STUDY ON PROTEIN REQUIREMENTS OF GROWING MALE THAI SWAMP BUFFALOES

Pattaraporn Tatsapong

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

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การศึกษาความต้องการโปรตีนของกระบือปลักไทยเพศผู้ระยะรุ่น

นางสาวภัทรภร ทัศพงษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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STUDY ON PROTEIN REQUIREMENTS OF GROWING MALE THAI SWAMP BUFFALOES

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

Thesis Examining Committee

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

_____
(Asst. Prof. Dr. Pramote Paengkoum)
Member (Thesis Advisor)

_____
(Assoc. Prof. Dr. Opart Pimpa)
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_____
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วัตถุประสงค์ของงานวิจัยนี้คือ การศึกษาผลของโปรตีนต่อการใช้ประโยชน์ได้ของไนโตรเจน, กระบวนการหมัก, จำนวนประชากรจุลินทรีย์ในรูเมน และการประเมินความต้องการโปรตีนเพื่อการดำรงชีพ และเพื่อการเจริญเติบโตของกระบือปลักไทยเพศผู้ระยะรุ่น

การทดลองที่ 1 กระบือปลักไทยเพศผู้ระยะรุ่นจำนวน 4 ตัว น้ำหนักตัวเฉลี่ย 209 ± 17.7 กิโลกรัม และอายุเฉลี่ยประมาณ 12 ถึง 18 เดือน ใช้แผนการทดลองแบบ 4 x 4 ลาตินสแควร์ กลุ่มทดลองคือ ระดับของโปรตีนหยาบในสูตรอาหาร 4 ระดับ คือ 5, 7, 9 และ 11 เปอร์เซ็นต์ของวัตถุแห้ง และทุกสูตรอาหารมีการสมดุลพลังงาน โดยให้มีพลังงานที่ใช้ประโยชน์ได้มากกว่าความต้องการเพื่อดำรงชีพ 20 เปอร์เซ็นต์ ผลการทดลองพบว่า ระดับโปรตีนหยาบในอาหารเพิ่มขึ้น มีผลทำให้ สมดุลไนโตรเจน, ความเข้มข้นของยูเรียในกระแสเลือด, แอมโมเนียไนโตรเจนและกรดไขมันระเหยได้ง่ายในรูเมน และการสังเคราะห์จุลินทรีย์เพิ่มขึ้นเป็นเส้นตรงอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) อย่างไรก็ตามระดับโปรตีนหยาบในอาหารที่เพิ่มขึ้นไม่มีผลทำให้ค่าความเป็นกรด-ด่าง, จำนวนประชากรของจุลินทรีย์ในรูเมน, การกินได้และการย่อยได้ของโภชนะเปลี่ยนแปลง ($P>0.05$) ยกเว้นการกินได้และการย่อยได้ของโปรตีนหยาบของกระบือ ผลจากการทดลองนี้ พบว่าความต้องการโปรตีนเพื่อการดำรงชีพของกระบือปลักไทยเพศผู้ระยะรุ่นคือ 4.63 กรัมของโปรตีนหยาบต่อกิโลกรัมน้ำหนักเมแทบอลิคต่อวัน

การทดลองที่ 2 กระบือปลักไทยเพศผู้ระยะรุ่นจำนวน 16 ตัว น้ำหนักตัวเฉลี่ย 233 ± 25.0 กิโลกรัม และอายุเฉลี่ยประมาณ 18 ถึง 24 เดือน ใช้แผนการทดลองแบบสุ่มในบล็อกสมบูรณ์ กลุ่มทดลองคือ ระดับของโปรตีนหยาบในสูตรอาหาร 4 ระดับ คือ 1.0, 1.4, 1.8 และ 2.2 เท่าของความต้องการโปรตีนหยาบเพื่อการดำรงชีพ และในทุกสูตรอาหารมีการสมดุลพลังงานโดยให้มีพลังงานที่ใช้ประโยชน์ได้เท่ากับความต้องการของสัตว์ที่โตวันละ 0.5 กิโลกรัมต่อวัน ผลการทดลองพบว่าระดับโปรตีนหยาบในอาหารเพิ่มขึ้น มีผลทำให้การกินได้และการย่อยได้ของโภชนะ, อัตราการเจริญเติบโต, สมดุลไนโตรเจน, จำนวนประชากรของจุลินทรีย์ในรูเมน, ความเข้มข้นของยูเรียในกระแสเลือด, แอมโมเนียไนโตรเจนและกรดไขมันระเหยได้ง่ายในรูเมน และการสังเคราะห์จุลินทรีย์ในรูเมนของลูกกระบือ เพิ่มขึ้นเป็นเส้นตรงอย่างมีนัยสำคัญทางสถิติ อย่างไรก็ตามระดับโปรตีนหยาบในอาหารที่เพิ่มขึ้นไม่มีผลทำให้ค่าความเป็นกรด-ด่างในรูเมนเปลี่ยนแปลง ผลจากการทดลองนี้ พบว่าความต้องการโปรตีนเพื่อการดำรงชีพของกระบือปลักไทยเพศผู้ระยะรุ่นคือ 5.41 กรัมของโปรตีนหยาบต่อกิโลกรัมน้ำหนักเมแทบอลิคต่อวัน และความต้องการโปรตีนเพื่อการเจริญ

เติบโตของกระบือปลักไทยเพศผู้ระยะรุ่นคือ 0.46 กรัมของโปรตีนหยาบต่อกรัมของอัตราการเจริญโตต่อวัน

การทดลองที่ 3 กระบือปลักไทยเพศผู้ระยะรุ่นจำนวน 24 ตัว น้ำหนักตัวเฉลี่ย 205 ± 45.6 กิโลกรัม และอายุเฉลี่ยประมาณ 12 ถึง 36 เดือน จัดการทดลองแบบ 2 x 3 แฟกทอเรียล ในแผนการทดลองแบบสุ่มในบล็อกสมบูรณ์ ปัจจัยศึกษาที่หนึ่งคือ ระดับของโปรตีนหยาบในสูตรอาหาร 2 ระดับ คือ 6 และ 12 เปอร์เซ็นต์โปรตีนหยาบ และปัจจัยศึกษาที่สองคือ ระดับพลังงานที่ใช้ประโยชน์ได้ 3 ระดับ คือ 1.0, 1.4 และ 1.8 เท่าของความต้องการเพื่อการดำรงชีพของพลังงานที่ใช้ประโยชน์ได้ ผลการทดลองพบว่า การเพิ่มขึ้นของทั้งระดับโปรตีนหยาบและระดับพลังงานที่ใช้ประโยชน์ได้ในอาหาร มีผลทำให้การกินได้และการย่อยได้ของโภชนะ, อัตราการเจริญเติบโต, ไนโตรเจนเมแทบอลิซึม, ความเข้มข้นของกรดไขมันระเหยได้ง่ายในรูเมน และการสังเคราะห์จุลินทรีย์ของกระบือ เพิ่มขึ้นเป็นเส้นตรงอย่างมีนัยสำคัญทางสถิติ และพบว่ามีปฏิกิริยาสัมพันธ์ระหว่างระดับโปรตีนหยาบและระดับพลังงานที่ใช้ประโยชน์ได้ต่อการกินได้และการย่อยได้ของโปรตีนหยาบ, ความเข้มข้นของกรดไขมันระเหยได้ง่ายในรูเมน และการสังเคราะห์จุลินทรีย์ อย่างไรก็ตามทั้งระดับโปรตีนหยาบและพลังงานที่ใช้ประโยชน์ได้ในอาหารที่เพิ่มขึ้นไม่มีผลทำให้ค่าความเป็นกรด-ด่างในรูเมน, จำนวนประชากรของจุลินทรีย์ในรูเมนเปลี่ยนแปลง ผลจากการทดลองนี้ พบว่าความต้องการโปรตีนเพื่อการดำรงชีพของกระบือปลักไทยเพศผู้ระยะรุ่นคือ 3.12 กรัมของโปรตีนหยาบต่อกิโลกรัมน้ำหนักเมแทบอลิคต่อวัน และความต้องการโปรตีนเพื่อการดำรงชีพของกระบือปลักไทยเพศผู้ระยะรุ่นคือ 0.61 กรัมของโปรตีนหยาบต่อกรัมของอัตราการเจริญโตต่อวัน

จากการทดลองทั้งสามงานนี้ สามารถนำข้อมูลมาประมวลและประเมินหาความต้องการโปรตีนของกระบือปลักไทยเพศผู้ระยะรุ่นได้ดังนี้ คือความต้องการโปรตีนเพื่อการดำรงชีพ และเพื่อการเจริญเติบโต คือ 4.64 กรัมของโปรตีนหยาบต่อกิโลกรัมน้ำหนักเมแทบอลิคต่อวัน และ 0.50 กรัมของโปรตีนหยาบต่อกรัมของอัตราการเจริญโตต่อวัน ตามลำดับ

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2552 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

PATTARAPORN TATSAPONG : STUDY ON PROTEIN REQUIREMENTS OF GROWING MALE THAI SWAMP BUFFALOES. THESIS ADVISOR : ASST. PROF. PRAMOTE PAENGKOUM, Ph.D., 201 PP.

SWAMP BUFFALO/PROTEIN REQUIREMENT/MICROBIAL PROTEIN SYNTHESIS/NITROGEN RETENTION/NITROGEN BALANCE

The objective of this study was to investigate the effects of dietary protein on nitrogen utilization, rumen fermentation, rumen microbes, and estimation of protein requirement for maintenance and growth of growing male Thai swamp buffaloes.

In experiment I, four growing male entire (bulls) swamp buffaloes, with an average initial weight of 209 ± 17.7 kg and approximate age of 12 to 18 months, were randomly assigned in a 4 x 4 Latin Square Design. The treatments consisted of four levels of crude protein (CP) in the diets (5, 7, 9 and 11% of dry matter, DM); all diets were isocaloric (20% above maintenance of metabolizable energy, ME). The results showed that increasing the level of CP in diet, nitrogen balance, concentration of blood urea nitrogen (BUN), ammonia-N (NH_3-N) and total volatile fatty acids (VFAs) in the rumen, and microbial N synthesis were noticed to have increased linearly ($P<0.05$). However, increasing the levels of CP in the diet did not significantly ($P>0.05$) affect ruminal pH, rumen microbial counts, nutrients intake, and digestibility, except for CP intake and digestibility of the buffaloes. These findings suggest that the CP requirements for maintenance of growing male Thai swamp buffaloes are 4.63 g CP/kg $W^{0.75}$/d.

In experiment II, sixteen growing male entire (bulls) swamp buffaloes, with an average initial weight of 233 ± 25.0 kg and approximate age of 18 to 24 months, were assignd in a Randomized Complete Block Design (RCBD). The treatments comprised four levels of CP for maintenance (M) in the diets of 1.0M, 1.4M, 1.8M and 2.2M of DM and all diets were isocaloric (0.5 kg, expected body weight gain of buffalo of ME for maintenance). The results indicated that as the levels of CP in diet increased, intake and digestibility of nutrients, growth rate, N balance, rumen microbial counts, concentration of BUN, NH_3-N and VFAs in the rumen, and microbial N synthesis were noticed to have also increased linearly ($P<0.05$). However, ruminal pH of the buffalo was not affected by increasing the levels of CP in the diet. These present findings suggest that the protein requirements for maintenance and growth of growing male Thai swamp buffaloes are 5.41 g CP/kg $W^{0.75}$ and 0.46 g CP/g ADG, respectively.

In experiment III, twenty-four growing male entire (bulls) swamp buffaloes, with an average initial weight of 205 ± 45.6 kg and approximate age of 12 to 36 months, were used in a RCBD with 2 x 3 factorial arrangement. Factor 1 had two levels of CP (6 and 12% of DM), and factor 2 had three levels of ME (1.0, 1.4 and 1.8 time of ME requirement for maintenance). Increasing the levels of either CP or ME significantly increased ($P<0.05$) ADG, nutrients intake and digestibility, VFAs concentration, N metabolism, and microbial N synthesis of buffaloes. Significant ($P<0.05$) interactions were found between levels of CP and ME for CP intake and digestibility, and VFA concentrations, N metabolism and microbial N synthesis of the buffaloes. However, ruminal pH and microbial counts of the buffaloes were not affected by increasing the levels of neither CP nor ME in the diet. From this study, it

was found that the protein requirements for maintenance and growth of growing male Thai swamp buffaloes are 3.12 g CP/kg $W^{0.75}$ and 0.61 g CP/g ADG/d.

Based on the three experiments conducted in this research, it can be concluded that the protein requirements for maintenance and growth of growing male Thai swamp buffaloes are 4.64 g CP/kg $W^{0.75}$ and 0.50 g CP/g ADG, respectively.

School of Animal Production Technology

Academic Year 2009

Student's Signature_____

Advisor's Signature_____

Co-advisor's Signature_____

Co-advisor's Signature_____

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Pattaraporn Tatsapong

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CHAPTER I

INTRODUCTION

Buffaloes can be categorized into two groups, namely Mediterranean and Asian buffaloes. The Asian buffalo (water buffalo) (*Bubalus bubalis*) species is further divided into two types (subspecies), the swamp buffalo (*B. bubalis carabanesis*) and the riverine (river) buffalo (*B. bubalis bubalis*) (En.wikipedia, www, 2010). The largest concentrations of swamp buffaloes are found in the rice-growing countries of South East Asia. Swamp buffaloes are light in weight, with a mature weight of approximately 700 kg for males and 500 kg for females and milk production average from 430 to 720 kg per lactation. The river buffalo is heavier than the swamp buffalo with a mature weight of approximately 1100 kg for males and 550 kg for females, and milk production ranging from 1000 to 2000 kg per lactation (Marai and Haeeb, 2009). River buffaloes are mainly found in India, Pakistan and in some countries of western Asia. Sarwar, Khan, Nisa, Bhatti and Shahzad, (2009) reported that the swamp and river buffaloes possess different genetics (48 vs. 50 chromosomes, respectively), morphology (body frame, body weight, horn shape and skin color) and behavior (wallowing in mud or water). They are reared for different purposes. Swamp buffaloes are stocky animals with marshy land habitats and are primarily used for draught power but are also used to produce meat and in some places small quantities of milk. River buffaloes are generally larger in size, heavier with curled horns and are primarily used for milk and meat production.

In the developing world, ruminants have an important role in the sustainability of village communities, and in many cases form the major source of income. Livestock production in tropical areas plays a crucial role, which extends beyond its traditional supply of meat and milk. Swamp buffaloes are used for multiple purposes such as draft power, means of transportation, capital, credit, meat, milk, social value, hides, and a source of organic fertilizer for seasonal croppings (Chantalakhana, 2001). Buffaloes raising as part of small-farm agricultural systems has positive effects on the concept of social and economic sustainability, and consequently on rural development (Wanapat, 1990).

In the Topics, most ruminants particularly buffaloes, are fed on low-quality roughages, agricultural crop-residues and industrial by-products which contain high levels of lingo-celluosic materials, low levels of fermentable carbohydrates and low levels of good-quality protein (Wanapat and Rowlinson, n.d.). Buffaloes are better converters of poor-quality fibous feeds into milk and meat, with better degradation of both crude protein and protein free dry matter than in cattle (Terramoccia, Bartocci, Amici, and Martillotti, 2000). Calabro et al. (2008) found that buffalo used a greater proportion of OM for biomass production at the expense of VFAs compared with cattle. The differences in buffalo and cattle rumen fermentation can be explained with a different microbial activity of the two ruminant breeds. There are different amounts of microbial population constituted by different species of bacteria and protozoa. Bacteria populations including *F. succinogenes, R. albus* and *R. flavefaciens* of buffaloes were significantly higher than those found in cattle fed similar diets (Wora-anu, 2006). The observations from Granum, Wanapat, Pakdee, Wachirapakorn and Toburan (2007) reported that the number of fungi (fungal zoospores) were

significantly higher in the rumen fluid of the buffalo than in those of cattle. Other works have also indicated that buffaloes have a better digestive ability than cattle to utilize poor quality roughage (Bartocci, Amici, Verna, Terramoccia and Martillotti, 1997; Agarwal, Kamra, Chatterjee, Ravindra Kumar, and Chaudhary, 2008; Hussain and Cheeke, 1996).

Buffaloes, like other domesticated ruminants, meet their protein and energy requirements from fermentation end-products (microbial protein and volatile fatty acids) (Sarwar et al., 2009). In order to meet their production potential, buffaloes have to consume the required amounts of energy and proteins (Chanjula, Wanapat, Wachirapakorn and Rowlinson, 2004). Protein and energy feeds are necessary for growing and finishing buffalo in order to maximize the potential of meat production (Basra et al., 2003b). Sarwar et al. (2009) suggested that balanced nutrition and better management can enhance buffalo productivity. In particular, protein is an essential nutrient for animal growth and development, and a sufficient protein supply is a crucial factor for normal growth (Hwangbo et al., 2009). Recent research on locally available feed resources such as crop residues, and industrial by-products, dietary addition of micronutrients, feed technology, use of performance modifiers and use of ruminally protected fat and protein sources, have shown a significant potential to improve growth, milk yield and reproductive performance of buffaloes (Sarwar et al., 2009; Verma Mehra, Dass and Singh, 1996). Animal growth and nutrition interact with one another in the sense that each can influence the other; the growth pattern of an animal determines its nutrient requirements (McDonald, Edwards, Greenhalgh, and Morgan, 1995). Hence, in these changing perspectives of livestock production, there is an absolute need to redefine the protein requirement of animals keeping in mind

their growth potential (Lohakare, Pattanaik and Khan, 2006). An optimum growth rate and feed utilization efficiency according to inherent genetic potentiality of a particular category of animal, can be achieved only through the accurate evaluation of their nutrient requirements (Paul and Patil, 2007).

Feeding standards for livestock varies between countries. Because of this, the same feed is often valued in a different manner. Even the underlying assumptions of the basal diet for cattle calves differ amongst countries, which can further influence the feed value of a particular feedstuff and of the nutrients contained in the feed. The main feeding standards having widespread acceptance for various categories of livestock are National Research Council (NRC) recommendation of the United States and Agricultural Research Council (ARC) recommendation of the United Kingdom and for developing countries (Kearl, 1982). Nutrient needs of buffaloes in tropical regions probably differ from cattle breeds of temperate regions because of differences in genetic make–up, breed size, production level, quality of feeds, climate conditions and digestive physiology (Paul and Patil, 2007; Singh, Kundu, Kushwaha and Maity, 2009).

Protein requirements for maintenance and growth are of fundamental importance in the correct feeding of any animals, and there is an urgent need to establish protein requirements of swamp buffaloes. Furthermore, research on the nutrient requirements of nutrient of growing Thai swamp buffaloes does not exist and the limited information available indicates that buffaloes are good at converting low quality feed into body weight gain. The present study was to designed to investigate protein requirements for maintenance and growth of growing Thai swamp buffaloes

employing regression analysis models on nutrient intake and performance data generated through a feeding trial conducted under a tropical environment.

1.1 Research Objectives

1.1.1 To determine the protein requirements for maintenance of growing Thai swamp buffaloes.

1.1.2 To determine the protein requirements for growth of growing Thai swamp buffaloes.

1.1.3 To study the effect of dietary crude protein on the performance of growing Thai swamp buffaloes.

1.2 Research Hypothesis

1.2.1 The protein requirements of growing Thai swamp buffaloes are the same as the recommendation by Kearl (1982) for buffaloes.

1.2.2 The protein requirements of growing Thai swamp buffaloes are different to the recommendation by NRC (1996) for beef cattle.

1.2.3 Dietary crude protein may increase microbial protein flow to the duodenum and improve the performance of growing Thai swamp buffaloes.

1.3 Scope and Limitation of the Study

This research was conducted to investigate the effects of dietary crude protein in total mixed ration on rumen fermentation, nutrient utilization, microbial protein synthesis, nitrogen metabolism and growth response of growing male Thai swamp buffaloes. In addition, the effects of dietary crude protein and energy levels on the performance and protein requirements of growing male Thai swamp buffaloes were studied.

