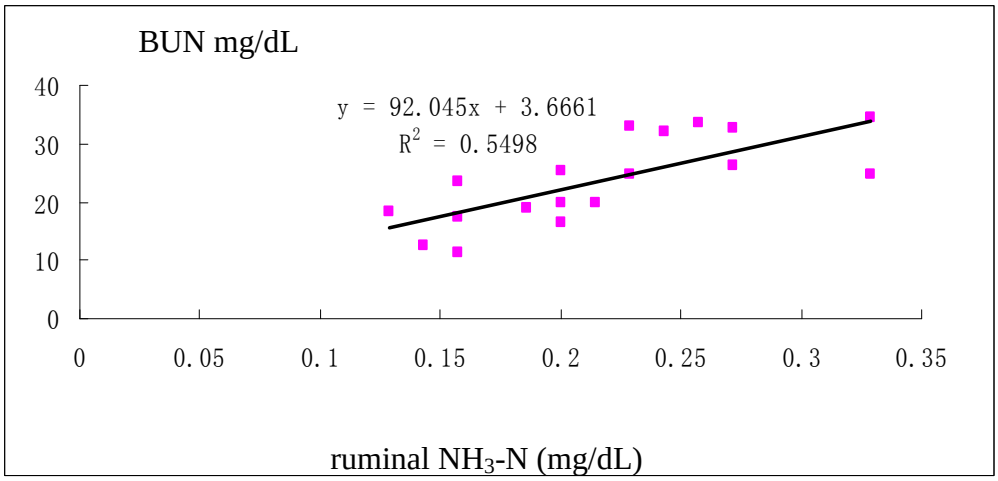

Figure 4.2 The linear regression relationship of blood urea nitrogen (BUN) and ruminal NH_3-N.

4.6 Conclusions

Thai-indigenous production performance was improved ($P<0.05$) linearly with increasing ratios of UIP to DIP. The ruminal fermentation decreased with increasing UIP levels of CP. Nitrogen (N) retained was improved by increasing UIP levels of CP. The efficiency of dietary N converted into microbe N synthesis decreased with increasing UIP level of CP in diet or increased with increasing DIP level of CP in diet.

The estimate of the MP requirement for 1 g/kg $BW^{0.75}$ gain was 0.34 g MP/kg $BW^{0.75}$ of Thai-indigenous growing cattle and MP maintenance requirement was 2.77 g/kg $BW^{0.75}$. The diet contained 10% CP of DM was enough to meet the protein requirement for growth and 6.5% DIP of DM diet can provide adequate N source for the requirement of ruminal microbes growth in growing Thai-indigenous beef cattle. The optimal diet contained 10% CP of DM and the ratio of UIP to DIP was 35:65.

Table 4.2 Effects of crude protein and undegradable intake protein levels on dry matter intake, organic matter intake and average daily gain.

	10% CP			12% CP			SEM	P-value							
	UIP level (%)			UIP level (%)				CP	UIP	CP× UIP	Contrast (UIP %)				
	15	25	35	15	25	35					15	25	35	L	Q
DMI (kg/d)	3.47	4.07	4.10	3.31	3.91	4.11	0.10	NS	*	NS	3.39^{B}	3.99^{A}	4.11^{A}	*	NS
DMI (% of BW)	2.40	2.75	2.79	2.47	2.62	2.73	0.06	NS	*	NS	2.43^{B}	2.68^{A}	2.75^{A}	*	NS
DMI (g/d/kg $BW^{0.75}$)	82.40	95.38	96.28	83.34	91.07	95.23	1.08	NS	*	NS	82.87^{B}	93.22^{A}	95.76^{A}	*	NS
OMI (kg/d)	3.25	3.78	3.79	3.10	3.62	3.79	0.09	NS	*	NS	3.17^{B}	3.70^{A}	3.79^{A}	*	NS
OMI (% of BW)	2.25	2.55	2.58	2.30	2.43	2.51	0.05	NS	*	NS	2.28^{B}	2.49^{A}	2.54^{A}	*	NS
OMI (g/d/kg $BW^{0.75}$)	77.04	88.50	89.05	77.85	84.39	87.77	0.97	NS	*	NS	77.45^{B}	86.44^{A}	88.41^{A}	*	NS
ADG (g/d)	150.79	511.90	646.83	277.78	396.83	595.24	26.17	NS	**	NS	214.28^{C}	454.37^{B}	612.02^{A}	**	NS
ADG (kg/d/kg $BW^{0.75}$)	3.72	12.23	15.01	7.03	9.23	13.65	0.51	NS	**	0.07	$5.38d^{C}$	10.73^{B}	14.32^{A}	**	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; CP = crude protein; UIP = undegradable intake protein; DMI = dry matter intake; DOMI = dry organic matter intake; ADG = average daily gain.

Table 4.3 Effects of crude protein and undegradable intake protein levels on nutrient digestibility.

	10% CP			12% CP			SEM	P-value							
	UIP level (%)			UIP level (%)				CP	UIP	CP× U IP	Contrast (UIP %)				
	15	25	35	15	25	35					15	25	35	L	Q
Digestibility (%)															
DM	60.5	60.7	62.3	63.1	59.8	62.4	0.64	NS	NS	NS	61.85	60.24	62.35	NS	NS
OM	64.6	64.5	65.3	66.7	63.5	65.9	0.60	NS	NS	NS	65.64	64.02	65.61	NS	NS
CP	60.5	56.7	58.9	67.9	63.8	63.8	0.72	NS	NS	NS	64.22	60.25	61.35	NS	NS
NDF	58.4	58.7	58.9	60.3	56.6	58.2	0.75	NS	NS	NS	59.33	57.61	58.58	NS	NS
ADF	40.2	39.5	43.0	41.5	44.7	43.7	1.32	NS	NS	NS	40.81	42.12	43.35	NS	NS

[A–C] Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; DM = dry matter; OM= organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Table 4.4 Effects of crude protein and undegradable intake protein levels on ruminal pH, ammonia nitrogen, volatile fatty acid and blood urea nitrogen.