1.4 Expected Results

1.4.1 To obtain the protein requirements for maintenance and growth of growing male Thai swamp buffaloes.

1.4.2 To know the effects of dietary protein on performance and growth response of growing male Thai swamp buffaloes.

1.4.3 Increased microbial protein synthesis, nutrient utilization and average daily gain of growing male Thai swamp buffaloes may occur when increasing the protein content in the diet.

CHAPTER II

LITERATURE REVIEW

2.1 Buffalo Production in Thailand

Thai swamp buffaloes have been raised from generation to generation as an important source of draft for rice and other field crop cultivation. Swamp buffaloes are mostly raised by smallholder farmers, who rely on subsistence farming. Buffaloes are usually looked after by the women, old folks and children, who are not employed otherwise. The buffaloes have an important role in the sustainability of mixed crop-livestock farming systems and are a major source of farmer's income (Chantralakna, 2001). Buffaloes provide meat, draft power, and manure as fertilizer. Buffalo meat is well-consumed by many local people (Wanapat, Sommart, Wachirapakorn, Uriyapongson and Wattanachant, 1994). Buffaloes have been incorporated as a vital component in these sustainable agricultural production systems. They continue to provide renewable resources in terms of draft power and manure, while meat or milk are secondary products in these systems. In Thailand, rice productivity during the last three or four decades has been sustained at a constant level, while chemical fertilizer use has been minimal. Thai farmers in rain-fed areas have been using cattle and buffalo manure to maintain soil fertility. Manure is spread on to paddy fields in the early rainy season and ploughed down before rice planting. Besides being used as fertilizer, in India and Pakistan, manure has also been used for other purposes such as burning fule, and construction materials. These features are among the many

complicated and delicate interrelationship between crops and animals in sustainable agricultural production systems on small farms in tropical rural areas.

About 60-70% of Thailand's buffaloes are raised by farmers in the Northeast (NE) region of Thailand (Chantalakhana, 2001). Village farmers generally raise 2 to 5 buffaloes and grazing areas are available in paddy fields. In general, buffaloes and village cattle depend mainly on rice straw and stubble for feed, while other crop residues such as corn stalks, cassava and kenaf leaves, also provide substantial sources of roughage, especially during the dry season. Animals are generally kept under the house during the night where they are more protected from thieves.

As reproductive efficiency is low with a long production cycle, the buffalo population has dramatically decreased in recent years, from 3.3 million in 1996 to 1.5 million in 2003 (AOE, 2005). The rate of the decline was 11.5% per year. The major constraints leading to low productivity can be demonstrated by Mondal, Prakash and Palta (2007), who explained that late maturity, silent heat, coupled with poor expression of oestrus, irregular oestrous cyclicity, seasonality in breeding, anoestrus, low conception rate, long postpartum interval and repeat breeding are peculiar problems in buffaloes. The decline in buffalo number is also partly related to on increased use of small (two-wheel) tractors in villages. The Thai government has supported the mechanization in agricultural production by offering loan credits to farmers for small tractor purchase through the government's Bank of Agriculture and Agricultural Cooperatives (BAAC). Farmers also sell some of their buffaloes for cash.

Other factors affecting the changes in buffalo production were classified as internal and external. Internal factors have been the role of mechanization, farmer's debts, lack of household labor, grazing area factors, breedable bulls and the attitude of

the younger generation towards buffalo raising. External factors have included government policies and socioeconomic factors (Skunmun, Poondusit, Koga and Chantalakhana, 2000).

Another important aspect of sustainable integrated farming systems practiced by rural smallholders is the utilization by ruminant animals of crop wastes and by-products, such as rice straws, maize and sorghum stovers, pineapple wastes, sweet potato vines and groundnut vines. In some areas, where farmers do not raise buffaloes or other ruminants, they burn straw and stubble in order to dispose of them; this is wasteful, creates pollution, and sometimes causes serious accidents. Buffalo production offers an opportunity for poverty reduction in rural areas where living conditions and resources such as water and soil are less favorable, such as that in Northeast Thailand (Chantalakhana, 2001).

Nowadays, following the period after the 1997-1998 economic crisis in Thailand, a self-sufficient economy, a concept strongly emphasized by His Majesty the King of Thailand, has been promoted. In the mixed crop-livestock farming systems in rural areas, such as in the North-East (NE) region, buffaloes can be a major component contributing to the self-sufficiency features of the farming systems. Buffaloes are now of more interest to farmers, because the current supply of live buffaloes does not meet the demands within the country. As a result, the prices of live buffaloes have risen. The price is now nearly the price of crossbred beef cattle (35 vs. 43 bath/kg, for buffalo and beef cattle, respectively) (OAE, 2010). The numbers of buffalo have now increased by 0.2 million head from 2003 to 2005 (OAE, 2005). However, the number of buffaloes in Thailand form 2006 to 2009 are only 1.4 million head (DLD, 2009), lower than half the number twenty years ago. Consequently, one

to increase buffalo numbers solution is to produce buffaloes for sustainability before turning it into an industry. It could be possible to develop sustainable village buffalo systems and develop on industrial production system later.

2.2 Role of Protein and Amino Acids

Protein and amino acids play a prominent role in animal physiology. In addition to their obvious role as a part of the protoplasm and a constituent of hormones and enzymes, they constitute a large proportion of the dry weight of muscle, skin, blood and body secretions. However, it would be most difficult to pinpoint specific functions of proteins and amino acids because almost every organ/system in the body utilizes proteins or amino acids (Marai and Haeeb, 2009).

Protein is an essential nutrient for animals. This nutrient, however, cannot be synthesized in sufficient quantities by animals to meet their requirements. Fortunately, it is synthesized by plants and stored in plant cells. Through this means, a source of protein is provided for use by ruminants. Dietary source of nitrogen include nucleic acids, amino acids, protein, peptide, amines, amides, nitrates, nitrites, urea, and ammonia (Kearl, 1982), all proteins commonly used to feed cattle degrade into two or more fractions during rumen fermentation. With the exception of some proteins and N associated with ADF, these N source are readily soluble and susceptible to degradation in the rumen. Huntington and Archibeque (1999) noted that evolution of symbiosis among ruminal microbes and their host, as well as symbiosis among the microbes themselves, has placed ammonia as a major component of N metabolism in ruminants.

2.3 Protein Metabolism

The remaining dietary proteins and microbial proteins synthesized in the rumen, which escape ruminant breakdown and endogenous secretions, are present in the small intestine (Figure 2.1). Amino acids and peptides are made available for absorption, through hydrolytic and enzymatic processes occurring in the abomasums and upper small intestine. A primary requirement for protein metabolism is for the resynthesis of tissue protein and other nitrogen-containing-constituents, such as enzymes, hormones and milk. True absorption of amino acids of dietary and microbial origin lies, somewhere between 80 and 90%, although over processing (e.g. overheating) can reduce digestibility. Theoretically, formation of tissue protein is the reversal of the hydrolysis observed in the digestion process (Marai and Haeeb, 2009). Blood plasma proteins are primarily manufactured in the liver. A second fate of absorbed amino acids is that of deamination. Both the kidneys and the liver deaminate amino acids. The enzyme involved in this process is amino acid oxidase which is involved in the formation of keto acids plus free ammonia. Keto acids can be converted to fat and/or carbohydrate, re-synthesized into amino acids or oxidized to carbon dioxide and water. The liver represents the primary site of amino acid deamination and urea formation through an active urea cycle (Figure 2.1).

In ruminants, urea that enters the blood pool is synthesized from ammonia absorbed to blood circulation from the gastrointestinal tract and from the deamination processes of amino nitrogen breakdown. Functionally, urea leaves the body fluid pool by three main routes into urine: the rumen, the abomasums and intestine (Smith, 1989). In addition, the increase in urinary nitrogen excretion, as indicated by a negative nitrogen balance (Paengkoum and Tatsapong, 2009), may also contribute to

the decrease in rumen ammonia-N as a result of the decrease in each of serum urea level under such conditions. Paengkoum, Liang, Jelan and Basey (2006b) reported that urea was normally high when protein was in excess in the diet or there was a low energy/protein ratio. The average high level of urea was due to the low energy/protein ratio and to gluconeogenesis by protein degradation in conditions of insufficient energy for growth or milk production (Campanile, Filippo, Di Palo, Taccone and Zicarelli, 1998).

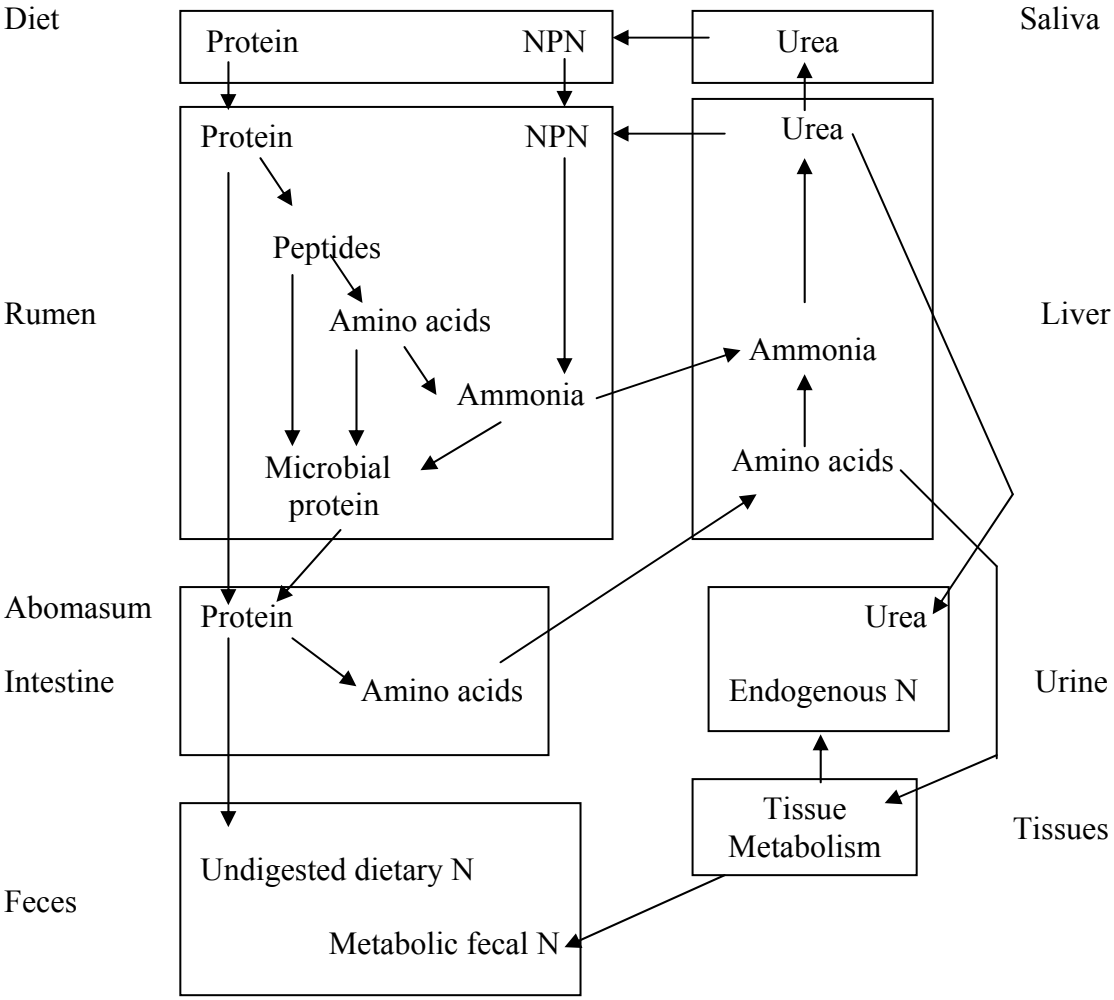

Figure 2.1 Fate of dietary crude protein in the ruminant animal.

Source : Adaptation from McDonald et al., 1995

Obitsu and Taniquchi (2009) demonstrated that lactating dairy cows show a higher gut entry of urea compared with growing cattle, while sheep and buffalo probably have higher abilities to reabsorb urea from the kidney compared with cattle.

Nitrogen retention indicates that more protein had been built than lost. The increase in body weight represents the increase in total body solids (dry body weight) and in the total body water. The total body solids includes dry body tissue, which could be fat, protein (lean body mass) and bones.

2.4 Microbial Protein in Rumen

In ruminants, their capacity to use their diet effectively relies on the presence of microorganisms in the rumen, reticulum and omasum to digest feedstuffs (Ørskov, 1992). The microbial processes of the rumen confer the ability to convert fibrous feeds and low-quality protein, even non-protein-nitrogen, into valuable nutrients for the ruminant animal (Dewhurst, Davies and Merry, 2000). It has been reported that duodenal flow of CP encompasses 3 major fractions: rumen undegradable protein (RUP), microbial protein and endogenous protein (Johnson, Harrison and Riley, 1998). These nitrogen sources are digested and absorbed mainly as amino acids for ruminants (Obitsu and Taniguchi, 2009; Clark, Klusmeyer and Cameron, 1992). The contribution of each fraction to the total flow is directly related to the diet composition and dry matter intake (DMI) and varies widely, with the microbial protein fraction usually supplying the majority of the proteins (Dewhurst et al., 2000; Clark et al., 1992). Endogenous protein originates from various sources, including mucoproteins, saliva, sloughed epithelial cells, and enzyme secretions into the abomasums or the lumen of the gastrointestinal tract (Ørskov, 1992). Nitrogen

recycling between the body and gut of ruminants plays a key role in the adaptation to such diverse nutritional conditions, and it is the nitrogen source for microbial protein synthesis in the rumen (Obitsu and Taniguchi, 2009). Whilst the rumen has many advantages, particularly when animals are offered low-quality feeds, it can be a major cause of inefficiency of nitrogen utilization in ruminants. Over half of the amino acids absorbed by ruminants, often two-thirds to three-quarters, derive from microbial protein (MCP). Therefore microbial protein must be considered as an important protein source (Devant, Ferret, Calsamiglia, Casals and Gasa, 2001; Clark et al., 1992). In order to maximize ruminal microbial production, both the ratio of available protein to energy sources for rumen microbes, and synchronizing energy and protein supply in the rumen are important (Obitsu and Taniguchi, 2009). Clark et al. (1992) demonstrated that protein and carbohydrate in feeds that are not degraded in the rumen, increase the quantity of dietary protein that passes to the small intestine but may decrease the quantity of microbial protein that is synthesized in the rumen. As a result, microbial protein synthesis in the rumen is often the main component of metabolizable protein supply in ruminants (Moorby, Dewhurst, Evans and Danelon, 2006). Microbial protein may also be more important than increased rumen undegradable protein (RUP) supply, in improving the protein status in lactating dairy cows (Broderick, 2003).

2.5 Measurement of Microbial Protein Synthesis

There are six methods of measurement of microbial protein synthesis (MPS) (Ørskov, 1992); a) use of a protein-free purified diet, b) use of diamino pimelic acid (DAPA), c) duodenal nucleic acid as a marker for microbial N, d) amino acid profile

in postruminal digesta, e) use of isotopes ^{35}S or ^{15}N and ^{32}P for determination of microbial N, and finally f) urinary purine derivatives as an estimate of microbial N supply. Therefore, the measurement of microbial protein flow *in vivo* require surgically cannulated animals, which is expensive, an increase in animal care concerns, and may affect dry matter intake and milk yield. The methods generally used in determining microbial protein production depend on the use of natural microbial makers, such as RNA (ribonucleic acid) and DAPA or isotopes ^{35}S or ^{15}N and ^{32}P. These techniques are complex, tedious and difficult to practice extensively under field conditions and require ruminally and post ruminally fistulated animals. Consequently, the method based on measurement of urinary purine derivatives (PD) over comes the problems of earlier methods. It is simple and non-invasive and has the potential to be further simplified for use under farm conditions. The urinary excretion of purine derivatives (PD: allantoin, uric acid, hypoxanthine, and xanthine) appears to be a reliable method to estimate the microbial N flow to the duodenum. The principle is that the duodenal flow of nucleic acids and their derivatives is mainly of microbial origin, which are, to a large extent, digested and absorbed in the small intestine, purine bases are catabolized to their PD, and excreted in the urine. Therefore, microbial N flow can be estimated from the quantitative excretion of PD in urine (IAEA, 1997).

2.6 Relationship Between Duodenal Purine Flow (PB) and Urinary PD Excretion

The urinary excretion of PD has been found to be linearly correlated with purine base (PB) input. The relationship between daily urinary PD excretion (mmol/d)

and duodenal PB flow (mmol/d) have been observed in Kedah-Kelantan cattle (Pimpa, Liang, Jelan and Abdullah, 2001), in swamp buffaloes (Pimpa, Liang, Jelan and Abdullah, 2003), in goats (Belenguer, Yanez, Balcells, Ozdemir Baber and Gonzalez Ronquillo, 2002), in dry cows (Orellana Boero, Balcells, Martin-Orue, Liang and Guada, 2001), in dairy cows (Gonzalez-Ronquillo, Baleclls, Guada and Vicente, 2003). The urinary PD originates from absorbed purine and endogenous contribution of PD from body tissue. A linear relationship was found between the excreted PD and the infused PB, where the intercept represents the endogenous contribution and the slope the incremental response. In Kedah-Kelantan cattle, the relationship between the excreted PD and the PB (adenosine 46% and guanosine 54%) flow was Y= 0.847X + 7.146 (Pimpa et al., 2001), suggesting that 0.85 of the supplied exogenous PB were excreted in urine, with an endogenous excretion of 7.15 mmol/d. Whereas, Orellana Boero et al. (2001) reported that 0.84 of the supplied exogenous PB was excreted in the urine of dry cows, with an endogenous excretion of 32.7 mmol/d. In buffaloes, the linear model was Y= 0.12X + 12.78 (Pimpa et al., 2003), suggesting that only 12% of supplied exogenous PB (adenosine 50% and guanosine 50%) was excreted in the urine, with an endogenous excretion of 12.78 mmol/d (0.207 mmol/kg $W^{0.75}$). Based on this study, in buffaloes, the lower recovery rate of PD in the urine of buffaloes could be due to a lower absorption rate at the small intestine and/or recycling of plasma PD. However, this speculation requires further investigation.

In goats, urinary PD excretion responded linearly to incremental supply of purine bases as yeast-RNA throughout the abomasal cannula, with recovery averaging 76% and endogenous excretion of 0.202 mmol/kg $W^{0.75}$ (Belenguer et al., 2002). In

sheep, endogenous excretion were similar to these obtained in goats but were lower than reported in cattle (Orellana Boero et al., 2001; Chen, Ørskov and Hovell, 1990; Verbic, Chen, MacLeod and Ørskov, 1990). Jetana, Abdullah, Halim, Jalaludin and Ho (2000) found a positive linear correlation (r = 0.73) between urinary allantoin and duodenal purines (μmol/d/kg $W^{0.75}$) in sheep. Urinary excretion of PD closely reflected changes in duodenal flow of PB as a result of feed restriction (Gonzalez-Ronquillo, Baleclls, Belenguer, Castrillo and Mota, 2004). Total excretion of PD in urine plus milk was linearly related to the duodenal input of purine bases, but differed between lactation stages resulting in a lower equimolar recovery in early rather than in late lactation (Gonzalez-Ronquillo et al., 2003). Consequently, the relationships between microbial yield of purines from the rumen and urinary excretion of PD may differ between different breeds of ruminants.

2.7 Estimating Microbial N Flow from Urinary PD Excretion

The purine derivative method uses the end-products of the absorbed purines in the urine as the microbial marker. The main advantage is that cannulation of the animal is not required and the experimental procedure is greatly simplified (only a total collection of urine).

A positive relationship was found to exist between microbial N flow and purine derivative excretion in urine (Johnson et al., 1998; Moorby et al., 2006). The true amino acid N in microbial N is 80%, and about 15% consists of nucleic acid N and 5% is composed of other non-protein N forms. Many reports have confirmed that approximately 85% of the total purines absorbed is excreted via urine; approximately 15% is excreted via other routes or recycled in the saliva and milk (Gonzalez-

Ronquillo et al., 2003). Endogenous losses of purine derivatives increase with body weight and the intestinal supply of energy (VFA) and protein do not modify urinary excretion of purine N. Consequently, the calculation of the amount of microbial N can then be made from the estimated purine absorption (X) into the duodenum that then predicts the urinary excretion of purine derivative. However, the purine derivative should not be used to predict microbial N flow across different physiological states of animal and breeds because of differences of endrogenous losses.

Intestinal flow of Microbial N (g N/d) is estimated by the following formula:

Microbial N (g N/d) = 70X/0.83 x 0.116 x 1000 = 0.727X. Where, X is purine absorption (mmol/d) (Chen et al., 1990; Verbic et al., 1990), and intestinal absorbed purine (mmol/d) is estimated by the following formula: $Y = 0.85X + 0.385 \text{ kg } W^{0.75}$. Where, Y is the purine derivative excretion (PD) (mmol/d) and X is the purine absorption (mmol/d), and kg $W^{0.75}$ is the metabolic body weight (kg) of animals. Furthermore, the following assumptions are made:

1. The mean endogenous contribution to urinary PD excretion from the degradation of tissue nucleic acids, based on 14 observation with cattle, is 0.385 mmol/d/kg $W^{0.75}$
2. The recovery of absorbed purines as urinary PD is assumed to be 85%, with the other 15% being lost via non-renal routes, i.e. via saliva and milk.
3. For the digestibility of microbial purines in the intestines, a mean value of 0.83 is assumed.
4. The ratio of purine N to total N in mixed rumen microbes is taken as 0.116, assuming no effect of dietary treatments.
5. The N content of purines is 70 mg N/mmol.

2.8 Source of Dietary Protein for Rumen Microbes

The most important source of N for the rumen microbes is normally dietary protein and non-protein N (NPN) (Ørskov, 1992). Therefore, using urea in the diets is similar to using degraded intake protein. The rumen microflora are highly proteolytic, and thus ensure that most of the protein entering the rumen are degraded to peptides and amino acids, which are then subsequently deaminated. Similarly, most of NPN entering the rumen is degraded to ammonia, which is required for the synthesis of microbial protein. Therefore, the extent to which the uptake by bacteria is in the form of ammonia or amino acids or even peptides as N sources are required for their cells synthesis. Moreover, it would be extremely useful if the rumen bacteria degraded protein only to the extent required to optimize their cell yields (Ørskov, 1992). However, a suitable for microbial growth would also depending on the synchrony between availability fermentable carbohydrates and degradable protein in the rumen. Consequently, dietary protein escaping ruminal degradation, and microbial protein synthesized in the rumen and endogenous proteins, are the sources of metabolizable protein used by animal for their production (Brito, Broderick and Reynal, 2006). Therefore in diet formulation for lactating dairy cows sufficient RDP must be provided to meet the requirements of rumen microorganisms, in order to optimize ruminal fermentation, so that microbial growth is maximized.

Sources of protein such as soybean meal, fish meal, and casein have been found to not affect microbial protein synthesis (MPS) and total PD but affect RDP and cause high levels of CP in the diet or urea, it opposed, indicating an abundance of protein degradation product (peptide, amino acids, and ammonia). NRC (1988) recommended that nitrogen from urea is only 80%, used as efficiently as nitrogen

from degraded intake protein. So there is a limitation in urea use, which should only be used when the supply of ruminally available protein is inadequate. Thus, feeding true protein supplements, rather than NPN, may result in an increased supply of microbial protein (Brito, Broderick, Olmos Colmenero and Reynal, 2007).

Overfeeding CP was found to reduce profit margins because of the relatively high cost of protein supplements and the poor efficiency of nitrogen use by dairy cows fed high protein diets (Broderick, 2003). However, there have been reports that a proportion of RDP may flow out of the rumen in the liquid and solid phase, reducing its availability for utilization by ruminal microbes (Griswold, Apgar, Bouton and Firkins, 2003). Therefore, protein supplement may be ineffectively utilized in the rumen if appropriate energy sources are not available. In energy-defict diets, additional energy input is necessary to optimize microbial protein synthesis. As a result, the slow degradation of fiber by cellulolytic bacteria in the rumen requires a good synchrony between energy produced and ammonia-N released. Ways to increase cellulolytic bacteria activity will improve digestion and increase protein microbial supply to the host animal. Consequently, supplementing the rumen with protein and energy sources will enhance microbial growth and digestion, and thus increase protein and energy supplies to the animal (Jetana et al., 2000). To achieve maximal rumen microbial growth, synchronizing the release of carbohydrate and N has been suggested as an important factor. Fadel Elseed (2005) reported that cotton-seed meal supplementation given twice daily rather than once daily to sheep, increased total PD and microbial supply N.

Synchronous diets produce high microbial yields (Dewhurst et al., 2000). It is envisaged that MPS will be maximized by synchronizing the availability of

fermentable energy and degradable nitrogen in the rumen. Consequently, it is possible to alter the synchronicity of diets, either by changing dietary ingredients, or by altering the relative times of feeding ingredients or dosing specific forms of energy and N into the rumen, or a combination of both approaches. However, it is not possible to identify whether an increase in MPS through feeding ingredients is an effect of synchrony or a factor associated with the manipulation of the ingredients (level and type) themselves (Dewhurst et al, 2000).

2.9 Effect of Dietary RDP on Microbial Protein Supply

Rumen microbial growth is dependent on the availability of nitrogen in the form of peptide, amino acid and ammonia. Also high concentrations of RDP (rumen degradable protein) and NSC (nonstructural carbohydrate) in diets produce greater excretion of urinary PD and microbial protein supply to the duodenum, so that efficiency of microbial protein synthesis is based on the digestibility of organic matter in the rumen (Johnson et al., 1998; Griswold et al., 2003). The work of Martin-Orue, Balcells, Guada and Fondevila (2000) reported that the excretion of PD (76.8 vs. 87.3 mmol/d) also responded to the level of effective rumen degradable protein (ERDP) supply in the diet (0 to 75 g/kg concentration), but microbial N supply no affected. In contrast, Gressley and Armentano (2007) reported that high RDP did not affect on excretion of PD and microbial protein supply (Brito et al., 2006), indicating that there may be a lack of available energy. Dewhurst et al. (2000) explained that the effect of protein sources on microbial protein synthesis is more complicated than synchrony effect or a limitation imposed by ERDP. However, different microbial protein

synthesis responses to protein supplements have been found in different types of energy supplied in concentrates (Clark et al., 1992).

2.10 Effect of Microbial N Supply on Animal Performance

Reducing CP concentration and increasing ruminal undegradable protein supply did not affect animal performance or estimated duodenal flow of microbial protein in rapidly growing heifers fed high-concentrate diets (Devant, Ferret, Calsamiglia, Casals and Gasa, 2000). Richardson, Wilkinson and Sinclair (2003) reported that growing lambs fed a barley based diet had a higher level of PD and microbial N production. These results indicated that neither dietary synchrony nor energy source significantly influenced growth rate. Dietary protein from raw and dry roasting and supplementation of legume seeds did not affected the flow of microbial N into the duodenum and PD excretion. But the ADG was higher than when no seeds were supplemented (Yu, Egan, Boon-ek and Leury, 2002). Paengkoum, Liang, Jelan and Basery (2006a) reported that microbial N supply increased with increasing urea supplementation up to 30 g/kg oil pram fronds (OPF) and thereafter, decreased, and the ADG of goats followed the same pattern (Table 2.1). Moreover, the supplementation of energy enchanced utilization of urea and resulted in higher animal performance as a consequence of improved ruminal fermentation and microbial yield (Paengkoum et al., 2006b). Jetana et al. (2000) report that microbial N supply was not affected by protein supplements (soy bean meal and fish meal) in sheep fed guinea grass, but tended to be affected by energy supplements (corn flour and paper pulp). Similarly, Devant et al. (2001) found that the microbial N supply and ADG were not affected by protein supplements.

Table 2.1 Effect of varying protein diets on microbial N supply (MN, g of N/d), average daily gain (ADG, g/d) and total PD excretion (mmol/d).

Items	**Total PD**	**MN**	**ADG**	**References**
High protein + HD	70	369	1.17	Devant et al.
High protein + LD	71	377	1.21	(2000)[1]
Low protein + HD	66	349	1.21	
Low protein + LD	64	333	1.23	
SEM	5.5	35.1	0.05	
g urea/kg steamed oil palm fronds				Paengkoum et
10	2.96^{c}	1.4^{c}	22.4^{b}	al. (2006a) [2]
20	4.95^{b}	3.9^{ab}	47.1^{a}	
30	6.60^{a}	5.5^{a}	48.6^{a}	
40	3.54^{bc}	2.3^{c}	20.2^{b}	
50	2.84^{c}	1.5^{c}	-5.8^{c}	
SEM	0.365	0.40	1.02	
Low energy + Urea 3%	7.05^{ab}	5.9^{b}	35.7^{bc}	Paengkoum et
Low energy + Urea 4%	5.42^{c}	4.3^{c}	-8.9^{d}	al. (2006b) [2]
Low energy + Urea 5%	3.60^{d}	2.5^{d}	-66.1^{d}	
High energy + Urea 3%	9.30^{a}	7.9^{a}	85.7^{a}	
High energy + Urea 4%	6.54^{b}	4.5^{b}	51.8^{ab}	
High energy + Urea 5%	4.35^{cd}	3.3^{cd}	5.4^{cd}	
SEM	0.42	0.40	14.81	

Table 2.1 Effect of varying protein diets on microbial N supply (MN, g of N/d), average daily gain (ADG, g/d) and total PD excretion (mmol/d) (Count.)

Items	Total PD	MN	ADG	References
Oat straw and alfalfa hay on seed	11.6	10.0	123	Yu et al. (2002) [3]
Raw whole lupin seeds	12.9	11.0	161	
Roasting whole lupin seeds	12.4	10.6	172	
Raw whole faba beans seeds	14.2	12.2	178	
Roasting whole faba beans seeds	13.9	11.9	180	
SEM	1.24	1.09	8.0	

[a] Mean within column are significantly different (P<0.05) (within experiment)

[1]In growing heifers and ADG (kg/d)

[2]In growing goats

[3]In growing lambs

HD = High degradability; LD = Low degradability; SEM = Standard error of mean

Milk yield was positively correlated with urinary excretion of PD as an increase in rumen microbial synthesis usually implies an increase in energy and protein supply to the host animal and, consequently, an improvement in milk yield (Table 2.2). Thus, milk production increased due to a high energy balance in animals, synchrony of available fermentable carbohydrates, adequate RDP, and increased microbial N supply to the duodenum. The consequence of increasing concentrate in

diet was to increase microbial N synthesis, and milk yield was increased linearly with the increasing microbial N supply to the duodenum (Moorby et al., 2006).

Table 2.2 Effect of varying protein diets on microbial N supply (MN, g of N/d), milk yield (kg/d) and total PD excretion (mmol/d) in lactating cows.

Items	**Total PD**	**MN**	**Milk yield**	**References**
Dietary crude protein, % of DM				Olmos
13.5	314	425	36.3	Colmenero
15.0	333	416	37.2	and Broderick
16.5	341	476	38.3	(2006a;b)
17.9	336	480	36.6	
19.4	339	480	37.0	
SEM	12	34	1.01	
Low protein	522	334	34.3	Sannes et al.
Low protein + sucrose	500	293	33.2	(2002)
High protein (urea)	527	317	33.6	
High protein (soy bean meal)	538	341	3.7	
SE	17.4	12.8	0.2	
Solvent soybean meal 3.7%	610	528	38.8	Olmos
Solvent soybean meal 9.6 %	652	562	40.0	Colmenero
Solvent soybean meal 4.6%	632	557	40.3	and Broderick
Solvent soybean meal 11.7 %	674	590	40.1	(2006c)
SE	35	36	1.3	

Table 2.2 Effect of varying protein diets on microbial N supply (MN, g of N/d), milk yield (kg/d) and total PD excretion (mmol/d) in lactating cows (Count.).

Items[1]	**Total PD**	**MN**	**Milk yield**	**References**
Low RDP	450	-	29.7	Gressley and
Low RDP + inulin	487	-	31.3	Armentano
High RDP	501	-	30.4	(2007)
High RDP + inulin	481	-	29.3	
SED	18	-	0.7	
Urea	402	223	32.9 [b]	Brito and
Solvent soybean meal	432	245	40.1 [a]	Broderick
Cottonseed meal	424	240	40.5 [a]	(2007)
Canola meal	378	205	41.1 [a]	
SED [1]	23.0	17.0	11.0	

[a] Mean within column are significantly different ($P<0.05$) (within experiment); -: not detected

[1]RDP = Rumen degradable protein; SED = Standard error of the least squares means difference; SEM = Standard error of mean; SE = Standard error.

However, increasing intake ad libitum, while increasing microbial N supply, did not affect on milk yield (Gonzalez-Ronquillo et al., 2004). Similarly, Olmos Colmenero and Broderick (2006b; 2006c; Sannes, Messman and Vagnoni (2002) found that the PD excretion was related to milk production, and was not affected by increasing CP in the diet, but microbial N synthesis increased due to high protein in the diets (Ipharraguerre, Clark and Freeman, 2005).

2.11 Contribution of Recycled N to Rumen Synchrony

Large amounts of the N incorporated into bacterial protein can originate from endogenous urea (Figure 2.1). Ammonia and microbial protein produced in the gut and urea synthesized in the liver are major plays in N-recycling transactions (Obitsu and Taniguchi, 2009). Huntington and ArchibeZque (1999) noted that nitrogen recycled to the digestive tract as urea in saliva or urea transported from blood, range from 10 to 40% of N consumed in feed. The recycled N can be as high as 70% of the N delivered to the rumen at extremely low intakes of dietary protein (5% CP), but at 20% CP in the diet, endogenous N contribution decreases to 11% of the ruminally available N (Asplund, 1994). The amount of recycled N incorporated into bacterial N was only about half as high for the low concentrate (34 to 40%) compared to the high concentrate diet (15 vs. 30% of bacteria N). This relatively high estimate of recycled N for high concentrate diet was supported by the large increases in N flow over dietary N at the duodenum. The greater quantity of recycled urea into the rumen on high concentrate diet was associated with a more active fermentation and a lower concentration of ruminal NH_3-N, which would stimulate greater passage of urea from the blood through the rumen wall, despite the diets being isonitrogenous (Asplund, 1994). However, most buffaloes are fed on low-quality roughage, agriculture crop-residues and industrial by-products, which basically contain low levels of fermentable carbohydrates and low levels of good-quality protein (Wanapat and Rowlinson, n.d.). As a result, considering the potential for a substantial influx of endogenous urea into the rumen of buffaloes at low levels of energy and protein intake, the need for a constant supply of NH_3 from degradable intake protein to supply microbial needs becomes less important. However, N recycling via saliva and blood in buffaloes were

higher than in cattle (Obitsu and Tinaguchi, 2009). There are three important priorities of urea metabolism aspects: maximizing microbial function in the rumen; optimizing amino acid supply to the host ruminant, and minimizing negative environmental effects of cycling N through ruminant production systems (Huntington and ArchibeZque, 1999).

2.12 Quality and Level of Dietary Protein on Performance

Buffaloes, like other domesticated ruminants, meet their protein and energy requirements from fermentation end products (microbial protein and volatile fatty acids) (Sarwar et al., 2009). Hayashi, Maharjan and Kumagai (2006) suggested that while different supplies of CP, NDF and TDN might affect milk yield and nutritional status in cattle and buffalo, it was more likely those were affected by the lower supplies to CP and TDN for cattle that calve in the fodder-shortage periods and the lower 305-day milk yield of cattle.

The increased undegradable protein in the small intestine in buffalo does not positively influence the digestibility of non structural carbohydrate (NSC), but does increase that of cellulose (Bartocci and Terramoccia, 2006). While, protein supplementation of wheat straw as a basal diet increased intake, digestibility and metabolizable energy, a maximum response could only be obtained when soybean meal was used as a supplement due to increased digestible crude protein intake (Mehra et al., 2006). Reddy (1996) concluded that supplementation of deoiled rice bran at 1000 g/d, along with a poultry droppings molasses mineral mixed with rice straw, maximizes the utilization of rice straw in buffaloes, due to the increased intake and digestibility of crude protein. Supplementation with legume straw (blackgram)

increased the protein digestibility, N retention and rumen ammonia-N concentration in murrah buffaloes bulls fed rice straw-poultry droppings-based diet (Reddy, 1997). Buffaloes fed a fish meal diet had lower urinary N excretion, higher N retention and higher rumen ammonia-N concentration than those fed a soyabean meal or groundnut cake diet (Prakash, Reddy, Reddy and Krishna, 1996). Furthermore, Nisa et al. (2006) reported that ensilation of urea treated wheat straw (UTWS) with 9% corn steep liquor (CSL) seemed take a more effective was improve the nutritive value of wheat straw and increase milk yield (Sarwar, Khan and Nisa, 2004). The slower release of fiber bond N from UTWS ensiled with CSL might have synchronized with fiber fermentation and thus were utilized by the rumen micro-flora. This information is needed to match proteins with carbohydrates to better synchronize the release of protein and energy for microbial synthesis (Sarwar et al., 2004).

These degradation characteristics can be affected by many factors, such as rate of passage, liquid dilution, amount of protein consumed, processing of the protein source, dry matter consumption, pH in the rumen, and ionicity of rumen contents. The fermentability of substrate has a large influence on rumen microbial yield, which depends largely on availability of energy and protein to the microbial population (Hosamani, Mehra and Dass, 1998; Chanjula et al., 2004). Moreover, urea treated wheat straw ensiled with acidified molasses can be included at up to 60% DM of lactating buffalo rations without any adverse effect on productivity (Khan and Sarwar et al., 2006). Similarly, replacing diets based on urea treated rice straw with cassava hay, increased ammonia-N concentrations and cellulolytic bacteria, and OM and CP digestibility were improved as levels of cassava hay increased in the diets (Chanjula et al., 2004). A linear increase in fiber digestibility, greater microbial counts and a linear

reduction in N retention was found in buffalo bulls fed with increasing of ruminally degradable protein (RDP) in the diet (Javiad, Nisa, Sarwar, and Aasif Shahzad, 2008). However, recently study by Nisa, Javiad, Shahzad and Sarwar (2008) also demonstrated that the DM and NDF intakes decreased, while their digestibility in lactating buffaloes increased, when RDP contents were increased from 50 to 82% in the dietary CP. The data from Kumar, Tiwari and Kumar (2005) found that increasing UDP level from 41 to 48% of CP in concentrate mixtures, can maintain a consistently high milk production. From the published data it can be concluded that supplementing buffalo diets with RUP or ammonia nitrogen sources can increase the efficiency of N utilization by increasing the flow of N and amino acids to the small intestine (Sarwar et al., 2009).