	10% CP			12% CP			SEM	P-value							
	UIP levels (%)			UIP levels (%)				CP	UIP	CP× U IP	Contrast (UIP %)				
	15	25	35	15	25	35					15	25	35	L	Q
pH															
0 hr	6.93	7.13	7.03	7.33	7.07	7.07	0.09	NS	NS	NS	7.13	7.10	7.05	NS	NS
4 hrs	6.93	6.83	6.73	6.73	7.00	6.80	0.14	NS	NS	NS	6.83	6.92	6.77	NS	NS
NH3-N (mg/dL)															
0 hr	2.10	1.24	2.19	2.19	1.43	1.90	0.09	NS	**	NS	2.14^{A}	1.33^{B}	2.01^{A}	NS	**
4 hrs	2.76	2.00	2.29	3.24	2.00	2.67	0.15	NS	*	NS	3.00^{A}	2.00^{B}	2.48^{B}	NS	*
BUN (mg /dL)															
0 hr	31.05	15.01	14.69	24.91	22.35	21.99	0.55	NS	**	**	27.98^{A}	18.68^{B}	18.34^{B}	**	*
4 hrs	34.58	22.22	24.10	34.64	24.38	24.16	1.00	NS	**	NS	34.61^{A}	23.30^{B}	24.13^{B}	**	*
Total VFA (mM)															
0 hr	69.13	60.68	73.79	53.47	62.09	59.42	2.11	*	NS	NS	61.30	61.38	66.61	NS	NS
4 hr	75.60	71.32	70.79	88.41	73.30	71.44	2.38	NS	*	NS	82.00^{A}	72.31^{B}	71.15^{B}	NS	NS
Acetate (%molar)															
0 hr	72.00	75.00	75.00	76.14	75.88	76.11	1.81	NS	NS	NS	74.07	75.44	75.56	NS	NS
4 hrs	74.36	73.49	75.06	76.45	75.92	74.65	1.77	NS	NS	NS	75.40	74.71	74.87	NS	NS
Propionate(%molar)															
0 hr	15.48	14.90	12.96	12.84	12.17	12.29	0.80	*	NS	NS	14.16	13.53	12.62	NS	NS
4 hrs	13.50	17.30	12.64	14.11	12.47	13.74	0.72	NS	NS	NS	13.80	14.88	13.19	NS	NS
Butyrate (%molar)															
0 hr	12.52	10.10	12.04	11.02	11.96	11.60	0.50	NS	NS	NS	11.77	11.03	11.82	NS	NS
4 hrs	12.15	9.22	12.30	9.45	11.61	11.62	0.34	NS	NS	NS	10.80	10.42	11.96	NS	NS
C2:C3 (0 hr)	4.96	5.25	5.99	6.24	6.48	6.42	0.32	NS	NS	NS	5.60	5.87	6.20	NS	NS
C2:C3 (4 hrs)	5.63	4.58	5.96	5.54	6.50	5.54	0.32	NS	NS	NS	5.59	5.54	5.75	NS	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; CP = crude protein; UIP = undegradable intake protein; NH_3-N = ammonia nitrogen; VFA = volatile fatty acid; BUN = blood urea nitrogen.

Table 4.5 Effects of crude protein and undegradable intake protein levels on counts of protozoa, fungi and bacteria.

	10% CP			12% CP			SEM	P-value							
	UIP levels (%)			UIP levels (%)				CP	UIP	CP× UIP	Contrast (UIP %)				
	15	25	35	15	25	35					15	25	35	L	Q
Protozoa (10^5 cells/ml)															
0 hr	1.26	0.74	0.83	1.39	1.11	0.14	0.15	NS	NS	NS	1.31	0.93	0.49	*	NS
4 hrs	1.25	0.85	0.93	1.48	1.31	0.98	0.14	NS	NS	NS	1.37	1.08	0.96	NS	NS
Fungi (zoospore,10^6 cells/ml)															
0 hr	1.12	0.58	0.97	0.43	1.13	0.37	0.88	NS	NS	NS	0.78	0.86	0.67	NS	NS
4 hrs	1.70	1.27	0.50	1.22	2.27	1.10	0.17	NS	NS	NS	1.46	1.77	0.80	NS	NS
Bacterial (10^9 cells/ml)															
0 hr	3.00	2.21	2.50	2.79	2.13	2.17	0.25	NS	NS	NS	2.90	2.17	2.34	NS	NS
4 hrs	4.16	3.33	3.33	4.29	4.13	3.55	0.34	NS	NS	NS	4.23	3.73	3.44	NS	NS
Proteolytic bacteria (10^5 cells/ml)															
0 hr	3.60	3.93	15.08	2.17	2.25	18.00	1.98	NS	*	NS	2.88^{B}	3.09^{B}	16.54^{A}	*	NS
4 hrs	3.33	4.50	14.30	2.50	2.08	16.92	1.63	NS	*	NS	2.92^{B}	3.29^{B}	15.61^{A}	**	NS
Amylolytic bacteria (10^5 cells/ml)															
0 hr	1.17	12.17	19.67	2.17	10.25	12.68	1.74	NS	*	NS	1.67^{B}	11.2^{AB}	16.18^{A}	*	NS
4 hrs	0.08	5.92	12.00	0.92	2.17	9.25	1.53	NS	*	NS	0.50^{B}	4.04^{AB}	16.63^{A}	*	NS
Cellulolytic bacteria (10^7 cells/ml)															
0 hr	15.67	12.33	6.00	18.00	11.00	6.33	1.24	NS	*	NS	16.83^{A}	11.67^{AB}	6.17^{B}	**	NS
4 hrs	16.67	14.00	6.67	24.00	9.33	7.67	1.51	NS	*	NS	20.33^{A}	11.67^{B}	7.17^{B}	**	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; CP = crude protein; UIP = undegradable intake protein;

Table 4.6 Effects of crude protein and undegradable intake protein levels on nitrogen metabolism.

	10% CP			12% CP			SEM	P-value								
	UIP level (%)			UIP level (%)				CP	UIP	CP× U IP	Contrast (CP)		Contrast (UIP)			
	15	25	35	15	25	35					10%	12%	15%	25%	35%	L	Q
N intake (g/d)	59.3	72.6	71.2	63.7	82.7	90.6	1.26	**	**	NS	67.7^{B}	79.0^{A}	61.5^{B}	77.6^{A}	80.9^{A}	**	*
Fecal N excretion (g/d)	16.1	22.7	22.3	15.7	25.9	28.6	0.71	*	**	NS	20.3^{B}	23.4^{A}	15.9^{B}	24.3^{A}	25.5^{A}	**	*
Fecal N excretion (%,of N intake)	27.1	31.2	31.3	24.6	31.3	31.6	0.78	NS	**	NS	30.0	29.7	25.5^{B}	31.8^{A}	32.3^{A}	**	NS
Urine N excretion (g/d)	22.4	21.0	19.7	27.1	26.1	25.3	0.90	*	NS	NS	21.0^{B}	26.2^{A}	24.8	23.6	22.5	NS	NS
Urine N excretion (%,of N intake)	37.8	29.0	27.7	42.6	31.6	27.9	1.46	NS	**	NS	31.1	34.8	41.6^{A}	30.0^{B}	27.2^{B}	**	NS
N-retained (g/d)	20.9	28.9	29.2	20.9	30.6	36.7	1.10	NS	**	NS	26.31	29.38	20.9^{B}	29.8^{A}	32.9^{A}	**	NS
N-retained (%, of N intake)	35.2	39.8	41.0	32.8	37.1	40.5	1.54	NS	*	NS	38.9	35.5	32.8^{B}	38.3^{A}	40.5^{A}	*	NS

$^{A–C}$ Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; CP = Crude protein; UIP = Undegradable intake protein; N= nitrogen.

Table 4.7 Effects of crude protein and undegradable intake protein levels on purine derivative excretion and microbe N synthesis.

	10% CP			12% CP			SEM	P-value								
	UIP level (%)			UIP level (%)				CP	UIP	CP× U IP	Contrast (CP)			Contrast (UIP)		
	15	25	35	15	25	35					10%	12%	15%	25%	35%	L	Q
Allantoin (mmol/d)	53.12	58.22	55.97	54.87	67.91	64.25	3.47	NS	NS	NS	55.8	62.3	54.0	63.1	60.1	NS	NS
Allantoin (%, of N intake)	89.59	80.22	78.65	86.18	82.13	70.95	3.93	NS	NS	NS	83.1	81.9	90.6	81.5	75.9	NS	NS
Uric acid (mmol/d)	8.50	6.82	7.34	10.78	8.54	11.67	0.97	NS	NS	NS	7.6	10.3	9.6	7.7	9.5	NS	NS
Uric acid (%, of N intake)	14.33	9.39	10.31	16.94	10.32	12.89	1.20	NS	NS	NS	11.2	13.5	14.6	12.2	10.3	NS	NS
Creatinine (mmol/d)	45.46	46.69	38.75	29.78	40.95	37.52	6.70	NS	NS	NS	43.6	36.1	37.6	43.8	38.1	NS	NS
Creatinine (%, of N intake)	76.67	64.33	54.44	46.78	49.52	41.43	7.8	NS	NS	NS	64.2	42.2	58.1	55.0	47.0	NS	NS
Tot PD (mmol/d)	61.62	65.03	63.31	65.65	76.45	75.92	4.05	NS	NS	NS	63.3	72.7	63.6	70.7	69.6	NS	NS
Tot PD (%, of N intake)	103.92	89.61	88.96	103.12	92.45	83.83	1.31	NS	NS	NS	94.6	95.4	105.2	91.5	88.1	NS	NS
PD absorbed (mmol/d)	53.03	54.00	51.65	57.81	68.50	66.16	4.56	NS	NS	NS	52.9	64.2	55.4	61.3	58.9	NS	NS
PD absorbed (%, of N intake)	89.44	74.41	72.58	90.81	82.84	73.05	5.36	NS	NS	NS	78.8	84.1	91.0	79.0	74.4	NS	NS
MNS (g/d)	38.55	39.26	37.55	42.03	49.80	48.10	3.32	NS	NS	NS	38.5	46.6	40.3	44.5	42.8	NS	NS
MNS (%, of N intake)	65.02	54.10	52.76	66.02	60.22	53.11	3.89	NS	NS	NS	57.3	61.2	66.2	57.7	54.1	NS	NS

A–C Means in same row with different superscript letters differ (P<0.05); SEM = Standard Error of Means; NS = Not Significantly different (P>0.05); * Means Significantly different (P<0.05); ** Means Significantly different (P<0.01); L=Linear, Q = Quadratic; CP = crude protein; UIP = undegradable intake protein; PD = purine derivative

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CHAPTER V

OVERALL DISCUSSION AND IMPLICATIONS

5.1 The optimal crude protein concentration requirement for growing Thai-indigenous beef cattle

The optimal CP concentration may correlate with beef cattle breed, protein sources and quality, basis of roughage and beef cattle growth periods. Although early data indicated the importance of protein in terms of maintenance and production status, as well as chronological age (NRC, 1924), optimizing CP levels is still a focus of modem research. In Experiment 1, diets containing 4.3%, 7.3% and 10.3% CP of DM, was fed to Thai-indigenous. The results indicated that dry matter intake (DMI), microbial nitrogen synthesis and N retained increased linearly ($P<0.05$) with increasing dietary CP levels. However, in Experiment 2, when the Thai-indigenous cattle were fed 10% and 12% CP of diet , the DMI, microbial nitrogen synthesis and N retained and average daily gain (ADG) were not affected by CP levels. This finding means that 10% CP of diet can provide adequate CP to meet the growing Thai-indigenous beef cattle maintenance and growth requirements when fed rice straw as roughage.

From the results we can know that the optimal CP level was 10% CP of DM in growing Thai-indigenous beef cattle. Similarly, Vasconcelos, Greene, Cole, Brown,

and McCollum (2006) observed the performance was not affected significantly ($P>0.05$) in steer fed 10.0%, 11.5% and 13.0% CP of DM and reported 10.0% might be optimum CP level in steers. On the other hand, the optimum CP requirement of growing Thai-indigenous beef cattle was lower than recommended by numerous researchers. Thomson, Prestoon, and Bertle (1995) suggested that the optimal CP level was between 12% and 13% of DM. Galyean and Gleghom (2001) reported that the mean CP level formulated by consulting nutritionists in the major cattle feeding areas of the U.S. was 13.3% of DM. Cole, Greene, McCollum, Mantgomeyer, and McBride (2003) demonstrated that steers fed the 14% CP diet tended ($P<0.1$) to have greater ADG and gain: feed (G: F) than steers fed the 12% CP diet. Gleghorn, Elam, Galyean, Duff, Cole, and Rivera (2004) and Cole, Clark, Todd, Richardson, Gueye, and McBride (2005) suggested that dietary CP concentration requirements of beef cattle for maximum rate of gain were approximately 11.5% of diet DM during the later stages of feeding.