2.13 Protein Requirements of Buffaloes

The scientific data required to explain the protein requirements and their utilization from different source at various physiological stages in buffaloes is scarce. In contrast to high producing western dairy cattle, where much attention has been paid to develop energy and protein standards and nutrient requirement models, no such planned effort has been made to establish protein or energy needs in buffaloes (Sarwar et al., 2009). The host ruminant animal, unlike the microbes which supply most of its protein, has a fluctuating demand for protein. The fluctuations in dietary demand are due to normal physiological changes in the animal (Kearl, 1982). The protein requirement includes that needed for maintenance and production. The N maintenance requirement consists of urinary endogenous N, scurf N (skin, skin secretions, and hair), and metabolic fecal N. The N requirement for production includes the protein

needed for conceptus, growth, and lactation (NRC, 2001). Bartocci, Tripaldi and Terramoccia (2002) pointed out that the protein requirements of Mediterranean buffaloes bred for dry production was 78.96 g/kg DM and for those bred for a milking, which oscillates between 7 to 12 kg/d, the protein requirement was 101.64 to 150.76 g/kg DM. In early lactating *Nili ravi* buffaloes, a crude protein intake of 140 g/kg DM, was needed to produce a milk yield of 14 kg/d (Shahzad, Sarwar, and Nisa, 2007), 146 g/kg DM to produce a milk yield of 12.6 kg (Nisa et al., 2004), 150 g/kg DM to produce a milk yield of 13.1 kg (Sarwar et al., 2004), and 160 g/kg DM to produce a milk yield of 15.6 kg/d (Khan and Sarwar et al., 2006). Similarly, Campanile et al. (1998) pointed out that an increase of CP/DM (12%) increased milk yield and milk protein levels of buffalo cows, and it is necessary to increase the dietary protein concentration to meet their requirements. The high level energy/protein (0.94 milk FU/kg DM, 158 g/kg DM) diet fed lactating Mediterranean buffaloes showed that the greater milk yield (11.6 kg/d) was due to increased crude protein and NSC intake (Bartocci, Terramoccia and Tripaldi, 2006). In addition, it was found that a 400 g urea molasses mineral block (UMMB) is the optimum amount to maximize utilization of nutrients from wheat straw based diets to meet maintenance requirements of adult male buffaloes of 69.8 g CPI/kg DM (Verma, Mehra and Dass, 1998) and 78.9 g/kg DM (Hosamani et al., 1998). Resulted has found that the daily protein requirement of buffalo calves were 12.6 g CP/kg $W^{0.75}$ (Nair, Verma, Dass and Mehra, 2004), and 5.0 g DP/kg $W^{0.75}$ (Sahoo, Elangovan, Mehra and Singh, 2004), and buffalo bulls required 7.8 g CP/kg $W^{0.75}$ (Nisa et al., 2006), 7.3 g CP/kg $W^{0.75}$ (Verma et al., 1996), 8.0 g CP/kg $W^{0.75}$ (Khan and Iqbal et al., 2006), 5.3 g CP/kg $W^{0.75}$ (Granum et al., 2007) and 13.8 g CP/kg $W^{0.75}$ (Chanjula et al., 2004).

Metabolizable protein (MP) **requirements**

The metabolizable protein (the quantity of true protein or amino acids absorbed) requirements can be met with a knowledge of the dietary escape protein and microbial protein production (Wilkerson, Klopfenstein, Britton, and Miller, 1993). The metabolizable protein requirements that are currently in use were established from empirical formulas using N loss and tissue accretion. The NRC requirements (1996) for MP were based on the factorial method. The factors included were metabolic fecal losses, urinary losses, scurf losses, growth, fetal growth, and milk. Wilkerson et al. (1993) reported the calculated metabolizable protein flow using weighted regression analysis (r^2 = 0.69, n = 45) to determine the metabolizable protein requirements for maintenance of growing cattle (3.8 g/kg $W^{0.75}$/d), where body weight (W) is expressed in kilograms) and growth (305 g/kg of live weight gain). However, NRC (1985) recommended that the maintenance requirement of beef cattle is a single function of body size (3.25 g MP/kg $W^{0.75}$/d) based on N balance studies with nonproducing animals. Assuming CP x 0.64 (CP converted to bacteria crude protein (BCP): 80 percent true protein x 80 percent digestibility) = MP, and their diets were high in roughage and were based on the assumption that 0.13 TDN = BCP. If actual BCP synthesis efficiency was less than 0.13, the estimate of the maintenance would be less than 3.8 g MP/kg $W^{0.75}$ (NRC, 1996) and therefore microbial production may have been overestimated, resulting in higher requirements for metabolizable protein (Wilkerson et al., 1993).

The NRC (1985) and ARC (1984) used the factorial approach to determine the maintenance metabolizable protein requirements. Endogenous protein losses are merged into one estimate as a function of metabolic fecal losses to indigestible DMI

and endogenous urinary losses. Surface protein losses are a small part of the maintenance protein requirement with little difference between the NRC (1985) and the ARC (1984) system. The efficiencies of MP to net protein (NP) for maintenance of 0.67, for gain of 0.5 and to NP for milk of 0.65 were assumed (NRC, 1985; 1988; 2001).

Protein requirements can be determined through nitrogen balance studies. In these studies, healthy adult animals should be fed an adequate amount of energy and other nutrients in diets that contain different levels of protein. The minimum protein intake that will support nitrogen equilibrium is the maintenance requirement. Protein requirements can be determined through the regression equation, the relationship between nitrogen intake and average daily gain (Figure 2.2). Therefore, the maintenance requirement increases with body size. However, it is difficult to determine the precise protein requirements because protein can be used as a source of energy whenever an animal experiences an energy shortage.

Protein requirements for maintenance

For maintenance nitrogen balance in buffaloes, protein must be provided in a efficient amount to allow for metabolic fecal losses and provide for growth, production and (or) reproduction (Kearl, 1982). NRC (1996; 2001) reported that metabolic fecal, urinary, and scurf losses represent the requirement needed for maintenance. The maintenance requirement increases with body size, and decreases as the animal approaches maturity due to the decreasing protein content protein in the body tissues (Table 2.3). Every animal, regardless of the diet or the physiological function being performed, will have urinary nitrogen losses. This loss is reasonability constant per unit of body size (kg $W^{0.75}$). Fecal losses, normally will vary with the

composition of the maintenance diet and the metabolic fecal nitrogen. The metabolic fecal portion in the feces contains substances that indicate where in the animal's body such as bacterial residues, cells from the walls of the gastro intestinal tract and in residues of the digestive juices and other secretions (Kearl, 1982). Although the metabolic fecal nitrogen may be relatively constant in terms of body size, and the total fecal nitrogen depends on the digestibility of the dietary protein provided to the animal, it is difficult to measure fecal and urinary losses independent of each other. It is also difficult to separate microbial (complete cells or cell walls) losses in the feces from true metabolic fecal losses. For most beef cows, MP and CP requirements, using the calculation based on indigestible dry matter intake, are unrealistically high (NRC, 1985). The high requirement can be attributed to the fact that nitrogen is being excreted in the feces as microbial protein rather than as urea in the urine as a result of microbial growth in the postruminal digestive tract. NRC requirements (1996) based on MP requirement state that the CP intake needed can be estimated by dividing the total MP requirement by 0.67, which is based on 80 percent of the MP from MCP (microbial protein) and 20 percent from UIP (undegraded intake protein). The CP required is determined as MP/0.67.

The digestible protein (DP) requirement for maintenance of buffaloes recommended by Kearl (1982) : DP = 2.54 g/kg $W^{0.75}$/d.

The metabolizable protein (MP) for maintenance of beef cattle recommended by NRC (1996) : MP = 3.8 g/ kg $W^{0.75}$/d.

The metabolizable protein (MP) for maintenance of dairy cows recommended by (NRC, 2001) : $MP_M = MP_U + MP_{SH} + MP_{MFP}$

Where,

$MP_U = 4.1\ BW^{0.50}$

$MP_{SH} = 0.3\ BW^{0.60}$

$MP_{MFP} = \{(DMI \times 30) - 0.50((bactMP/0.8) - bact\ MP)\} + MP_{ENDO}/0.67$

Where,

MP_U = endogenous urinary protein requirement

MP_{SH} = scurf and hair (skin, skin secretion, hair) protein requirement

MP_{MFP} = metabolic fecal protein requirement

$MP_{ENDO} = 0.4 \times 1.9 \times DMI\ (kg/d) \times 6.25$

Table 2.3 Daily nutrient requirements for maintenance of growing buffaloes

Weight	**Dry matter**		**Energy requirements**			**Protein requirements**		
kg	**kg/d**	**% BW**	**ME, Mcal**	**TDN, kg**	**ME/kg DM**	**Total CP, g**	**DP, g**	**CP, %**
100	2.4	2.4	3.95	1.09	1.65	163	80	6.79
150	3.3	2.2	5.36	1.48	1.62	223	109	6.75
200	4.1	2.0	6.65	1.84	1.62	288	135	7.02
250	4.8	1.9	7.86	2.17	1.64	327	160	6.81
300	5.6	1.9	9.01	2.49	1.61	377	183	6.73

Source : Kearl, 1982.

Protein requirements for growth

The digestible protein requirement for growth varing with weight and age of animals. The dietary digestible protein requirements of growing, non-pregnant buffaloes and pregnant buffaloes during the first 7 months of the gestation period.

Using the value of 0.238 g/kg $W^{0.75}$/d of body weight gain by Kearl (1982) are as follows :

DP requirement for growth (g/d) = 0.238 g LWG + 0.6631 kg LW – 0.01142 kg LW^2

Where,

LWG = live weight gain

LW = live weight

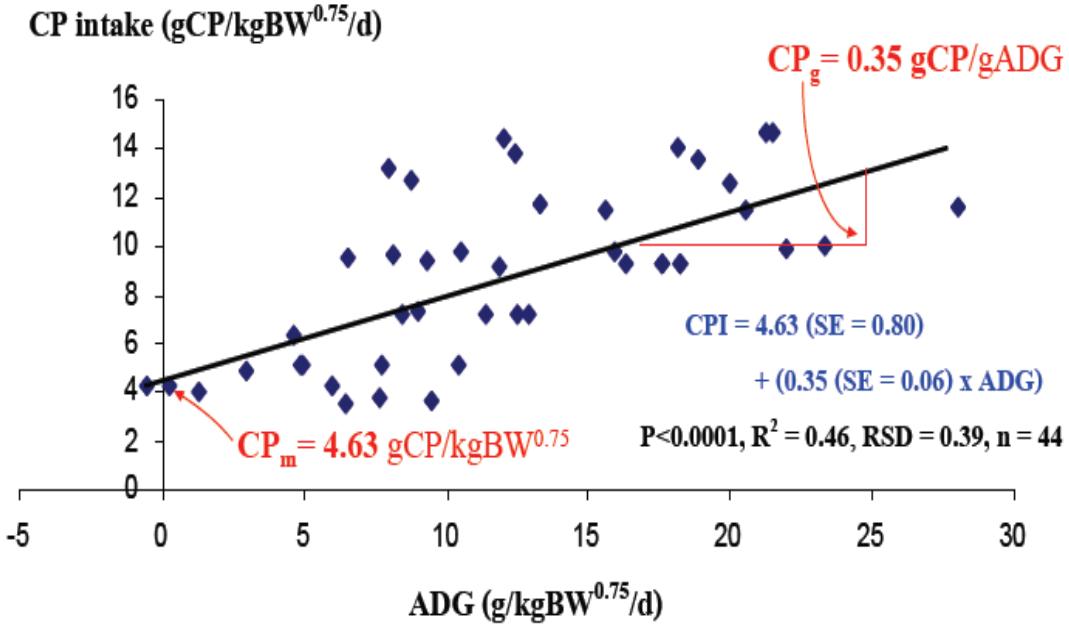

Figure 2.2 CP requirement of growing Brahman cattle for maintenance and gain were 4.63 g CP/kg $W^{0.75}$ and 0.35 g CP/g ADG

Source : Chaokaur et al., 2007.

Protein composition of weight gain decrease and fat composition increases with increase in rate of gain and BW (Wilkerson et al., 1993). It has been proposed (NRC, 1985) that the change in protein composition due to body size and rate of gain,

results in a lower metabolizable protein requirement. The metabolizable protein requirement for gain remains constant and that metabolizable protein utilization changes as body size and rate of gain varing, the efficiency of MP declines with increased rate of gain and body size (Wilkerson et al., 1993). However, the protein requirements are differences in breeds of animal (Table 2.4).

Table 2.4 Protein requirement for maintenance and for growth in different breeds.

Species	**Protein for maintenance (unit/kg $W^{0.75}$)**	**Protein for growth (unit/g ADG)**	**References**
Buffaloes	3.26 g DP	-	Granum et al. (2007)
	2.21 g DP	-	Verma et al. (1998)
	2.45 g DP	0.46 g DP	Hosamani et al. (1998)
	2.54 g DP	0.238 g DP	Kearl (1982)
Beef cattle	4.63 g CP	0.35 g CP	Chaokaur et al. (2007)
	3.8 g MP	0.305 g MP	NRC (1996); Wilkerson et al. (1993)
Dairy cattle	4.3 g MP	0.303 g MP	NRC (2001)
	3.5 g CP	-	NRC (1988)
Growing goats	4.3 g MP	0.318 g MP	Luo et al. (2004)
Mature goats	3.35 g MP	-	Luo et al. (2004)
sheep	3.25 g MP	-	NRC (1985)

CHAPTER III

EXPERIMENT I

EFFECT OF DIETARY CRUDE PROTEIN LEVELS ON THE PERFORMANCE OF GROWING MALE SWAMP BUFFALOES

3.1 Abstract

This experiment was conducted to investigate the effects of dietary crude protein on nutrient intake and digestibility, nitrogen utilization and the estimation of protein requirement for maintenance of growing male swamp buffaloes. Four growing bulls swamp buffaloes with average initial weight 209 ± 17.7 kg (12 to 18 months old) were used. The animals were assigned to a 4 x 4 Latin Square Design and received four diet treatments of crude protein (CP) levels in the diets of 5, 7, 9 and 11% of dry matter (DM). All diets were isocaloric (20% above maintenance of ME). The results showed that CP intake and CP digestibility of buffaloes increased ($P<0.01$) with increasing CP content in diet. However, increasing the levels of dietary protein did not significantly ($P>0.05$) affect feed intake and digestibility of nutrient, body weight change, and ruminal pH. As the level of CP in diets increased, blood urea nitrogen, ruminal ammonia N, total volatile fatty acid, nitrogen (N) absorption, and N excretion increased linearly ($P<0.01$). Total purine derivatives (PD) excretion and microbial nitrogen supply to the duodenum of buffaloes were significantly

different ($P<0.01$), when dietary protein levels increasing. Ruminal microbe populations were not significantly different ($P>0.05$) with increasing CP levels in the diet. The relationship between N balance (NB) and N intake (NI) (g/kg $W^{0.75}$) of male swamp buffaloes was NB = 0.883NI - 0.653 ($R^2 = 0.855$; $P<0.001$; n = 32). These findings suggest that the nitrogen requirements for maintenance of growing male Thai swamp buffaloes are 0.74 g N or 4.63 g CP/kg $W^{0.75}$/d.

3.2 Introduction

In order to meet their production potential, ruminants have to consume the required amounts of nutrient from the diet. The nutrition of young buffalo males is important as it plays a role in the onset of puberty when they are raised for breeding and it influences the quantity and quality of the meat they produce. Dietary protein supply is one of the factors that influence the productivity of animals. Feeding high levels of protein may be effective in promoting rapid liveweight gains, especially in growing buffaloes (Basra et al., 2003b). Currently, there is insufficient information concerning the effect of protein on nutrient digestibility and nitrogen metabolism in Thai swamp buffaloes. Chantiratikul, Chumpawadee, Kanchanamayoon and Chantriratikul (2009); Chumpawadee, Chantiratikul, Rattanphun, Prasert and Koobkaew (2009) found that CP digestibility and N retention of Thai indigenous heifers increased with increasing dietary CP levels.

A study of the nutritional requirements of buffalo is necessary because currently the NRC (1996 and 2001) suggesting for beef or dairy cattle are not suitable for buffaloes. Although, the nutrition requirements of river buffaloes have been determined by Kearl (1982), they may not be accurately applied to swamp buffaloes.

Basra et al. (2003b) reported that *Nili-ravi* buffalo male calves have lower protein requirements than cattle calves, but the CP requirements for growth may be the same as suggested for Holstein Friesian calves (Basra et al., 2003a). However, the digestible CP requirement for maintenance of buffaloes was 2.54 g/kg $W^{0.75}$/d (Kearl, 1982), and the metabolizable protein (MP) requirement for maintenance was between 4.03 to 6.3 g/kg $W^{0.75}$/d for growing *Nili-ravi* buffaloes from 125 to 400 kg (Paul and Patil, 2007). The optimum fattening performance of 15 months old *Nili-ravi* buffalo males was obtained by providing 10.22% CP (Mahmoudzadeh, Fazaeli, Kordnejad and Mirzaei, 2007), and 12% CP for 11 to 12 months old buffaloes (Tipu, Mirza, Ahmad, Tauqir and Mushtad Aziz, 2009). The nutrition needs of buffaloes probably differ from breeds found in temperate countries, because of differences in genetic make-up, mature body size, growth rate, composition of body tissue, quality of feed and climatic conditions (Kearl, 1982). Currently, research on the requirements of nutrient for Thai swamp buffalo does not exist and adequate information on the nutritional requirements of growing male buffaloes is lacking.

3.3 Objective

This experiment was conducted to determine the effects of increasing dietary protein levels on nitrogen utilization, nitrogen balance and estimation of protein requirements for the maintenance of growing male Thai swamp buffaloes.

3.4 Materials and Methods

Experimental location

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

Animals, diet and experimental design

Four growing male (bulls) swamp buffaloes, 12-18 months of age, with an average initial weight of 209 ± 17.7 kg were randomly assigned into a 4 x 4 Latin Square Design to receive four levels of crude protein (5, 7, 9 and 11% CP) in their diets. The energy contents of the diets were formulated to contain a metabolizable energy intake of 20% above maintenance (M) as determined by a review of literature of the M = 1.62 Mcal/kg DM (Kearl, 1982), and calculated from 1 kg TDN = 3.62 Mcal. All animals were fed rice straw as roughage and cassava pulp and soybean meal as energy and protein sources according to the respective treatments. In order to increase the palatability of the diet, molasses was added at 3.4%. All concentrate diets were mixed daily and fed twice daily at 0800 h in the morning and 1600 h in the evening. During the experimental period, the animals were kept in well ventilated shed on a concrete floor with individual feeding and watering.

For the rest period, all animals were fed *ad libitum* with rice straw and 1.5% BW of concentrate (12% CP).

Animals were weighed before started and the end of each period, before feeding and watering (fasting overnight; shrunk body weight) to record body weight for feed formulated during the experiment, and for calculation of live-weight changes.

Experimental period

The experiment was from May 2008 to September 2008. The experiment were 4 periods, and 28 days for each period. The animals were given 14 days for adaptation to diets at the start of each period, followed by a 7 days collection period and a 7 days rest period. Four days before the end of experiment during the final rest period the animals were fasted to measure N-retention.

During the experiment, the average temperature ranged from 23.3 to 33.0°C.

Data collection and sampling procedures

Feed offered and refusals were weighed and recorded daily and sampled every 3 days throughout the experiment to analyze chemical composition. The feces and urine were collected daily from day 3 to 7 of each collection period. Animals were kept on metabolism cage with the future board and plastic bag under the cage for collection of the urine and feces, respectively. The feces were weighed and mixed well and a 10% sub sample was taken and frozen. At the end of each collection period, the daily fecal samples were bulked for each animal. Ten percent of each mixed bulked sample was taken for chemical analysis and calculations of digestibility of DM, OM, CP, NDF and ADF were done. Urine samples were acidified with 25% H_2SO_4 to kept the final pH of the urine below 3 (to prevent ammonia losses during the day) and then weighed and, it was sampled for determination of urine nitrogen and purine derivative excretion. Urine was diluted five times with distilled water and mixed thoroughly and stored at -20°C for later analysis for PD and creatinine according to the technique of Balcells, Guada, Peiro and Parker (1992). Purine derivatives and creatinine concentrates in urine were analyzed using a HPLC, C18

reversed phase column with a UV detector wave length 205 nm (Hewlett-Packard HPLC system HP series 1100, agilent series 1100).

Feed, refusal and fecal samples were dried at 60°C and analyzed for proximate principles (AOAC, 1990) and fiber analysis determined by the methods of Van Soest, Robertson and Lewis (1991).