Changes in feed processing and application of implant practices have influenced the determination of CP levels in beef cattle diets. Carbohydrate digestion in the rumen is the most accurate predictor of microbial protein synthesis (Russell, 1992), and as feedstuffs are more thoroughly processed, an increased need of dietary CP is necessary (Cooper, Milton, Klopfenstein, and Jordan, 2002). Gleghorn et al. (2004) reported CP concentration above 13% seemed detrimental to ADG. Furthermore, Erickson, Klopfenstein, Milton, and Herold (1999), Cooper et al. (2002), and Trenkle (2002) reported no adverse effects on cattle performance when dietary CP concentrations were decreased during the later stages of the feeding period with dry-rolled corn-based diets

5.2 The maintenance and growth protein requirement

5.2.1 The maintenance protein requirement in growing Thai-indigenous beef cattle

The maintenance protein requirement was 3.54 g CP/kg $BW^{0.75}$ or 2.27 MP/kg $BW^{0.75}$ (2.27 = 3.54x0.64, according to NRC, 1996) estimated by linear regression between nitrogen intake and nitrogen retained, which was lower than the crude protein requirement for *Bos Taurus* cattle suggested by NRC (1996) (5.94 g CP/kg $BW^{0.75}$) and ARC (1980) for British breed (4.42 g CP/kg $BW^{0.75}$). It was also lower than the figures 5.07 CP/kg $BW^{0.75}$ or 3.25g MP/kg $BW^{0.75}$ recommended by the Institute National de la Recherche Agronomique (INRA, 1988).

5.2.2 The growth protein requirement for growing Thai-indigenous beef cattle

Metabolizable protein provided by microbial synthesis and undegradable intake protein (NRC, 1996), the requirement of MP was estimated by linearly regression between MP intake (MP intake = ((UIP + microbe synthesis protein) ×0.8) and body weight gain, for 1 g/kg $BW^{0.75}$ gain was 0.34 g MP/kg $BW^{0.75}$ of growing Thai-indigenous cattle. It was lower than the figures suggested by Tangjitwattanachai and Sommart (2009) who reported that requirement for 1 g/kg $BW^{0.75}$ gain of Brahman was 0.56 g CP/kg $BW^{0.75}$ or 0.36 g MP/kg $BW^{0.75}$ (MP=CPx0.64, NRC, 1996) and Brahman crossbred was 0.59 g CP/kg $BW^{0.75}$ or 0.38 g MP/kg $BW^{0.75}$ (MP=CPx0.64, NRC, 1996). Thus, the efficiency of MP is greater than that on Brahman and Brahman crossbred.

5.3 The degradable intake protein requirement and optimizing the ratio of UIP to DIP for ADG

In Experiment 2, the ruminal fermentation and ruminal NH_3-N were not affected by 10% and 12% CP of DM diet. Furthermore, the ruminal fermentation, ruminal NH_3-N, microbial growth and ADG were not limited by 35% UIP of CP diet for growing Thai-indigenous beef cattle. This result indicated that 10% CP of DM and 35% UIP of CP diet containing 6.5% DIP of DM (10%×(1-35%)) can provide adequate N source for the requirement for ruminal fermentation and ruminal microbes growth. Thus, 6.5% DIP of DM is enough to meet the growth requirement for growing Thai-indigenous beef cattle. However, the NRC. (2000) suggested that the minimum requirement of DIP is 6.8% of dietary DM. Therefore, the Thai-indigenous DIP requirement was low than NRC (2000) recommendation. Moreover, recent research indicated that the optimum level of supplemental DIP varies with degree of corn processing. Cooper et al. (2002) compared three levels of processing, namely, steam flaking, dry rolling, and high moisture harvesting and grinding, which were fed in conjunction with varying levels of urea as the source of supplemental CP. The results showed that optimum levels of DIP were 6.3%, 10.1%, and 8.3% (DM) for dry rolled, high moisture, and steam-flaked diets, respectively.

The optimal ratio of UIP to DIP for ADG was 35 to 65 for growing Thai-indigenous. However, diets containing DIP to UIP ratio 55 to 45 are considered optimum regarding N retention in buffalo calves (Sultan, Javaid, Nadeem, Akhter, and Musfaha, 2009). Furthermore, Haddad, Mahmoud, and Talfaha (2005) reported that

the optimal ratio of UIP to DIP was 23 to 77 in lambs. Therefore, the optimal ratio of UIP to DIP may be variable of different ruminant species/breeds.

The optimal diet contained 10% CP level of DM and the ratio of UIP to DIP was 35: 65 for growing Thai-indigenous beef cattle. According to table 4.2, the DMI was 4.11 kg/d when the cattle was fed the diet contained 10 % CP and 35 % UIP (UIP: DIP= 35: 65). Thus, we can calculate CP intake was 411 g/d or 10.1g/d/kg $BW^{0.75}$, UIP intake was 143. 9 g/d or 3.54 g/d/kg $BW^{0.75}$ and DIP intake was 267.2 g/d or 6.67 g/d/kg $BW^{0.75}$ of growing Thai-indigenous beef cattle fed this optimal diets.

5.4 Application of the result

Good feeding management is efficient feeding management. To realize the maximal economic gains from Thai-indigenous beef cattle products, we should know their protein requirement. Providing adequate DIP is necessary for maximum microbial CP synthesis and a balance of DIP and UIP is necessary for maximum productivity (Shain, Stock, Klopfenstein, and Herold, 1998). Furthermore, the proper ratio of DIP to UIP should be used to maximize performance (Stock, Merchen, Klopfenstein, and Poos, 1981; Milton, Brandt, and Titgemeyer, 1997a, b).

For this reason, the result of protein requirement can be included in a table of protein requirement for Thai feeding standards. Moreover, it can guide producers in estimating and formulating the dietary protein, balancing the UIP and DIP requirement in Thai-indigenous beef cattle diet and obtaining maximal economic productivity of Thai-indigenous beef cattle.

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APPENDIX

1) The determination of purine derivative (PD) by High Performance Liquid Chromatography (HPLC)

Column : Hypusil ODS (length : 250 mm, diamenter : 4.0 mm), particle sige : 5.0 μ m. Solution A : $NH_4H_2PO_4$. Solution B : $NH_4H_2PO_4$ +ACN, pH: 6.0-6.3. The wave peak area of PD standard and sample as fellowing :