At the end of each collection period, rumen fluid was taken (200 ml) from the middle part of the rumen by a stomach tube connected with a vacuum pump at 0 (just before feeding) and 4 h after morning feeding. Ruminal pH was measured immediately after sampling using a portable pH meter and the samples were then filtered through a nylon bag. Five ml of 1M H_2SO_4 was added to 50 ml of rumen fluid to terminate fermentation. The mixture was centrifuged at 3,500 rpm for 15 minutes and the clear supernatant was stored in bottles at -20°C until analyzed for ruminal ammonia nitrogen (NH_3-N) (Bremner and Keeney, 1965) and volatile fatty acids (VFA) concentrations, which were determined by Gas Chromatography (GC) (Hewlett-Packard GC system HP6890 A; Hewlett-Packard Avondale, PA) equipped with a 30 m x 0.25 mm x 0.25 µm film (DB-FFAP). A second portion, of one ml of ruminal fluid, was fixed with 9 ml of 10% formalin solution in normal saline (0.85% NaCl) (10 times dilution) for direct total cell counts of bacteria, protozoa and fungal zoospore using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco). Another portion was used to culture groups of bacteria using the roll-tube method described by Hungate (1969) for identifying bacteria groups of cellulolytic, proteolytic, amylolytic. The direct total cell counts of bacteria were determined at 0 and 4 h post feeding. One ml of sample ruminal fluid from the second portion was added with 9 ml of distilled water (the final dilution is 100 times). A few drops of

fluid were transferred to a thoroughly cleaned haemacytometer and covered with a cover slip. The counts were made from 20 squares fields (small square field; one of sixteen) of haemacytometer under microscope lens 100x and calculations were made according to the following equation:

Total bacterial cells/ml of rumen liquor = N x DF x SF

where N is the average number of bacteria counted per square field, DF is the dilution factor (1×10^2) and SF is the square factor (4×10^6).

The protozoal and fungal zoospore counts were determined at 0 and 4 h post feeding. A few drops of ruminal fluid from the second portion were transferred to a haemacytometer and covered with a cover slip. The counts were made from the central big square field (for protozoa) and medium square field (one of twenty-five of square; for fungal zoospore) of harmacytometer under microscope lens 10x or 40x and calculations were made according to the following equation:

Protozoa/ml of rumen liquor = N x DF x SF

where N is the average number of protozoa counted per square field, DF is the dilution factor (1 x 10) and SF is the square factor (1×10^4).

Fungal zoospore/ml of rumen liquor = N x DF x SF

where N is the average number of fungal zoospore counted per square field, DF is the dilution factor (1 x 10) and SF is the square factor (2.5×10^5).

Blood samples were collected from the jugular vein into tubes (10 ml per sample) at the same time with rumen fluid sampling to determine blood urea (BUN) concentration. The blood samples were centrifuged at 3,500 rpm for 20 minutes and serum was separated and stored frozen at -20°C until analyzed for BUN (Crocker, 1967).

Data analysis and calculations

The nitrogen requirements for maintenance were estimated by determining the average nitrogen balance (NB) of buffaloes fed different levels of protein. In order to estimate dietary N requirement for maintenance, the NB and N intake were inserted into a regression equation; Nitrogen balance = N balance index x (N intake) – N loss at zero N intake, where N requirement (Nm) for maintenance equals to N intake when N balance is zero.

The supply of microbial N was then calculated from the microbial purine absorbed (PB) using the following factors: digestibility of microbial purines is assumed to be 0.83, the N content of purine is 70 mg N/mmol and purine N: total N in mixed rumen microbes is taken as 11.6:100 (Chen and Gomes, 1992), equation: Microbial N (g N/d) = 70PB/(0.116 x 0.83 x 1,000) = 0.727PB, where, PB = the corresponding amount of microbial purines absorbed (mmol/d).

The calculation of daily purine absorption (PB, mmol/d) was done by using equation according to Pimpa et al. (2003); PB = PD (mmol/d) - $0.2W^{0.75}/0.12$, where PD represents daily urinary PD (mmol/d) excretion.

Data statistical analysis

All data in this experiment were statistically analyzed as a 4x4 Latin Square Design using the general linear model (GLM) procedure of the Statistical Analysis System Institute (SAS) (1996). Except for BUN and butyrate (4 h post feeding) was statistically analyzed as covariance, there was adjusted by BUN and butyrate (0 post feeding) (covariate), respectively. Duncan's New Multiple Range Test and Orthogonal Contrast Analysis (Steel and Torie, 1980) were used to compare treatment

means. Unless otherwise noted, high significance was declared at $P<0.01$, significance was declared at $P\leq0.05$, and non-significance was declared at $P>0.05$.

The model of 4 x 4 Latin Square Design was:

$$Y_{ijk} = \mu + R_i + C_j + T_k + \varepsilon_{ijk}$$

Where; Y_{ijk} = The criteria under study, response of buffalo in row i, column j of treatment k,

μ = Over all sample mean,

R_i = Effect of row i,

C_j = Effect of column j,

T_k = Effect of treatment k and

ε_{ijk} = Random error

The model of covariance of 4 x 4 Latin Square Design was:

$$Y_{ijk} = \mu + R_i + C_j + T_k + \beta(X_{ijk} + x) + \varepsilon_{ijk}$$

Where; Y_{ijk} = The criteria under study, response of buffalo in row i, column j of treatment k,

X_{ijk} = The covariates, response of buffalo in row i, column j of treatment k,

μ = Over all sample mean,

x = Mean of X

R_i = Effect of row i,

C_j = Effect of column j,

T_k = Effect of treatment k,

β = The regression or slope, adjusted y by X and

ε_{ijk} = Random error

3.5 Results and Discussions

Chemical composition of dietary treatments

The chemical composition of dietary treatments and ingredients used in the experiment are shown in Table 3.1. All diet treatments had similar chemical composition, and only in crude protein levels differed. Crude protein concentrations in dietary treatments were 5.1, 7.09, 9.12 and 11.16% of DM and metabolizable energy (ME) concentrations in all diets were 2.14 Mcal/kg DM.

Live-weight change

Average body weight and body weight change of buffaloes were not significantly ($P>0.05$) different among dietary treatment (Table 3.2). These findings are in agreement with the previous work in *Nili ravi* buffalo calves fed dietary protein (12 to 18% of DM) (Basra et al. (2003a). These results are also similar to the work of Devant et al. (2000) who suggested that increasing CP concentration in the diets from 14 to 17% CP did not affected ADG of crossbred heifers.

Also Promkot and Wanapat (2005) found that ADG was not altered by increasing CP from 10.5 to 14.4% of DM in the diets of dairy cows. In contrast to previous reports, Chumpawadee et al. (2009) observed that in Thai-indigenous yearling heifers, the animals lost weight when they were fed low dietary protein during the experiment, even though they received 20% above maintenance of metabolizable energy (ME). The imbalance in nutrient intake to meet the minimum requirement of buffaloes for growth (Kearl, 1982), may explain the weight loss and not improved weight gain of growing buffaloes.

Table 3.1 Ingredients and chemical composition of dietary treatments

Ingredients	**Dietary Crude protein levels (%)**			
	5	**7**	**9**	**11**
Rice straw	66.22	65.48	65.39	65.78
Cassava pulp	26.04	22.50	18.18	13.38
Soybean meal	4.26	8.56	12.94	17.32
Molasses	3.37	3.36	3.38	3.40
Premix[1]	0.11	0.11	0.11	0.11
Total	100	100	100	100
Chemical composition (%)				
Dry matter	87.55	87.49	87.42	87.33
	% of dry matter			
Organic matter	87.30	87.69	87.55	87.36
Crude protein	5.10	7.09	9.12	11.16
Neutral detergent fiber	56.78	56.16	55.91	55.92
Acid detergent fiber	40.58	39.87	39.42	39.11
Acid detergent lignin	22.98	22.63	22.37	22.19
Hemicellulose	16.20	16.26	16.48	16.81
Cellulose	17.59	17.26	17.05	16.92
Total digestible nutrient[2]	58.93	59.17	59.18	59.05
ME, Mcal/kg DM[3]	2.13	2.14	2.14	2.14

[1]The premix provided per kilogram of DM: 10,000IU vitamin A; 2,000IU vitamin D_3; 20 IU vitamin E; 10 mg Cu; 80 mg Mn; 40 mg Zn; 50 mg Fe; 0.8 mg I; 0.3 mg Se; 0.3 mg Co

[2]Calculated from NRC (2001); TDN = tdNFC + tdCP + (tdFA x2.25) + tdNDF – 7

[3]ME = Metabolizable energy; calculated from Kearl (1982); 1 kg TDN = 3.62 Mcal

Table 3.2 Effect of dietary protein on body weight (kg) and body weight change of growing swamp buffaloes.

Items	Dietary Crude protein levels (%)				SEM[1]	Contrast		
	5	7	9	11		L	Q	C
Initial weight, kg	217.8	219.9	220.8	217.0	2.06	-	-	-
Final weight, kg	218.5	219.3	218.0	221.8	2.53	ns	ns	ns
Average weight, kg	218.1	219.4	219.4	219.4	1.99	ns	ns	ns
BW change, kg/d	0.04	-0.01	-0.13	0.23	0.12	ns	ns	ns

[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; ns = Not significantly different (P>0.05)

Intake and digestibility of nutrient

Intake of nutrient (DM, CP, OM, TDN, NDF and ADF) are shown in Table 3.3. All nutrient intake of buffaloes except CP, were not affected by increasing protein concentration in the diet. CP intake increased with increasing protein in the diet. Average daily DM intake of buffaloes was 3.86 kg/d or 68 g/kg $W^{0.75}$/d. This result is similar to the data from Basra et al. (2003a) who showed that daily DM intake for *Nili-ravi* buffalo calves was not affected by increasing CP content in the diet, and Yuangklang (2009); Chantiratikul et al. (2009) also showed that DM intake of Brahman and Thai-indigenous cattle, respectively, were not affected when fed with different CP levels. The daily CP intake in buffaloes, in terms of g/d and g/kg $W^{0.75}$, increased (P<0.05) with increasing CP levels in diet. These results are in agreement with previous studies in growing *Nili-ravi* buffalo (Basra et al., 2003a; 2003b), in growing indigenous heifers (Chantiratikul et al., 2009; Chumpawadee et al., 2009), in Korean black goats (Hwangbo et al., 2009) and in yearling indigenous Thai native

cattle (Paengkoum and Tatsapong, 2009) who all observed that CP or nitrogen intake was affected by dietary protein concentration. However, ADF tended to decline when CP levels in the diet increased. This is because with increasing CP content in the dietary treatment, the ADF proportion in the diet decreased (Table 3.1), whereas, the another nutrient remained constant among dietary treatments.

Nutrient digestibility of buffaloes fed with the different levels of dietary protein are presented in Table 3.4 and the result showed that digestibility of CP of buffaloes was more than two times higher in 11% CP diet compared to 5% CP diet. All another nutrient digestibility, except himicellulose, were not affected by increasing levels of CP in the diet. This result is in agreement with previous studies, where CP digestibility was significantly higher with increasing CP content in diets (Paengkoum and Tatsapong, 2009; Chantiratikul et al., 2009; Chumpawadee et al., 2009). Previous research in Thai-indigenous heifers (Chantiratikul et al., 2009) showed that DM, NDF and ADF digestibilities were not affected by dietary protein content. However, Chumpawadee et al. (2009) reported that in yearling heifers increasing dietary CP in significantly increased NDF and ADF digestibilities, but not DM and OM digestibilities. But, Paengkoum and Tatsapong (2009) found that DM and OM digestibilities increased, and NDF and ADF digestibilities did not differ as levels of dietary protein content increased. These contrasting results were possibly due to multiple factors such as feed composition, ration composition, preparation of feeds and balance of dietary protein, available carbohydrate and animal breeds (McDonald et al., 1995). Javaid et al. (2008) demonstrated that increasing the levels of dietary ruminally degradable protein (RDP) given to *Nili-ravi* buffalo bulls resulted in a linear decrease in CP and NDF digestibility, but an increase in DM digestibility.

Table 3.3 Effect of dietary protein on nutrient intake of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
Dry matter								
kg/d	3.84	3.89	3.85	3.84	0.04	ns	ns	ns
g/kg $W^{0.75}$	67.85	68.34	67.65	67.54	0.31	ns	ns	ns
%BW	1.77	1.78	1.76	1.76	0.01	ns	ns	ns
Crude protein								
g/d	196.3^{d}	275.9^{c}	350.2^{b}	429.4^{a}	4.66	**	ns	ns
g/kg $W^{0.75}$	3.47^{d}	4.85^{c}	6.15^{b}	7.55^{a}	0.04	**	ns	ns
OM, kg/d	3.38	3.41	3.37	3.36	0.03	ns	ns	ns
TDN, kg/d	2.28	2.31	2.28	2.26	0.02	ns	ns	ns
NDF, kg/d	2.18	2.18	2.16	2.15	0.02	ns	ns	ns
ADF, kg/d	1.56^{a}	1.55^{a}	1.52^{ab}	1.50^{b}	0.01	*	ns	ns
Hemicellulose, kg/d	0.62	0.63	0.64	0.65	0.01	ns	ns	ns
Cellulose, kg/d	0.67	0.67	0.66	0.65	0.01	ns	ns	ns

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly ($P<0.05$); * = Significantly different ($P<0.05$); ** = Significantly different ($P<0.01$); ns = Not significantly different ($P>0.05$)

[1]BW = Body weight; OM = Organic matter; ADF = Acid detergent fiber; NDF = Neutral detergent fiber; TDN = Total digestibility nutrient; SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Table 3.4 Effect of dietary protein on apparent nutrient digestibility (%) of growing swamp buffaloes.

Items[1]	Dietary Crude protein levels (%)				SEM	Contrast		
	5	7	9	11		L	Q	C
Dry matter	55.88	55.30	53.25	57.06	2.15	ns	ns	ns
Organic matter	64.71	64.31	62.91	66.11	1.75	ns	ns	ns
Crude protein	27.82^{d}	46.49^{c}	55.11^{b}	66.50^{a}	1.95	**	ns	ns
TDN	92.06	92.17	91.71	92.57	0.38	ns	ns	ns
NDF	47.51	46.03	43.68	49.30	2.48	ns	ns	ns
ADF	42.89	40.89	37.24	41.88	2.90	ns	ns	ns
Hemicellulose	59.02^{b}	58.14^{b}	58.84^{b}	66.53^{a}	1.85	*	ns	ns
Cellulose	46.21	46.19	41.11	44.33	3.3	ns	ns	ns

$^{a, b, c, d}$ Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]ADF = Acid detergent fiber; NDF = Neutral detergent fiber; TDN = Total digestibility nutrient; SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Blood urea nitrogen (BUN) and ruminal fermentation

Concentrations of BUN, ammonia nitrogen (NH_3-N), volatile fatty acids (VFA), and pH in rumen fluid were presented to monitor the ruminal fermentation pattern (Table 3.5). Valves were measured at 0 and 4 h post feeding. Ruminal pH was similar among diet 4h post feeding and the values were quite stable at 7.0 to 7.1. This finding was similar to that found by other researcher (Chantiratikul et al., 2009; Paengkoum and Tatsapong, 2009; Chumpawadee et al., 2009) who found that the ruminal pH was not affected by increasing dietary protein. Nisa et al. (2006); Khan

and Iqbal et al. (2006) reported that ruminal pH values at 12 h after feeding buffaloes 12% CP in the diet ranged from 7.0 to 7.2. Normally, ruminal pHs of buffaloes ranged between 6.7 and 7.0 which has been found to be optimal for microbial digestion of protein (Wanapat, 1999). Rate and extent of carbohydrates digestion normally influence ruminal pH and large amounts of soluble carbohydrates may reduce the ruminal pH. Animals in the current study received 20% above maintenance of energy.

Moreover, increasing dietary RDP usually reduces ruminal pH and increases ruminal NH_3-N (Javaid et al., 2008). In spite of the ammonia nitrogen in the rumen increasing linearly, with increasing protein content (Table 3.5), it did not alter ruminal pH. It was probably because the buffering capacity can maintain the pH in the rumen of buffaloes. Ruminal NH_3-N concentration were also different ($P<0.05$) at each time (0 and 4 h post feeding) of sampling. Values of ruminal NH_3-N ranged from 11.3 to 18.7 mg/dl which is the normal range considered optimum in swamp buffaloes (Wanapat and Pimpa, 1999). The increases in rumen NH_3-N levels also occurred as levels of BUN increased (Javaid et al., 2008; Wanapat and Pimpa, 1999) and both items were increased linearly as levels of dietary protein increased in the diets (Table 3.5).

Blood urea nitrogen concentrations ranged from 17.0 to 20.7 mg% in animals fed with dietary protein levels from 5 to 11% CP. Increased BUN concentrations were associated with the elevation of NH_3-N in the rumen and nitrogen intake (Figure 3.1). These results are in agreement with previous research in Thai-indigenous yearling heifers (Chumpawadee et al., 2009), Thai-indigenous steers (Paengkoum and Tatsapong, 2009), growing finishing Brahman cattle (Yuangklang, 2009) and Thai

native and Brahman crossbred cattle (Paengkoum and Yanee, 2009). The increase in ruminal NH_3-N when protein intake increased may have been related directly to protein degradation which is more rapid than synthesis, high dietary RDP (Javaid et al., 2008; Sultan, Javaid, Nadeem, Akhtar and Mustafa, 2009) or imbalance of fermentable energy, because ammonia will accumulate in the rumen fluid and the optimum concentration will be exceeded. Thus, ammonia is absorbed into the blood, and also excreted via urine in to high levels of urine nitrogen (Javaid et al, 2008; Yuangklang, 2009; Chantiratikul et al., 2009).

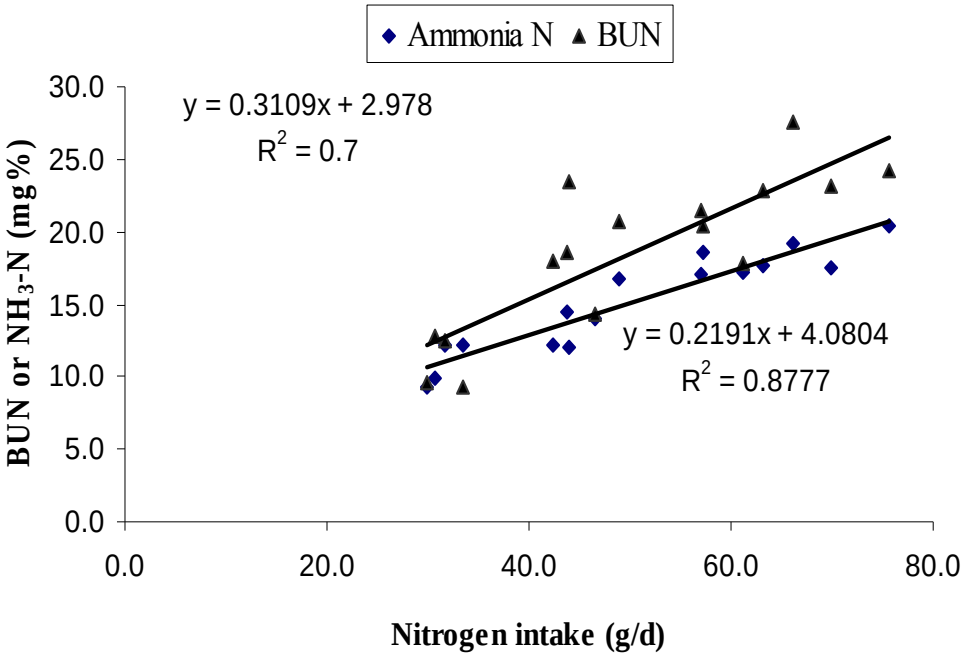

Figure 3.1 Relationship between nitrogen intake (g/d) and blood urea nitrogen (BUN) or ammonia nitrogen (NH_3-N) (mg%) in growing swamp buffaloes.

Total volatile fatty acids (TVFAs) concentrations in the rumen fluid were significantly different among treatment (P<0.05) 4 h post feeding, which ranged from 64.1 to 75.9 mM/l (Table 3.5). The values increased linearly (P<0.011) with increasing CP in the diet. These results are similar to Paengkoum and Yanee (2009) who studies in yearling Brahman x Thai native beef cattle. Generally, rate and extent of volatile fatty acid production are influenced by carbohydrate fraction and degradability of carbohydrate (McDonald et al., 1995). Acetic acid, however increased linearly with increasing protein levels in diet at 0 h post feeding, but did not increase at 4 h post feeding. Butyric acid was decreased at 0h post feeding when protein increased but increased at 4 h post feeding. However, propionic acid and the proportion of acetic and propionic acid were not affected by dietary protein concentrations. The results are in agreement with previous results observed in Thai-Indigenous heifers (Chumpawadee et al., 2009).