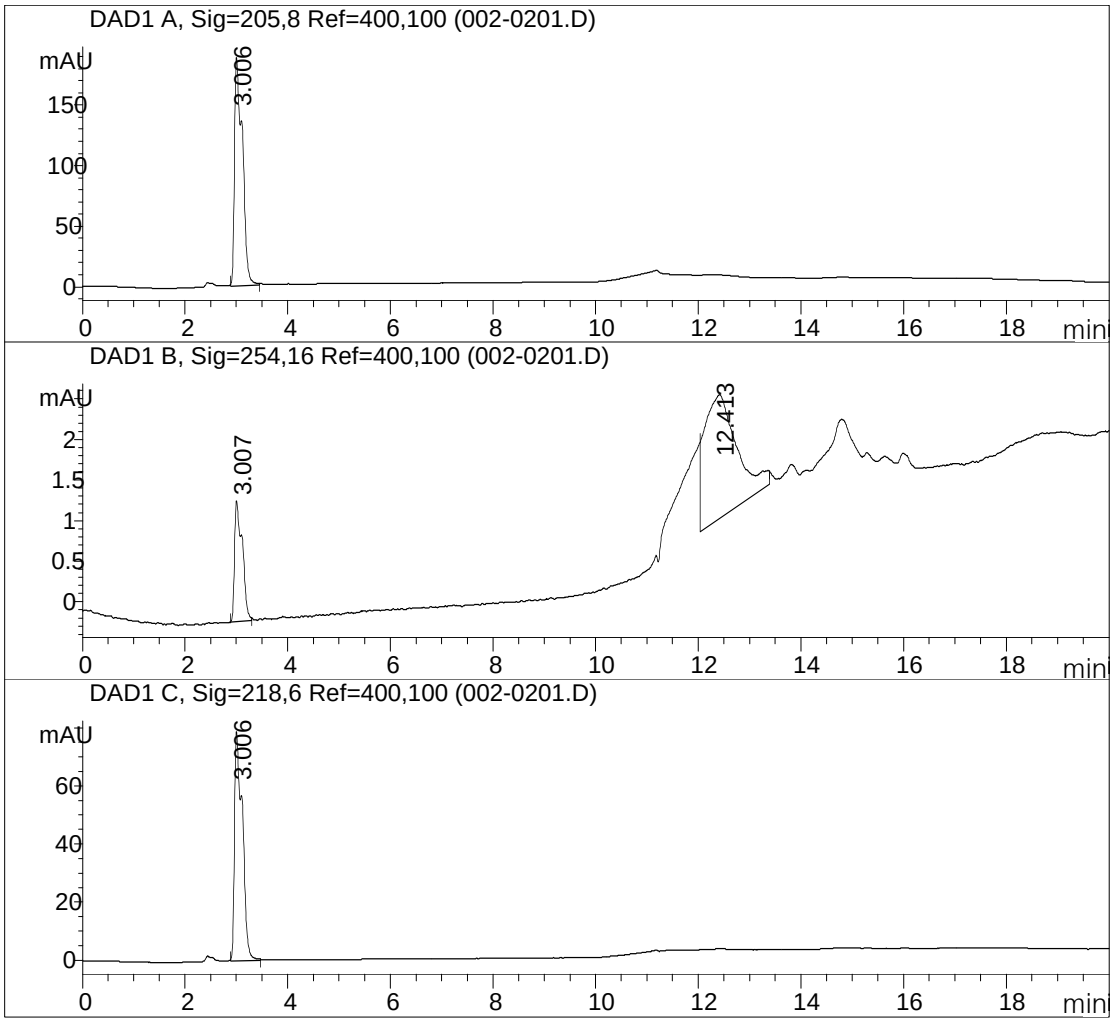

Figure A1 The peak area of standard of allantoin.

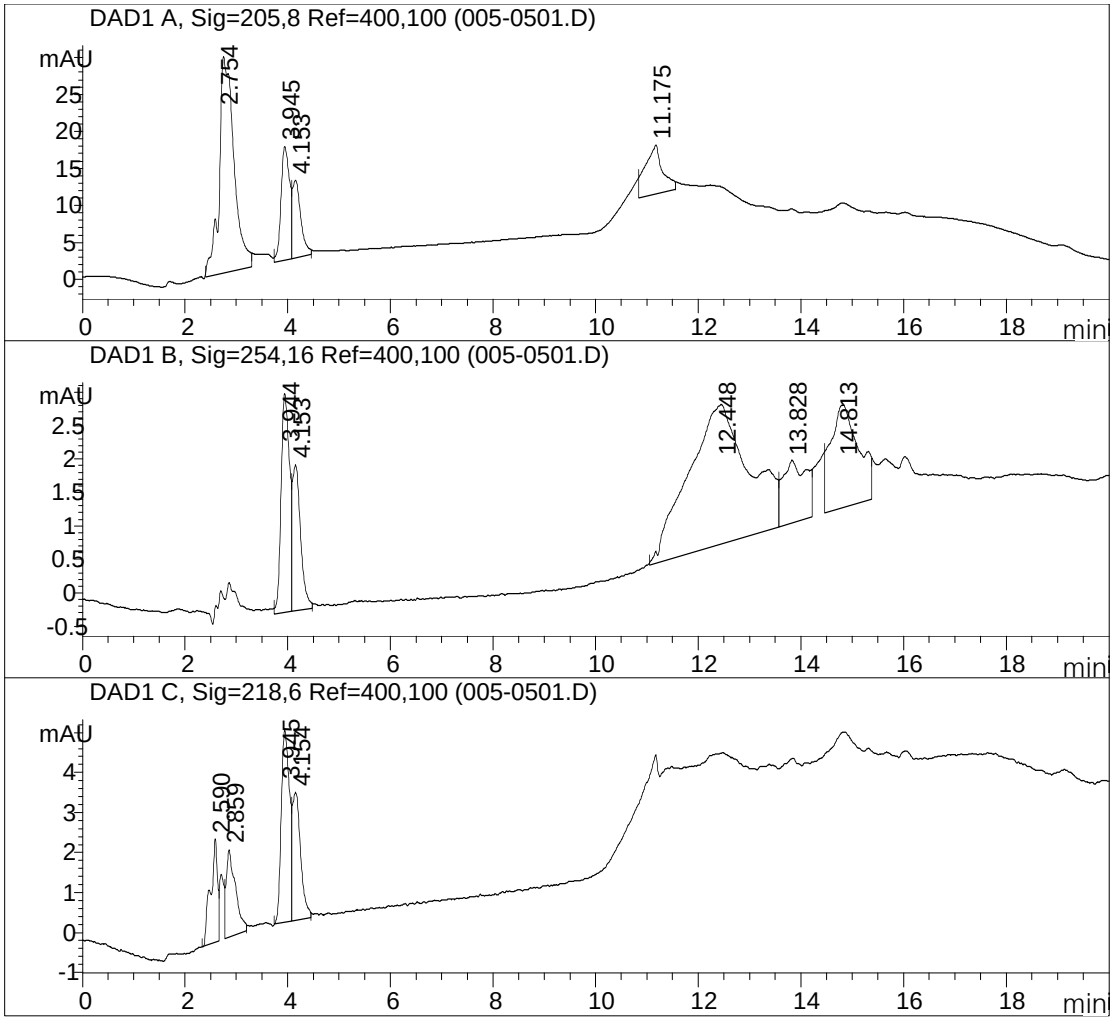

Figure A2 The peak area of standard of uric acid.

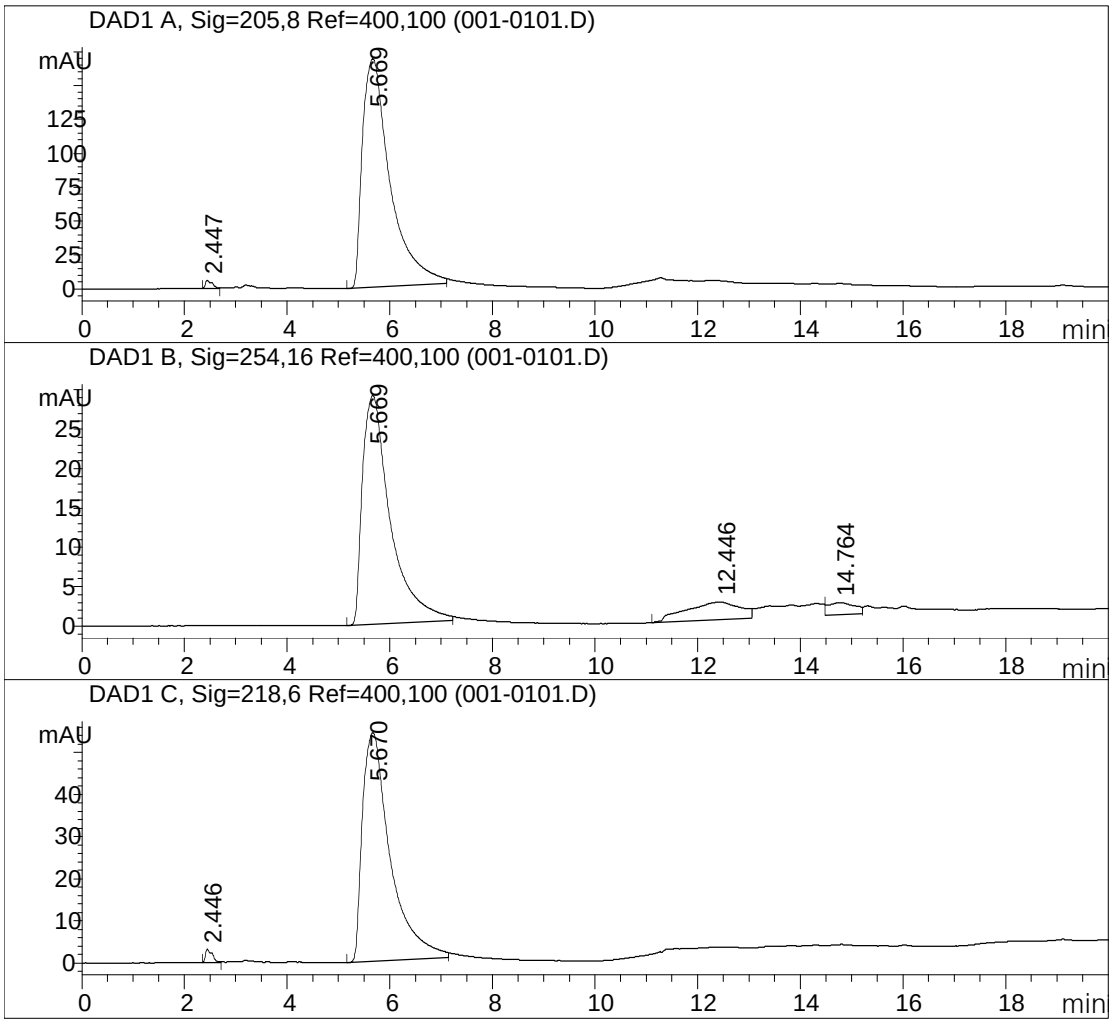

Figure A3 The peak area of standard of Creatinine.