Nitrogen balance

The nitrogen feces and nitrogen urine are presented in Table 3.6, and the nitrogen balance of buffaloes fed different dietary protein levels are given in Table 3.7. These results clearly showed that nitrogen intake, N balance, N absorption, and N excretion in urine increased significantly (P<0.01) when dietary protein levels increased. Similar results were found in male Thai native beef cattle (Paengkoum and Tatsapong, 2009; Sereethai, Thummasaeng and Suriyapat, 2009), in growing finishing Brahman cattle (Yuangklang, 2009) and in Thai-indigenous heifers (Chantiratikul et al., 2009). There was a high relationship between nitrogen intake and nitrogen absorption in swamp buffaloes (Figure 3.2).

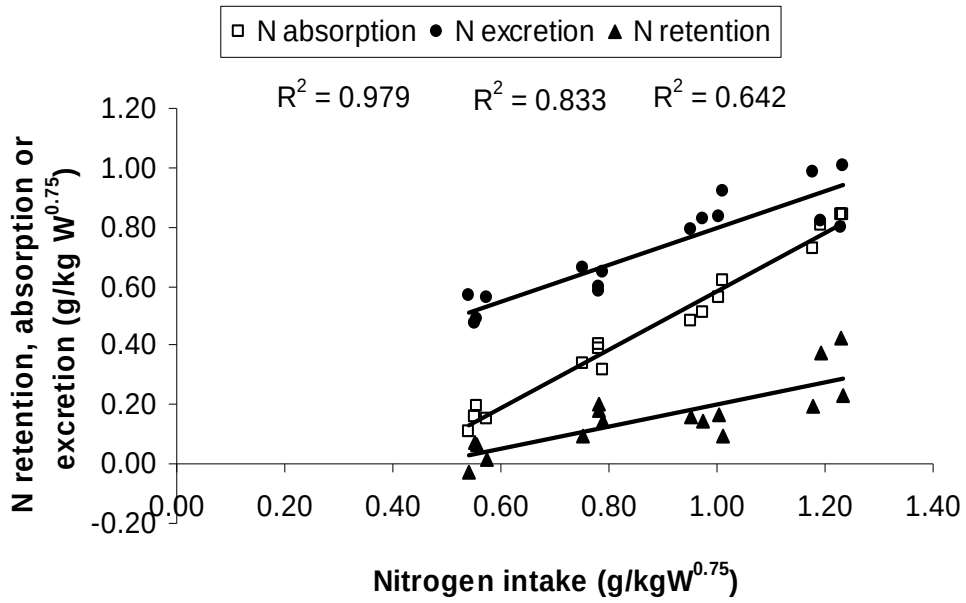

Figure 3.2 Relationship between nitrogen intake and nitrogen retention, absorption and excretion (g/kg $W^{0.75}$) in growing swamp buffaloes.

The present study found that nitrogen retention ranged from 0.03 to 0.31 g N/kg $W^{0.75}$ and N absorption ranged from 0.15 to 0.80 g N/kg $W^{0.75}$ when the buffaloes consumed dietary protein from 5 to 11% CP of DM. There was linear increase ($P<0.01$) in urinary N (6.93, 11.70, 22.93 and 28.52 g/d) by increasing (5, 7, 9 and 11% CP) protein content in the diets (Table 3.6). However, there was no significant difference ($P>0.05$) in fecal N among diets, which can be explained by the endogenous losses from digestive tracts may not be different in between animals (Kearl, 1982). N excretion through urine was found to increase due to more CP in the diet and greater N intake (Pimpa, Ruengsuwan and Pimpa, 2009; Mehra et al., 2006), was also directly related to RDP the ratio in dietary protein (Sultan et al., 2009), and high RDP ratio in dietary protein increased urine N but decreased N balance (Javaid et al., 2008).

Table 3.5 Effects of dietary protein on ruminal pH, concentrations of ruminal ammonia nitrogen, blood urea nitrogen (BUN) and volatile fatty acid of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
Ruminal pH								
0 h post feeding	7.03^{b}	7.33^{a}	7.18^{ab}	7.28^{a}	0.05	*	ns	*
4	7.05	6.88	7.12	7.10	0.10	ns	ns	ns
Ruminal NH_3-N (mg %)								
0 h post feeding	10.47^{c}	12.78^{b}	17.34^{a}	18.65^{a}	0.42	**	ns	*
4	11.33^{c}	13.59^{b}	17.52^{a}	18.73^{a}	0.45	**	ns	ns
Total volatile fatty acids (mM/L)								
0 h post feeding	66.98	65.13	66.15	68.82	1.52	ns	ns	ns
4	64.12^{b}	67.11^{b}	70.50^{ab}	75.95^{a}	2.02	**	ns	ns
Acetate (mol/100 mol)								
0 h post feeding	69.28^{b}	71.23^{ab}	71.08^{ab}	73.30^{a}	0.89	*	ns	ns
4	70.74	69.99	67.69	68.46	0.93	ns	ns	ns
Propionate (mol/100 mol)								
0 h post feeding	21.87	20.57	21.89	20.23	0.80	ns	ns	ns
4	21.43	22.34	22.52	22.49	0.68	ns	ns	ns
Butyrate (mol/100 mol)								
0 h post feeding	8.85^{a}	8.20^{ab}	7.04^{bc}	6.47^{c}	0.48	**	ns	ns
4	8.75^{b}	10.03^{a}	11.28^{a}	10.86^{a}	0.32	*	ns	ns

Table 3.5 Effects of dietary protein on ruminal pH, concentrations of ruminal ammonia nitrogen, volatile fatty acid and blood urea nitrogen (BUN) of growing swamp buffaloes (Cont.).

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
C_2:C_3	3.24	3.26	3.13	3.33	0.13	ns	ns	ns
Blood urea nitrogen (mg %)								
0 h post feeding	10.45^{c}	16.82^{b}	19.47^{b}	23.58^{a}	1.34	**	ns	ns
4	17.07^{c}	20.95^{a}	19.34^{b}	20.69^{a}	0.23	ns	**	**

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; C_2:C_3 = Acetate:Propionate

Normally, when protein degradation is more rapid than synthesis, ammonia will accumulate in the rumen liquid, absorbed into the blood, carried to the liver, converted to urea and then excreted in urine N (McDonald et al., 1995). Nitrogen endogenous losses were estimated from fasting N excretion by fasted animals during the last 4 days at the end of trial. Fecal nitrogen and urinary nitrogen losses were 0.116 ± 0.026 and 0.41 ± 0.036 g/kg $W^{0.75}$/d. These results are similar to Ørskov (1992), who reported that fasting N excretion was almost invariably about 40% higher than basal N excretion and fasting N excretion (mg/kg $W^{0.75}$) in sheep (371 and 33), goats (357 and 29), and cows (272 and 61) for urinary N and fecal N excretion, respectively.

Urinary purine derivatives excretion

Urinary purine derivative (PD) excretion, creatinine excretion and PDC index in buffaloes fed varying protein levels are presented in Table 3.8. These results showed that increasing dietary protein levels fed to buffaloes increased (P<0.01) total PD and allantoin. However, xanthine, hypoxanthine, uric acids and creatinine excretion in urine were not affected by increasing the levels of protein content in the diets. These results also agree with the work of Broderick, (2003) in dry cows, who found that increasing dietary CP (15.1 to 16.7% of DM) increased urinary PD.

Table 3.6 Effect of dietary protein on urine, feces and nitrogen excretion of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
Urine, L/d	5.20[b]	5.74[b]	6.54[b]	11.49[a]	1.15	**	ns	ns
Feces, kg/d	1.70	1.74	1.79	1.66	0.07	ns	ns	ns
Urine nitrogen								
g/d	6.93[b]	11.70[b]	22.93[a]	28.52[a]	1.76	**	ns	ns
g/kgW$^{0.75}$	0.12[b]	0.21[b]	0.40[a]	0.50[a]	0.03	**	ns	ns
Fecal nitrogen								
g/d	22.71	23.58	25.09	23.12	0.94	ns	ns	ns
g/kgW$^{0.75}$	0.40	0.42	0.44	0.40	0.02	ns	ns	ns

[a, b, c] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Table 3.7 Effect of dietary protein on N balance of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
Nitrogen intake								
g/d	31.40^{d}	44.16^{c}	56.04^{b}	68.71^{a}	0.75	**	ns	ns
g/kgW$^{0.75}$	0.55^{d}	0.78^{c}	0.98^{b}	1.21^{a}	0.01	**	ns	ns
Nitrogen excretion								
g/d	29.64^{b}	35.29^{b}	48.02^{a}	51.64^{a}	1.67	**	ns	ns
g/kgW$^{0.75}$	0.52^{c}	0.62^{b}	0.84^{a}	0.90^{a}	0.03	**	ns	ns
Nitrogen absorption[1/]								
g/d	8.69^{d}	20.57^{c}	30.94^{b}	45.58^{a}	1.09	**	ns	ns
g/kg W$^{0.75}$	0.15^{c}	0.36^{c}	0.54^{b}	0.80^{a}	0.02	**	ns	ns
Nitrogen retention[2/]								
g/d	1.76^{c}	8.87^{b}	8.02^{b}	17.06^{a}	1.36	**	ns	*
g/kg W$^{0.75}$	0.03^{d}	0.16^{b}	0.14^{b}	0.31^{a}	0.03	**	ns	*
Nitrogen endogenous loss, g/kgW$^{0.75}$ (during fasting)								
N loss in feces	0.12	0.12	0.12	0.12	-	-	-	-
N loss in urine	0.41	0.41	0.41	0.41	-	-	-	-

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels
[1/]Nitrogen absorption = Nitrogen intake – Nitrogen feces
[2/]Nitrogen retention = Nitrogen intake – Nitrogen excretion

But in other studies, increasing CP levels had no effect on excretion of total PD, allantoin and uric acid in lactating cows (Olmos Colmenero and Broderick, 2006c; 2006b), in growing steers (Rouzbehan Galbraith, Topps and Rooke ,1996), and in crossbred heifers (Devant et al., 2000). Protein source (soybean meal vs. fish meal and corn gluten meal with or without urea) and the RDP in the diets had no effect on urinary excretion of PD in heifers (Devant et al., 2001), and in lactating dairy cows (Gressley and Armentano, 2007; Sannes et al., 2002). Buffaloes fed with the 9 to 11% CP in the diets increased total PD and allantoin, it is possibly that buffaloes may be maximum efficiency of their CP requirement when compared to other breed.

In this present study, the amounts of allantoin, uric acid, hypoxanthine and xanthine excretion in urine of buffaloes ranged from 76.1-80.2, 8.5-11.1, 4.2-4.7 and 6.4-8.7%, respectively. These ranges were within the ranges reported by Chen and Gomes (1995) for allantoin (60-80%), uric acids (10-30%), xanthine plus hypoxanthine (5-10%).

Uric acid excretion in the present study (1.8 to 2.2 mmol) was low, but within the range (1.5 to 4.2 mmol) reported by Liang et al. (1994) in buffaloes. Xanthine and hypoxanthine are often hardly detectable in the urine of cattle and buffalo (Liang et al. 1994; Pimpa et al. 2003), because of the presence of xanthine oxidase in the plasma of them (Dipu, George, Singh, Verma and Mehra, 2006; Chen, Ørskov and Hovell, 1990). Chen, Samaraweera, Kyle and Ørskov (1996) summarized that xanthine oxidase activities are usually higher in buffalo than those in cattle and sheep.

Table 3.8 Effect of dietary protein on urinary purine derivatives excretion and creatinine excretion in urine of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
Allantoin								
mmol/d	12.33^{b}	14.02^{b}	16.89^{a}	18.36^{a}	0.79	**	ns	ns
mmol/kg W $^{0.75}$	0.22^{b}	0.25^{b}	0.30^{a}	0.32^{a}	0.01	**	ns	ns
%	76.05	76.26	80.19	79.40	1.76	ns	ns	ns
Uric acids								
mmol/d	1.76	2.02	1.80	2.21	0.30	ns	ns	ns
µmol/kg W $^{0.75}$	31.00	35.50	32.00	38.75	3.18	ns	ns	ns
%	10.86	11.13	8.53	9.52	1.36	ns	ns	ns
Hypoxanthine								
mmol/d	0.71	0.75	0.93	1.09	0.17	ns	ns	ns
µmol/kg W $^{0.75}$	12.50	13.25	16.50	19.25	3.16	ns	ns	ns
%	4.38	4.22	4.43	4.69	0.93	ns	ns	ns
Xanthine								
mmol/d	1.41	1.55	1.43	1.48	0.12	ns	ns	ns
µmol/kg W $^{0.75}$	24.70	27.25	25.50	26.25	2.06	ns	ns	ns
%	8.71	8.39	6.85	6.39	0.74	*	ns	ns
Total Purine derivatives								
mmol/d	16.10^{c}	18.34^{bc}	21.06^{ab}	23.13^{a}	0.85	**	ns	ns
mmol/kg W $^{0.75}$	0.28^{c}	0.32^{bc}	0.37^{ab}	0.41^{a}	0.01	**	ns	ns

Table 3.8 Effect of dietary protein on urinary purine derivatives excretion and creatinine excretion in urine of growing swamp buffaloes (Cont.).

Items[1]	**Dietary Crude protein levels (%)**					**Contrast**		
	5	**7**	**9**	**11**	**SEM**	**L**	**Q**	**C**
Creatinine								
mmol/d	42.79	35.99	43.75	38.72	3.89	ns	ns	ns
mmol/kg $W^{0.75}$	0.75	0.63	0.77	0.68	0.07	ns	ns	ns
A:Creatinine	0.29^{b}	0.40^{ab}	0.40^{ab}	0.48^{a}	0.05	*	ns	ns
PD:Creatinine	0.39	0.52	0.50	0.60	0.06	ns	ns	ns
PDC index	21.72	29.46	28.43	34.03	3.43	ns	ns	ns

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; A = Allantoin; PD = Purine derivative

Urinary excretion of creatinine was not significantly (P>0.05) affected by different CP levels. Several studies have shown that feed intake and nutrient intake did not affect creatinine excretion in urine in swamp buffaloes (Pimpa, Liang, and Balcells, 2007; Pimpa et al., 2003), in murrah buffaloes (Dipu et al., 2006) and in crossbred bulls (George, Dipu, Mehra, Verma and Singh, 2006). It has been reported that the creatinine excretion in urine is breed or species specific and is more closely correlated with muscle mass than body weight and does not depend on various dietary intake (Dipu et al., 2006; George et al., 2006). The ratios of allantoin and creatinine, PD:C and PDC index increased (P<0.01) with increasing allantoin concentration and were closely related to increased dietary protein content. Other studies have found that

allantoin:creatinine, PD:C ratios and PDC index were positively increased with increasing feed intake (George et al., 2006; Dipu et al., 2006).

Microbial nitrogen and microbial synthesis efficiency

Microbial purine base flow (PB), microbial nitrogen flow (MN) and microbial synthesis efficiency were affected by increased protein content in the diet (Table 3.9). Microbial nitrogen synthesis efficiency in terms of g N/kg of DOMR, OMI, DMI and TDNI increased ($P<0.01$) with increasing the levels of dietary protein, but microbial nitrogen synthesis efficiency in term of g N/kg of CPI was not changed. The results from this study were similar to those from Sannes et al. (2002) who showed that microbial protein synthesis increased 12% by increasing CP levels (17.0 to 19.4% of DM). Another study found that microbial protein synthesis and efficiency increased with increasing N intake (Brito et al., 2007). However, Reynal, Broderick, Ahvenjarvi and Huhtanen (2003) and Olmos Colmenero and Broderick, (2006a; 2006c) demonstrated that microbial synthesis and efficiency were not affected by increasing the CP content of the diet. In addition, Rouzbehan et al. (1996) showed that microbial nitrogen supply was unaffected by increasing nitrogen intake in growing steers. Devant et al. (2000) pointed out that increasing protein concentrations and ruminal degradability in crossbred heifers diets did not affect on microbial protein synthesis and efficiency. It is possible that low degradability of available N may contribute to the limitation in N supply for microbial growth. However, Chikunya, Newbold, Rode, Chen and Wallace (1996) observed that the microbial yield in sheep (estimated in vivo from urinary excretion of purine derivatives) increased more with casein than urea supplementation. Broderick and Reynal (2009) also found that microbial nonammonia N and efficiency were not changed by increasing RDP from urea.

Supplementation with true protein has been shown to be necessary to obtain sufficient microbial protein (Brito et al., 2007). Efficiency of the microbial growth and microbial protein production may be improved by balancing the overall daily ratio of ruminally available energy and N intake in the diet (Chumpawadee, Sommart, Vongpralub and Pattarajinda, 2006). However, microbial yield in the rumen depends on many factors such as the availability of carbohydrates and nitrogen in the rumen (Hoover and Stokes (1991), ruminal pH, physiological effects, sources and levels of nitrogen components (Olmos Colmenero and Broderick, 2006a; Brito et al., 2007) and stabilizing ruminal fermentation and synchronization for rapid fermentation with the more degradable starch and protein (Herrera-Saldana, Gomez-Alarcon, Torabi and Huber, 1990; Obitsu and Taniguchi, 2009).

Ruminal microbial populations

Table 3.10 shows the effect of dietary protein on rumen microbial ecology. The microbial counts ranged from 5.2 to 7.3 x 10^5 cells/ml rumen liquor for protozoa, 9.5 to 11.4 x 10^8 cells/ml for bacteria and 1.4 to 3.7 x 10^7 cells/ml for fungal zoospores. These results similar to microbial counts found by Chanjula et al. (2004) and Javaid et al. (2008), but the numbers of fungal zoospores in this study were higher than those reported by Chanjula et al. (2004), Granum et al. (2007) and Nisa et al. (2006), due possibly to different diet (also roughage) and rumen conditions. Normally, the fungal zoospores was greater in the ruminants fed with rice straw than that fed hay. In the present study, the numbers of total bacteria, protozoa and fungi (fungal zoospores) were not significantly different ($P>0.05$) in rumen fluid of the buffaloes when fed dietary protein from 5 to 11% CP.

Table 3.9 Effect of dietary protein on microbial purine base (PB), microbial nitrogen flow to duodenum and microbial protein synthesis efficiency of growing swamp buffaloes.

Items[1]	**Dietary Crude protein levels (%)**				**SEM**	**Contrast**		
	5	**7**	**9**	**11**		**L**	**Q**	**C**
PB flow, mmol/d	39.83^{c}	58.08^{bc}	80.59^{ab}	97.89^{a}	6.81	**	ns	ns
Microbial N supply								
g N/d	28.96^{c}	42.22^{bc}	58.95^{ab}	71.17^{a}	4.95	**	ns	ns
g N/kg DOMR	20.68^{c}	30.58^{bc}	43.29^{ab}	49.56^{a}	4.33	**	ns	ns
g N/kg OMI	8.68^{b}	12.49^{b}	17.53^{a}	21.32^{a}	1.41	**	ns	ns
g N/kg DMI	7.63^{b}	10.95^{b}	15.36^{a}	18.62^{a}	1.24	**	ns	ns
g N/kg CPI	149.19	154.06	169.09	166.55	21.47	ns	ns	ns
g N kg TDNI	12.83^{b}	18.49^{b}	25.84^{a}	31.52^{a}	2.08	**	ns	ns

[a, b, c] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels;. DOMR = Digestibility of organic matter fermented in rumen; DMI = Dry matter intake; CPI = Crude protein intake; TDNI = Total digestible nutrient intake; OMI = Organic matter intake

However, Chanjula et al. (2004) and Granum et al. (2007) found that total protozoal counts dramatically decreased, and fungal zoospores increased with increasing protein intake by supplementation of cassava hay in the diets. It is possible due to ratio of concentrate and roughage, protozoa increased with increasing concentrate. The level of crude protein in the diet had a cubic effect (P<0.05) on amylolytic bacteria populations at 4 h post feeding (cubic maxima at 11% CP), and

quadratic effect ($P<0.011$) on cellulolytic bacteria populations at 0 h post feeding (quadratic maxima at 11% CP). Moreover, amylolytic bacteria counts at 4 h post feeding increased linearly with increasing dietary protein. However, proteolytic bacteria counts were not affected by increasing protein intake. Chanjula et al. (2004) also found that cellulolytic, amylolytic and proteolytic bacteria counts did not differ with increasing protein intake by cassava hay supplementation.