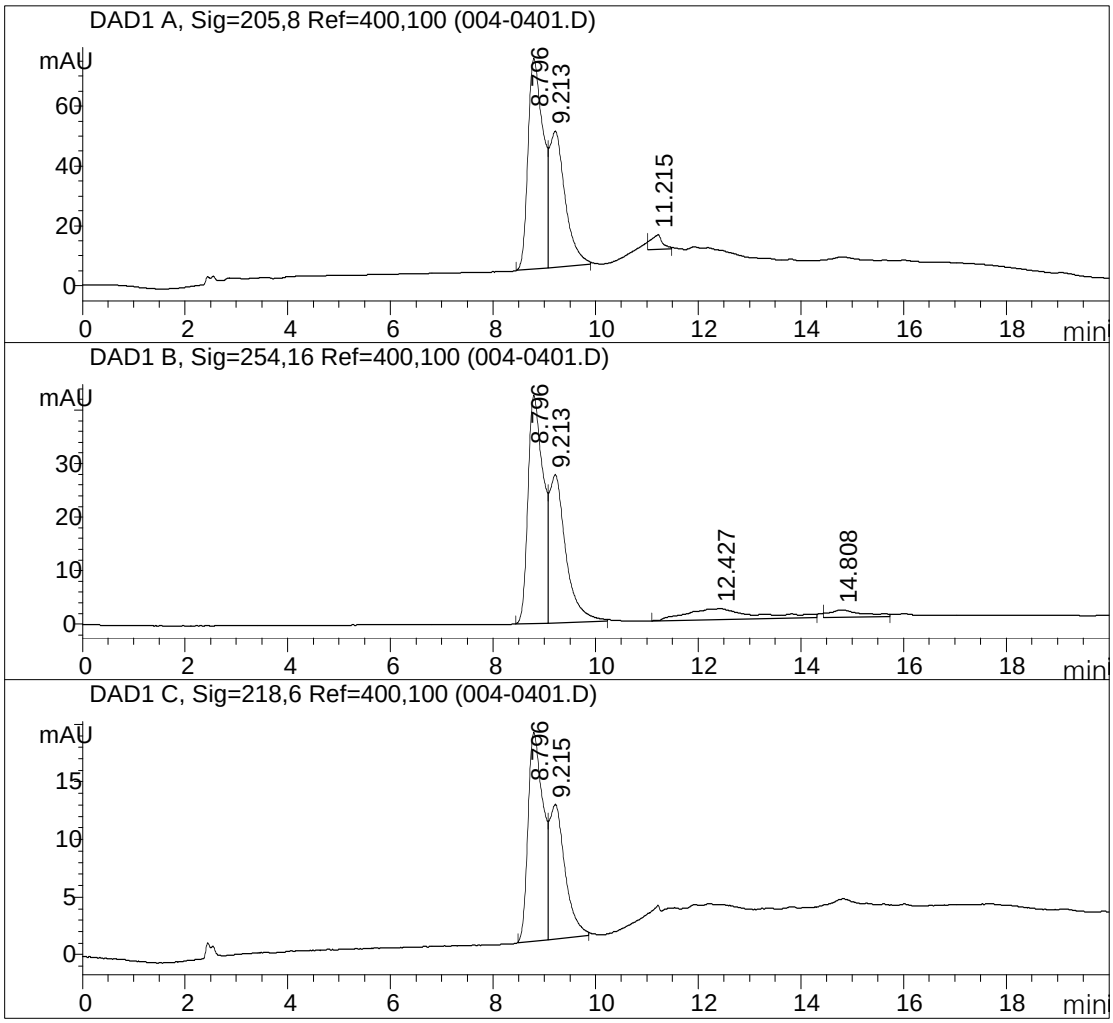

Figure A4 The peak of standard of hypoxanthine.

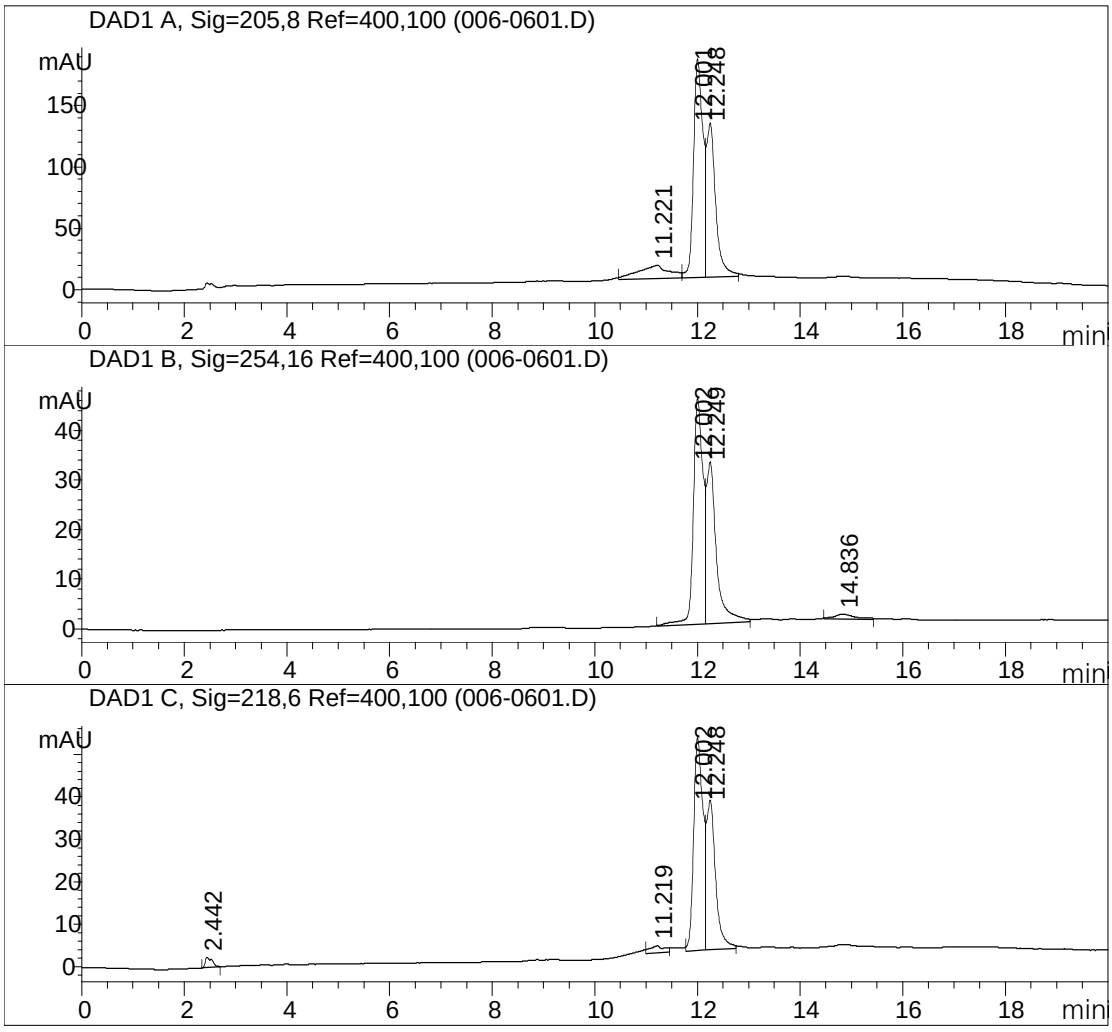

Figure A5 The peak area of standard of xanthine.