Normally, the response of ruminal microbes indicate an optimal NH_3-N level in the rumen for ruminal microbial growth. Increased microbial counts were due to increasing levels of ruminal NH_3-N which has also been reported in *Nili-ravi* buffaloes (Javaid et al., 2008). In the present study, ruminal ammonia nitrogen levels ranged from 11 to 18 mg/dl and pH levels ranged from 7.0 to 7.2, and were within the optimal range for microbial growth (Wanapat and Pimpa, 1999). Maximum microbial counts are reached when ruminal ammonia level ranged from 10 to 25 mg/100 ml (Ørskov, 1992). Wanapat and Pimpa (1999) suggested that an optimum ruminal NH_3-N concentration was higher than 13.6 mg/dl in swamp buffaloes.

Similar findings have been reported by Javaid et al. (2008), who found hat bacteria and protozoa populations increased when dietary RDP increased from 66 to 82% and microbial counts decreased when ruminal NH_3-N concentration exceeded above 37.18 mg/dl. It has been also reported that synchronizing the rate of degradation of dietary energy and nitrogen release improves microorganism populations (Chumpawadee et al., 2006). Based on this study, when buffaloes were fed isocaloric diets, it is possible that energy was a limiting nutrient for growth of ruminal microbes (Clark et al., 1992).

Table 3.10 Effects of dietary protein on ruminal microbe populations of growing swamp buffaloes.

Items[1]	Dietary Crude protein levels (%)				SEM	Contrast		
	5	7	9	11		L	Q	C
Protozoa (10^5 cells/ml)								
0 h post feeding	7.47	5.44	5.69	7.28	1.49	ns	ns	ns
4	7.28	5.19	6.50	5.91	2.22	ns	ns	ns
Fungal zoospores (10^7 cells/ml)								
0 h post feeding	4.40	2.05	2.00	2.33	0.71	ns	ns	ns
4	3.70	1.42	1.42	1.89	0.78	ns	ns	ns
Bacteria (10^8 cells/ml)								
0 h post feeding	11.63	11.01	11.91	12.71	1.38	ns	ns	ns
4	9.54	11.38	10.73	9.88	2.00	ns	ns	ns
Amylolytic bacteria (10^5 CFU/ml)								
0 h post feeding	7.06	13.94	3.31	6.00	5.87	ns	ns	ns
4	3.56^{b}	14.19^{ab}	2.25^{b}	29.50^{a}	4.93	*	ns	*
Cellulolytic bacteria (10^7 CFU/ml)								
0 h post feeding	25.67^{ab}	12.67^{b}	12.67^{b}	30.33^{a}	4.02	ns	**	ns
4	21.67	30.33	16.00	24.33	7.74	ns	ns	ns
Proteolytic bacteria (10^5 CFU/ml)								
0 h post feeding	28.56	38.00	14.69	23.44	8.41	ns	ns	ns
4	15.93	18.06	34.13	35.88	10.9	ns	ns	ns

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly ($P<0.05$); * = Significantly different ($P<0.05$); ** = Significantly different ($P<0.01$); ns = Not significantly different ($P>0.05$)

[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; CFU = Colony forming unit

Protein requirement of buffaloes

The values of N balance regressed linearly for the determination of dietary nitrogen requirement for maintenance (Figure 3.3). The regression equation between N balance and N intake of buffaloes was N balance (g N/kg $W^{0.75}$) = 0.883N intake – 0.653 (R^2 = 0.855; P<0.001; n = 32). This equation was be used to estimate the N requirement for maintenance, with the N intake at which N balance equal to zero was 0.74 g N/kg $W^{0.75}$/d. Consequently, the nitrogen requirements for the maintenance of growing swamp buffaloes were 0.74 g N/kg $W^{0.75}$ or equivalent to 4.63 g CP/kg $W^{0.75}$/d or approximately 6% of dietary crude protein. These finding are in an agreement with previous reports in yearling Thai native cattle (4.36 g CP/kg W $^{0.75}$/d) (Sereethai et al., 2009), in Thai southern native cattle (0.17-0.78 g N/kg $W^{0.75}$) (Pimpa et al., 2009), and in Thai-indigenous heifers (176 g CP/d or 4.5 g CP/kg $W^{0.75}$/d) (Chantiratikul et al., 2009). The current results for the protein requirement for maintenance are approximately 12% and 14% lower than Kearl (1982) recommendation for growing domestic buffaloes (5.24 g CP/kg $W^{0.75}$) and growing crossbred cattle (5.36 g CP/kg $W^{0.75}$).

But the current results are in an agreement with Basra et al. (2003b) and Tauqir, Tipu, Fayyaz and Mushtag Aziz (2006a) who found that protein requirements of *Nili-ravi* buffalo calves are lower than dairy cattle calves recommendation by NRC (2001). In contrast, Tauqir et al. (2009b) suggested that the CP requirements of *Nili-ravi* buffalo calves is higher than those recommended by NRC (2001) for dairy cattle. The buffaloes seem to have a lower requirement for protein than those cattle, it may explained that buffaloes used a greater proportion to the OM for biomass production at the expense of VFAs compared with cows (Calabro et al., 2008). Protein

requirement for maintenance of animals appears to depend on temperate zone, climatic conditions (Marai and Haeeb, 2009), breeds, mature body size, composition of body tissue, feed quality and growth rate (Kearl, 1982; NRC, 1996).

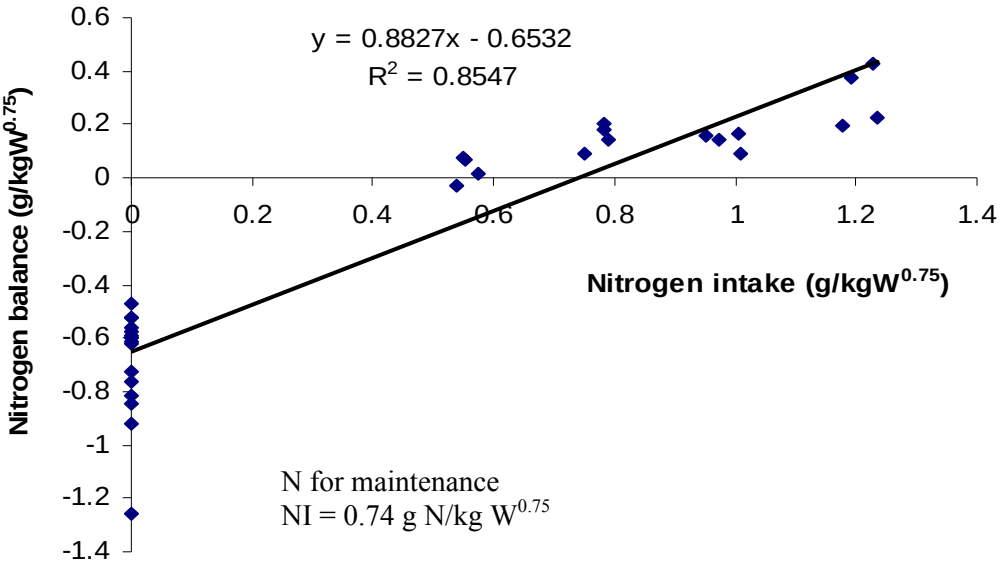

Figure 3.3 Relationship between N balance and N intake (g/kg $W^{0.75}$) in growing swamp buffaloes.

3.6 Conclusions

From this study it can be concluded that increasing dietary protein significantly increased ($P<0.01$) CP intake and digestibility, nitrogen balance, microbial nitrogen supply and efficiency ($P<0.01$) in growing male Thai swamp buffaloes. The protein requirements for maintenance of growing male Thai swamp buffaloes were 4.63 g CP/kg $W^{0.75}$/d.

CHAPTER IV

EXPERIMENT II

EFFECT OF DIETARY CRUDE PROTEIN ON THE GROWTH PERFORMANCE OF GROWING SWAMP BUFFALOES

4.1 Abstract

This experiment was conducted to investigate the effects of dietary crude protein on nitrogen utilization, and nutrient digestibility and to estimate the protein requirement for the maintenance and growth of growing swamp buffaloes. Sixteen growing male swamp buffaloes, 18 to 24 months old, with an average initial weight of 233 ± 25.0 kg, were assigned in a Randomized Complete Block Design (RCBD) with four dietary treatments, levels of crude protein (CP) for maintenance (M) 1.0, 1.4, 1.8 and 2.2 of dry matter (DM). All diets were isocaloric (0.5 kg, expected body weight gain of buffaloes of ME for maintenance). The results showed that the average daily gain (ADG) of buffaloes increased ($P<0.01$) with increasing CP content in diets. Increasing the levels of dietary protein significantly ($P<0.01$) altered intake and digestibility of nutrient of buffaloes, but did not alter ($P>0.05$) ruminal pH in buffaloes. As the level of CP in diet increased, the concentrations of blood urea nitrogen, ruminal ammonia N, total volatile fatty acids, and urinary N, faecal N, N absorption and N balance increased linearly ($P<0.01$). Increasing dietary protein

significantly increased ($P<0.01$) urinary PD excretion and the microbial N supply to the duodenum of growing Thai swamp buffaloes. Total counts of bacteria, amylolytic and cellulolytic bacteria increased ($P<0.05$) with an increase in dietary protein. However, protozoal, fungal zoospores and proteolytic bacterial populations were not significantly different ($P>0.05$) with increasing CP levels in the diet. The relationship between ADG (g ADG/kg $W^{0.75}$) and N intake (NI) (g/kg $W^{0.75}$) in growing swamp buffaloes was nitrogen intake (NI) = 0.073ADG + 0.866 ($R^2 = 0.577$; $P < 0.001$; n = 16). The present findings suggest that the nitrogen requirements for the maintenance and growth of growing male Thai swamp buffaloes are 0.866 g N or 5.41 g CP/kg $W^{0.75}$ and 0.073 g N or 0.46 g CP/g ADG.

4.2 Introduction

Swamp buffaloes are used for multiple purposes as draft power, transportation, capital, credit, meat, milk, social value, hides, and sources of natural fertilizer for cropping (Wanapat and Rowlinson, n.d.). In many places, buffaloes are preferred over cattle because of their superior quality of milk, better efficiency in utilization of nutrient from poor-quality fibrous tropical feeds and relatively better disease resistance and adaptability to tropical climates (Paul and Patil, 2007). In order to meet their production potential, buffaloes have to consume their required amounts of nutrient from their diets. The nutrition of young male buffalo is also important as it plays a major role in the onset of puberty when they are raised for breeding and it influences the quantity and quality of the meat they produced. Dietary protein supply is one of the factors that influence the productivity of animals and is supplied from microbial and dietary sources. Generally, microbial protein supplies 70 to 80% of the

required amino acids to ruminants and microbial yield in the rumen depends largely on the availability of carbohydrate and nitrogen (N) in the rumen (Chumpawadee et al., 2006). Feeding high levels of protein may be effective in promoting rapid live-weight gains, especially in growing buffalo (Basra et al., 2003b). Currently, there is insufficient information concerning the effects of protein on nutrient digestibility and nitrogen metabolism in Thai swamp buffaloes. A study of the nutritional requirements of buffaloes is necessary because the current standards of NRC (1996 and 2001) are used for beef or dairy cattle. Although, the nutrition requirements of buffalo have been determined by Kearl (1982), they can not be accurately applied for swamp buffalo. Basra et al. (2003b) reported lower protein requirements for *Nili-ravi* buffalo male calves than cattle calves, and found that the CP requirements for growth may be the same as for Holstein Friesian calves (Basra et al., 2003a). However, an optimum growth rate and feed utilization efficiency, according to inherent genetic potentiality of a particular category of animal, can only be achieved through an accurate evaluation of their nutrient requirements (Paul and Patil, 2007).

4.3 Objectives

This study was designed to determine the effect of dietary crude protein on the nitrogen utilization, and nutrient intake and digestibility, and to estimate the protein requirements for maintenance and growth of growing male Thai swamp buffaloes.

4.4 Materials and Methods

Experimental location

The experimental location was described in similar manner as that showed in Chapter III.

Animals, diets and experimental design

Sixteen growing male (bulls) swamp buffaloes, 18-24 months of age and 233 ± 25.0 kg initial weight, were allocated in a Randomized Complete Block Design. Animals were divided into 4 groups (4 animals per group) according to body weight and were assigned to treatments randomly within groups to evaluate the response to dietary protein on animal performances. The four treatment diets were formulated as follows; 1.0M, 1.4M, 1.8M and 2.2M of crude protein for maintenance, and was derived from experiment 1. The energy content of the diets were isocaloric and formulated to contain a metabolizable energy requirement equivalent to 0.5 kg, expected body weight gain of animals derived from Kearl (1982) and calculated from 1 kg TDN = 3.62 Mcal. The buffaloes were fed twice a day, at 0800 h in the morning and 1600 h in the evening. All animals were kept in a well ventilated shed and concrete floor with individual feeding and watering. Animals were dewormed for endo and ecto parasites with ivormed before commencing the experimentation. The animals were weighed monthly, before feeding and watering (fasting overnight; shrunk body weight) to record live weight changes for feed formulated during the study. All animals were fed rice straw as roughage, cassava pulp, ground corn and soybean meal as an energy and protein source according to the respective treatments. Ingredients and chemical composition of dietary treatments are presented in Table 4.1.

Experimental period

The experiment was from October 2008 to January 2009. The experimental period were 90 days for feeding period, including 7 days for collection period. The collection period was started after fed animal 45 days of the experiment, by rotation of four animals, they were kept on the metabolism cage, for collecting feces and urine, until all of them were collected feces and urine.

During the experiment, the average temperature ranged from 19.4 to 29.2°C.

Data collection and sampling procedures

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

Rumen fluid and blood samples from of all buffaloes were collected and analyzed in similar manner as that described in Chapter III.

The weight gain of buffaloes was calculated from the weighing done on a 16 h shrunk basis (fasted 16 h or over night) before and after the 90 days feeding trial.

Data analysis and calculations

The nitrogen requirement for growth was estimated by determining the average daily gain (ADG) of buffaloes fed different levels of protein. To estimate dietary N requirement for growth, the ADG and N intake were inserted into regression equation: ADG = ADG index x (N intake) – ADG at zero N intake, where the N requirement (Nm) for maintenance equals to N intake when ADG is zero. ADG index is a slope of the equation equals to N requirement for growth.

The supply of microbial N and microbial purine absorption (PB) were calculated in similar manner as that described in previous Chapter (Chapter III).

Statistical analysis

All data in this experiment were statistically analyzed as a Randomized Complete Block Design (RCBD) using the general linear model (GLM) procedure of the Statistical Analysis System Institute (SAS) (1996). Except for fungal zoospores (4 h post feeding) was statistically analyzed as covariance, there was adjusted by fungal zoospores (0 post feeding) (covariate). Duncan's New Multiple Range Test and Orthogonal Contrast Analysis (Steel and Torie, 1980) were used to compare treatment means. Unless otherwise noted, high significance was declared at $P<0.01$, significance was declared at $P\leq0.05$, and non-significance was declared at $P>0.05$.

The model was :

$$Y_{ij} = \mu + B_i + T_j + \varepsilon_{ij}$$

Where;

Y_{ij} = The criteria under study, response of buffalo in block i of treatment j,

μ = Over all sample mean,

B_i = Effect of block i,

T_j = Effect of treatment j and

ε_{ij} = Random error

The model of covariance of RCBD was :

$$Y_{ij} = \mu + B_i + T_j + \beta(X_{ij} + x) + \varepsilon_{ij}$$

Where;

Y_{ij} = The criteria under study, response of buffalo in block i of treatment j,

X_{ij} = The covariates, response of buffalo in block i of treatment j,

μ = Over all sample mean,

x = Mean of X

B_i = Effect of block i,

T_j = Effect of treatment j,

β = The regression or slope, adjusted Y by X and

ε_{ij} = Random error

4.5 Results and Discussions

Chemical composition of dietary treatments

The chemical composition of dietary treatments, and ingredients are shown in Table 4.1. All diet treatments had similar chemical compositions, but differed in crude protein levels. Crude protein concentrations in dietary treatments were 5.42, 6.96, 8.94 and 10.71% of DM and metabolizable energy (ME) concentrations in all diets were 2.20 Mcal/kg DM.

Table 4.1 Ingredients and chemical composition of dietary treatments.

Ingredients	**Crude protein for maintenance (M)**			
	1.0M	**1.4M**	**1.8M**	**2.2M**
Rice straw	61.90	63.10	66.79	65.50
Cassava pulp	14.88	15.36	14.02	12.38
Ground corn	22.76	17.81	11.02	10.81
Soybean meal	0	3.10	7.38	10.44
Urea	0.37	0.55	0.71	0.80
Premix[1]	0.09	0.08	0.08	0.08
Total	100	100	100	100
Chemical composition (%)				
Dry matter	87.37	87.45	87.49	87.65
	% of DM			
Organic matter	89.35	89.24	88.46	88.23
Crude protein	5.42	6.96	8.94	10.71
Neutral detergent fiber	56.94	55.16	57.70	57.02
Acids detergent fiber	36.73	36.15	38.43	38.56
Hemicellulose	20.21	19.01	19.26	18.46
Cellulose	16.24	15.43	16.62	16.19
Total nutrient digestible[2]	62.40	62.20	60.15	59.82
ME, Mcal/kg DM[3]	2.25	2.25	2.18	2.16

[1]The premix contained (per 5 kg kilogram) : vitamin A, 20 mIU; vitamin D3, 2 mIU; vitamin E, 20 IU; Mn, 80 g; Zn, 50 g; Fe, 120 g; Cu, 10 g; Se, 0.25 g; Co, 1 g; I, 2.5 g
[2]Calculated from NRC (2001); TDN = tdNFC + tdCP + (tdFA x2.25) + tdNDF – 7
[3]ME = Metabolizable energy; calculated from Kearl (1982); 1 kg TDN = 3.62 Mcal

Average daily gain and body weight

Initial, average and final body weight, average daily gain (ADG) and feed efficiency in growing swamp buffaloes fed different levels of dietary protein are presented in Table 4.2. The final weight and ADG of buffaloes fed with differences level of CP were significantly different ($P<0.05$) among treatments. The average daily gain throughout the 90 d of the experiment increased ($P<0.05$) with increasing protein content in the diet (-0.05 and 0.51 kg/d for the lowest and highest concentrate protein diet, respectively). These results are also similar to Chumpawadee et al. (2009) who found that ADG of Thai-indigenous yearling heifers increased when levels (6 to 12%) of dietary CP increased. However, Devant et al. (2000) found that increasing CP content of the diets (14 to 17% CP) did not affect ADG of crossbred heifers. Also from Promkot and Wanapat (2005) showed that ADG were not altered by increasing level (10.5 to 14.4%) of dietary CP, and Basra et al. (2003a) found no improvement in ADG, when CP concentration increased from 12 to 18% of DM in *Nili ravi* buffalo calves. It appears that crude protein levels higher than 10% do not improve ADG. It is possible that there is an optimum level of crude protein for buffaloes. At levels above 10% CP, ADG do not increase and there is no advantage in giving buffaloes more than 10% CP in their diet. None of the following researchers (Barsa et al., 2003a; Devant et al., 2000; Promkot and Wanapat, 2005) have got any increase in ADG with crude protein above 10% in diets.

The animals lost weight when they were fed with low dietary protein during the experiment. It is probably that the buffaloes received maintenance level of CP and its imbalance in nutrient intake to compare with minimum requirement of buffaloes for growth (experiment 1) may explain the weight loss.

Feed efficiency (ADG/DMI, CPI or TDNI) were greater (P<0.01) with increasing protein content in the diet. These observations were in disagreement with Basra et al. (2003a; 2003b) and Chantiratikul et al. (2009) who demonstrated that non-significant differences in daily feed efficiency of buffaloes fed different CP level in the diet. The differences from my results are probably due to the CP levels being much higher than those their optimum levels, and also no affect on ADG and DMI.