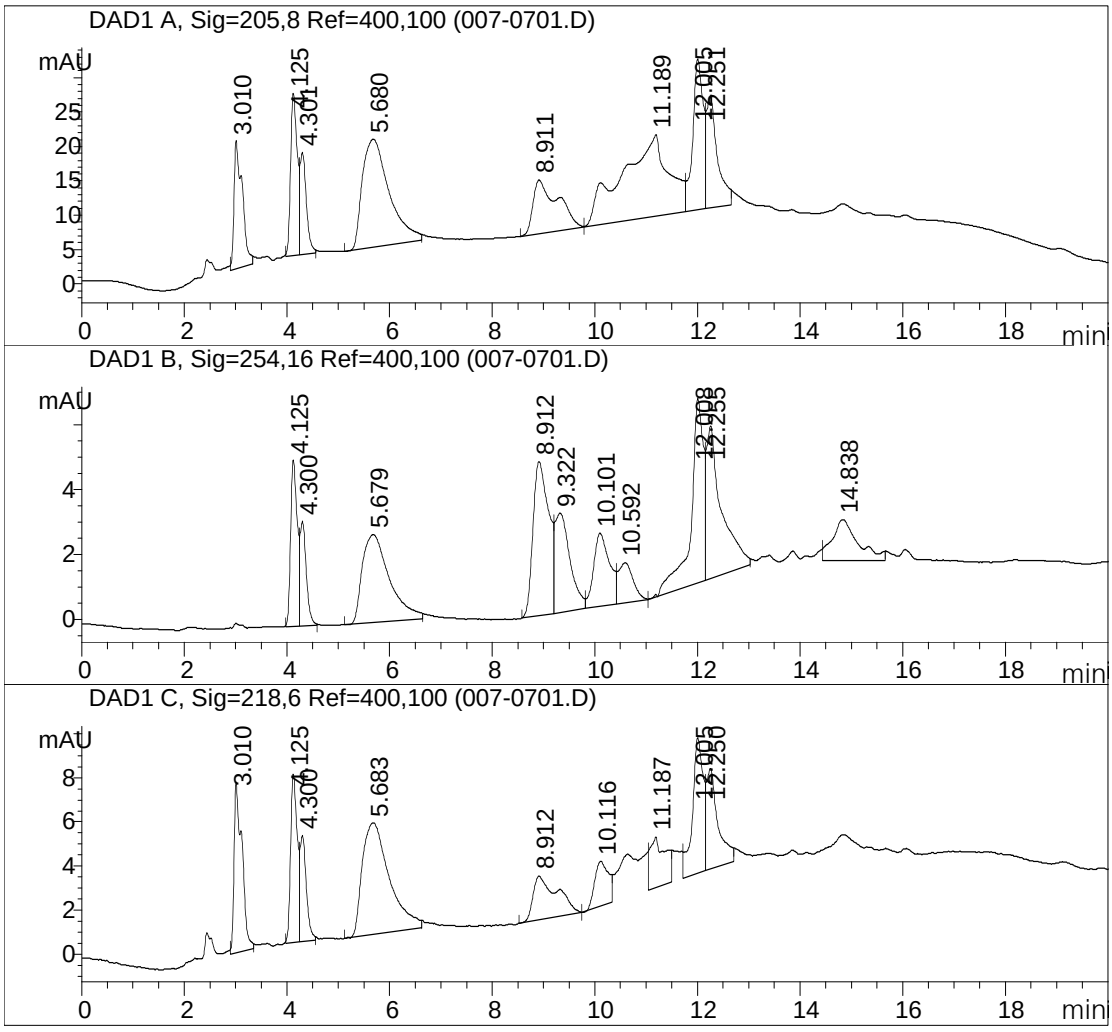

Figure A6 The peak area of standard of mixture.

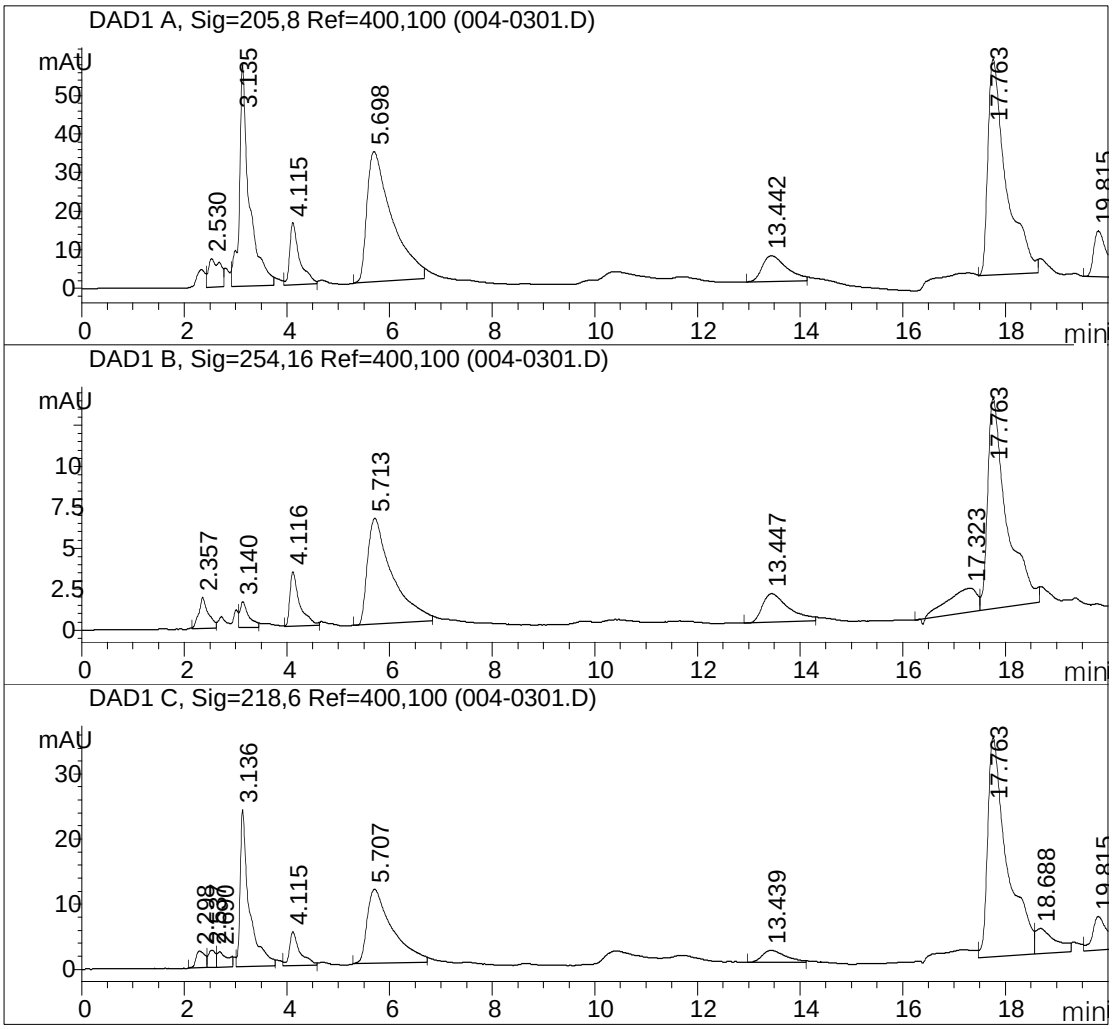

Figure A7 The peak area of sample.

BIOGRAPHY

Mr. Shengchang Chen, born on April 27th, 1971 in Tongren, Guizhou province, China. He has taught animal anatomy and animal nutrition since 1994 at the School of Animal Science, Guihzou University, China. He holds a BA degree in Veterinary Science from Northwest University of Agriculture and a M.S degree in Animal Nutrition from Guizhou University. Mr. Chen is currently a Ph.D. candidate in Ruminant Nutrition under the supervision of Asst. Prof. Dr. Pramote Paengkoum in the School of Animal Production Technology, Suranaree University of Technonogy, Thailand. To date, he has published seven research papers in the field of animal nutrition.