Table 4.2 Effect of dietary protein on body weight, average daily gain (ADG) and feed efficiency of growing swamp buffaloes.

Items[1]	**Crude protein for maintenance (M)**				**SEM**	**Contrast**		
	1.0M	**1.4M**	**1.8M**	**2.2M**		**L**	**Q**	**C**
INW, kg	233.8	234.0	234.0	232.8	6.74	-	-	-
FW, kg	229.3^{c}	248.3^{bc}	260.5^{ab}	279.0^{a}	8.50	**	ns	ns
AW, kg	231.5^{b}	241.1^{ab}	247.3^{ab}	255.9^{a}	7.34	*	ns	ns
ADG, kg/d	-0.05^{c}	0.16^{b}	0.29^{b}	0.51^{a}	0.05	**	ns	ns
ADG/DMI, g/kg	-14.20^{c}	31.89^{b}	54.49^{b}	92.07^{a}	9.91	**	ns	ns
ADG/CPI, g/g	-0.28^{b}	0.48^{a}	0.62^{a}	0.86^{a}	0.17	**	ns	ns
ADG/TDNI, g/kg	-22.49^{c}	51.14^{b}	90.65^{b}	153.73^{a}	16.36	**	ns	ns

[a, b, c] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)

[1]INW = Initial weight; FW = Final weight; AW = Average weight; ADG = Average daily gain; DMI = Dry matter intake; CPI = Crude protein intake; TDNI = Total digestibility nutrient intake; SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Intake and digestibility

Daily nutrient intakes including dry matter (DM), organic matter (OM), crude protein (CP), total digestible nutrient (TDN) and fiber fraction intake of buffaloes are presented in Table 4.3. All of the nutrient intake increased ($P<0.01$) with increasing dietary protein content. Crude protein intake (g/kg $W^{0.75}$) was 3.55 and 9.44 for the lowest and highest concentrate protein diet, 1.0 and 2.2 times CP for maintenance, respectively. These observations again were in disagreement with Basra et al. (2003a; 2003b) and Chantiratikul et al. (2009) who found that no differences in daily DM intake of calves fed different CP level in the diet. But the current results are in agreement with previous studies in growing *Nili-ravi* buffalo (Basra et al. 2003b; 2003c), in growing indigenous heifers (Chantiratikul et al., 2009; Chumpawadee et al., 2009), in Korean black goats (Hwangbo et al. (2009), in yearling indigenous Thai native cattle (Paengkoum and Tatsapong, 2009) who all found CP or nitrogen intake was sharply affected by dietary protein concentration. Feed intake can be controlled by many factors such as animal condition, nutrient requirements, rumen capacity, rumen metabolic level (VFAs), digestion rate (McDonald et al., 1995).

Nutrient deficiencies that reduce the activities of rumen microorganisms are liable to reduce feed intake. The suggested mechanism underling this effect is that high protein intake increases microbial fermentation in the rumen, which improves digestion of nutrient and also increases feed intake (Granum et al., 2007; Chumpawadee et al., 2006).

Table 4.3 Effect of dietary protein on nutrient intake of growing swamp buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Dry matter								
kg/d	4.17^{c}	4.97^{b}	5.25^{ab}	5.60^{a}	0.16	**	ns	ns
g/kg $W^{0.75}$	70.43^{c}	81.25^{b}	84.13^{ab}	87.67^{a}	1.94	**	ns	ns
% BW	1.82^{b}	2.06^{a}	2.12^{a}	2.20^{a}	0.05	**	ns	ns
Crude protein								
kg/d	0.21^{d}	0.33^{c}	0.46^{b}	0.60^{a}	0.01	**	ns	ns
g/kg $W^{0.75}$	3.55^{d}	5.42^{c}	7.34^{b}	9.44^{a}	0.21	**	ns	ns
TDN								
kg/d	2.57^{b}	3.12^{a}	3.15^{a}	3.35^{a}	0.09	**	ns	ns
g/kg $W^{0.75}$	43.57^{b}	50.93^{a}	50.49^{a}	52.51^{a}	1.31	**	ns	ns
OM, kg/d	3.70^{c}	4.44^{b}	4.65^{ab}	4.94^{a}	0.14	**	ns	ns
NDF, kg/d	2.45^{c}	2.77^{bc}	3.06^{ab}	3.21^{a}	0.11	**	ns	ns
ADF, kg/d	1.65^{c}	1.82^{bc}	2.07^{ab}	2.17^{a}	0.08	**	ns	ns
Hemicellulose, kg/d	0.80^{b}	0.96^{a}	1.00^{a}	1.03^{a}	0.04	**	ns	ns
Cellulose, kg/d	0.69^{c}	0.71^{bc}	0.87^{ab}	0.90^{a}	0.05	**	ns	ns

$^{a, b, c, d}$ Values on the same row under each main effect with different superscript differ significantly ($P<0.05$); * = Significantly different ($P<0.05$); ** = Significantly different ($P<0.01$); ns = Not significantly different ($P>0.05$)

[1]OM = Organic matter; ADF = Acid detergent fiber; NDF = Neutral detergent fiber; TDN = Total digestibility nutrient; SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Digestibility of nutrient of buffaloes fed different levels of dietary protein are shown in Table 4.4. Digestibility of CP was significantly higher ($P<0.05$) with increasing CP in the diets, but DM, OM, TDN and NDF were lower. Paengkoum and Tatsapong (2009) found that both CP and OM digestibility were higher as CP content increased in the diets. Paengkoum et al. (2006a) also found that nutrient digestibility increased with the addition of urea from 10 to 30 g/kg feed in growing goats.

In contrast, research in Thai-indigenous heifers (Chantiratikul et al., 2009) and in crossbred heifers (Devant et al., 2000) found that DM, NDF and ADF digestibilities were not affected by dietary protein content. However, Chumpawadee et al. (2009) reported that increasing dietary CP significantly increased NDF and ADF digestibilities, while DM and OM digestibilities were not affected in yearling heifers. These different results regarding effects of nutrient digestibilities were possibly due to multiple factors such as animal breed, feed quality, source of roughage, dietary CP levels and balance of dietary protein, balance of rumen degradable protein and available carbohydrate (McDonald et al., 1995; Kearl, 1982). Differences in digestive ability between sheep and cattle are small with most diets; however, highly digestible feed as cereal grains was better digested by sheep, and low-quality roughages was better digested by cattle (McDonald et al., 1995). The higher capacity of buffalo rumen population to degrade fibrous feedstuffs compared with the cow (Calabro et al., 2008).

Table 4.4 Effect of dietary protein on nutrient digestibility of growing swamp buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Apparent digestibilities (%)								
DM	60.81^{ab}	63.22^{a}	57.45^{ab}	56.40^{b}	1.77	*	ns	ns
OM	66.64^{ab}	69.73^{a}	65.14^{b}	63.02^{b}	1.26	*	ns	ns
CP	36.44^{c}	52.53^{b}	58.20^{ab}	63.30^{a}	2.76	**	ns	ns
TDN	91.08^{a}	91.92^{a}	89.36^{b}	88.59^{b}	0.47	**	ns	*
NDF	53.36^{ab}	59.95^{a}	47.85^{b}	44.82^{b}	3.19	*	ns	ns
ADF	51.41	50.36	45.92	43.97	2.67	ns	ns	ns
Hemicellulose	57.52^{ab}	67.82^{a}	51.65^{b}	47.26^{b}	4.60	*	ns	ns
Cellulose	49.71	44.07	41.08	40.74	4.53	ns	ns	ns

[a, b] Values on the same row under each main effect with different superscript differ significantly ($P<0.05$); * = Significantly different ($P<0.05$); ** = Significantly different ($P<0.01$); ns = Not significantly different ($P>0.05$)
[1]DM = Dry matter; OM = Organic matter; CP = Crude protein; ADF = Acid detergent fiber; NDF = Neutral detergent fiber; TDN = Total digestibility nutrient; SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Ruminal fermentation and blood urea nitrogen (BUN)

The ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) concentration, and pH in rumen fluid were presented to monitor the ruminal fermentation pattern and values measured at 0 and 4 h post feeding are showed in Table 4.5. Ruminal pH of buffaloes receiving all diets was similar regardless of dietary protein and the values were quite stable at 6.8 to 6.9. This data is in agreement

with previous reports by Chantiratikul et al. (2009); Paengkoum and Tatsapong (2009); Chumpawadee et al. (2009) who suggested that the ruminal pH was not affected by increasing dietary protein. Similar findings were reported by Wanapat, Pilajun and Kongmun (2009) in buffalo fed 15 to 30 g urea/kg concentrate. Ruminal pH was in the normal range from 6.8 to 6.9, which is according to Wanapat et al. (1994) who reported that the optimum level of pH in the rumen should be 6.2-6.9, which is optimal for rumen fermentation (Wanapat and Rowlinson, n.d.) and microbial digestion of protein (Wanapat, 1999; Wanapat et al., 1994). Ammonia nitrogen in the rumen linearly increased with increasing protein content (Table 4.5), but did not affect ruminal pH. It was possibly that the buffering capacity in rumen of animals may be maintained the ruminal pH and may be depended on ruminal NH_3-N.

Ruminal NH_3-N and BUN concentrations were linearly and positively correlated with dietary protein content ($P<0.01$), at each hour of sampling (Table 4.5). Average ruminal NH_3-N concentration in this experiment ranged from 10.8 to 22.1 mg/dl, within the normal range according to Wanapat and Pimpa (1999) who reported that ruminal NH_3-N concentrations ranging from 13.6 to 17.6 mg/dl in swamp buffaloes are considered optimum for microbial production. It has been reported that increasing protein intake increases ammonia nitrogen concentration in the rumen and BUN (Kim et al., 2009; Viswanathan and Fontenot, 2009). This is supported by the studies of Paengkoum and Tatsapong (2009) and Chantiratikul et al. (2009) where BUN was found to be highly correlated with dietary protein intake. Furthermore, the increases in ruminal NH_3-N levels were also associated with increasing levels of BUN (Javaid et al., 2008; Sultan et al., 2009; Kim et al., 2009).

Table 4.5 Effects of dietary protein on ruminal pH, ruminal ammonia nitrogen and blood urea nitrogen of growing swamp buffaloes.

Items[1]	**Crude protein for maintenance (M)**				**SEM**	**Contrast**		
	1.0M	**1.4M**	**1.8M**	**2.2M**		**L**	**Q**	**C**
Ruminal pH								
0 h post feeding	7.08	7.05	7.08	6.98	0.04	ns	ns	ns
4	6.88	6.75	6.70	6.75	0.07	ns	ns	ns
Ruminal NH_3-N (mg %)								
0 post feeding	10.39^{d}	12.44^{c}	16.14^{b}	18.44^{a}	0.26	**	ns	*
4	10.80^{d}	16.37^{c}	18.88^{b}	22.12^{a}	0.27	**	**	*
Blood urea nitrogen (mg%)								
0 post feeding	11.94^{d}	16.02^{c}	19.34^{b}	24.34^{a}	0.30	**	ns	ns
4	13.69^{d}	19.02^{c}	21.34^{b}	26.83^{a}	0.21	**	ns	**

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

The mean of BUN concentration in this study ranged from 13.7 to 26.8 mg/dl and, increased significantly with increasing protein content in the diet. These data are in agreement with other studies in Thai-indigenous yearling heifers (Chumpawadee et al., 2009), Thai-indigenous steers (Paengkoum and Tatsapong, 2009), growing finishing Brahman cattle (Yuangklang, 2009), Thai native and Brahman crossbred (Paengkoum and Yanee, 2009) and beef steers (Kim et al., 2009) who all found that increased BUN was associated with the elevation of NH_3-N in the rumen.

There was high relationship between nitrogen intake and BUN and ruminal NH_3-N (Figure 4.1). The increase in ruminal NH_3-N concentration when protein intake increased may have been related directly to more rapid protein degradation than synthesis (Paengkoum et al., 2006a), higher dietary rumen degradable protein (RDP) (Javiad et al., 2008; Sultan et al., 2009; Kim et al., 2009) or an imbalance of fermentable energy, so ammonia will accumulate in rumen fluid and the optimum concentration will be exceeded. Then, ammonia is absorbed into the blood, and also excreted via urine in to high levels of urine nitrogen (Paengkoum et al., 2006a; Chantiratikul et al., 2009).

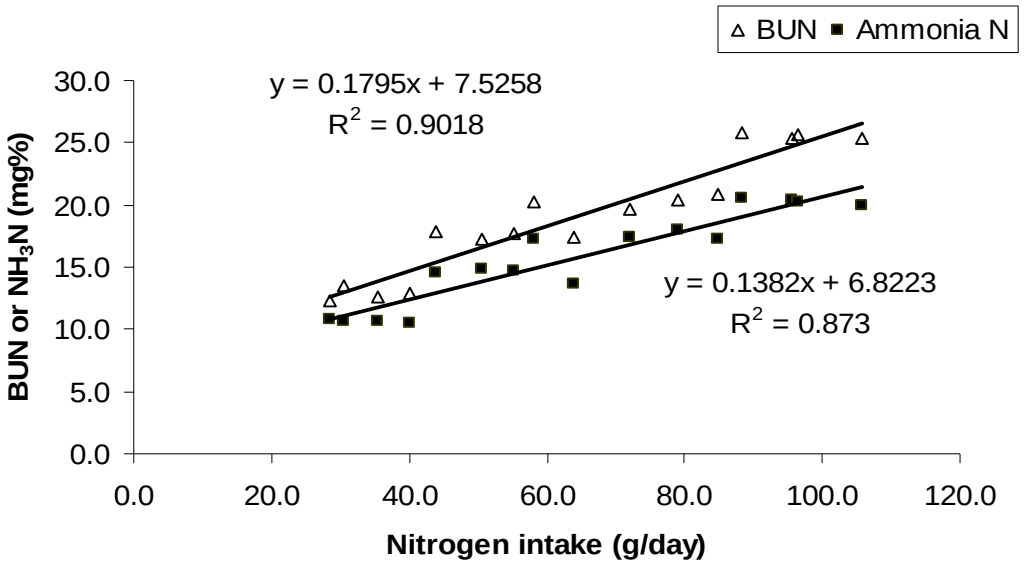

Figure 4.1 Relationship between nitrogen intake (g/d) and blood urea nitrogen (BUN) or ruminal ammonia nitrogen (NH_3-N) (mg%).

The average ruminal total volatile fatty acids (TVFAs) concentrations in growing swamp buffalo fed different levels of dietary protein are presented in Table 4.6. Average total VFAs concentrations in this study ranged from 79.0 to 85.0 mM/l

but average total VFA, and average molar proportion of VFA (acetate, propionate and butyrate) were not affected (P>0.05) by increasing protein concentration. This current data is in agreement with the work of Kim et al. (2009) and Viswanathan and Fontenot (2009) who found that total VFAs were not affected by increasing dietary protein levels. However, total VFAs at 4 h-post feeding of sampling increased linearly (P<0.05) as a consequence of feeding more CP in the diet. Similar results have been reported by Paengkoum and Yanee (2009) in their studies in yearling crossbred Brahman cattle. Molar proportions of acetate, propionate and butyrate (68, 20 and 11, respectively) were within the normal range for buffalo (Wanapat et al., 2009; Wanapat and Rowlinson, n.d.).

Molar proportions of propionate increased linearly (P<0.05), while the molar proportions of acetate decreased with increasing protein content in diet at 4 h-post feeding. This may be related to decease in NDF digestion (Table 4.4). The ratio of C_2:C_3 (acetate:propionate) declined with increasing protein content, as also found with in Thai-indigenous heifers (Chumpawadee et al., 2009). The rate and extent of volatile fatty acids production influenced by carbohydrate fraction and degradability of carbohydrate (McDonald et al., 1995).

Nitrogen balance

The volume of urine and amount of feces, N urine and N fecal in growing swamp buffalo fed different levels of dietary protein are given in Table 4.7. The amount of feces, nitrogen fecal and urine were significantly higher (P<0.05), at higher levels protein content in the diet.

Nitrogen intake, N excretion, N absorption and N retention of growing swamp buffalo fed different levels of dietary protein are presented in Table 4.8. Nitrogen

intake, N excretion, N absorption, and N retention increased linearly (P<0.01) with increasing dietary protein content. Similarly, urinary and fecal N excretion increased linearly (P<0.01) when protein content in the diet increased.

Table 4.6 Effects of dietary protein on ruminal total volatile fatty acids (VFA) and their proportion in growing swamp buffaloes.

Items[1]	**Crude protein for maintenance (M)**				**SEM**	**Contrast**		
	1.0M	**1.4M**	**1.8M**	**2.2M**		**L**	**Q**	**C**
Total volatile fatty acids (mM/L)								
0 post feeding	82.55	88.34	86.84	85.43	3.95	ns	ns	ns
4	79.01 [b]	81.56 [b]	81.56 [b]	85.00 [a]	0.86	**	ns	ns
Acetate (mol/100 mol)								
0 post feeding	68.80	69.09	69.63	68.75	0.56	ns	ns	ns
4	69.14[a]	68.43[a]	68.69[a]	65.96[b]	0.69	*	ns	ns
Propionate (mol/100 mol)								
0 post feeding	19.62	19.35	19.30	20.36	0.55	ns	ns	ns
4	18.78[b]	20.04[b]	19.95[b]	22.46[a]	0.61	**	ns	ns
Butyrate (mol/100 mol)								
0 post feeding	11.57	11.56	11.07	10.89	0.39	ns	ns	ns
4	12.07	11.53	11.36	11.57	0.34	ns	ns	ns
C_2:C_3	3.60[a]	3.51[ab]	3.54[ab]	3.15[b]	0.12	*	ns	ns

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; C_2:C_3 = Acetate:Propionate

Table 4.7 Effect of dietary protein on nitrogen excretion of growing male swamp buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Urine, L/d	3.55	3.62	4.48	4.80	0.57	ns	ns	ns
Feces, kg DM/d	1.67^{b}	1.83^{b}	2.23^{a}	2.44^{a}	0.12	**	ns	ns
Urine nitrogen								
g/d	8.08^{c}	10.06^{c}	20.93^{b}	32.55^{a}	2.17	**	ns	ns
g/kgW$^{0.75}$	0.14^{c}	0.16^{c}	0.34^{b}	0.51^{a}	0.36	**	ns	ns
Feces nitrogen								
g/d	21.79^{c}	25.19^{bc}	30.77^{ab}	35.17^{a}	2.12	**	ns	ns
g/kgW$^{0.75}$	0.36^{c}	0.41^{bc}	0.49^{ab}	0.55^{a}	0.03	**	ns	ns

$^{a, b, c, d}$ Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Nitrogen retention and N excretion in male Thai native beef cattle (Paengkoum and Tatsapong, 2009), in growing finishing Brahman cattle (Yuangklang, 2009), and in Thai-indigenous heifers (Chantiratikul et al., 2009) have also been found to increase with increasing CP content, due to greater N intake of animals as shown in Figure 4.2. The present study found that nitrogen retention ranged from 0.07 to 0.45 g N/kg $W^{0.75}$ and N absorption ranged from 0.21 to 0.96 g N/kg $W^{0.75}$ when the animals consumed different dietary protein levels from the maintenance to 2.2 times of maintenance.

Table 4.8 Effect of dietary protein on nitrogen balance of growing swamp buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Nitrogen intake								
g/d	33.65[d]	53.28[c]	73.41[b]	96.50[a]	2.36	**	ns	ns
g/kgW$^{0.75}$	0.57[d]	0.87[c]	1.17[b]	1.51[a]	0.03	**	ns	ns
Nitrogen excretion								
g/d	29.87[c]	35.24[c]	51.70[b]	67.73[a]	2.85	**	ns	ns
g/kgW$^{0.75}$	0.50[c]	0.57[c]	0.83[b]	1.06[a]	0.04	**	ns	ns
Nitrogen absorption[1/]								
g/d	11.86[d]	28.10[c]	42.64[b]	61.33[a]	2.33	**	ns	ns
g/kgW$^{0.75}$	0.21[d]	0.46[c]	0.68[b]	0.96[a]	0.04	**	ns	ns
Nitrogen retention[2/]								
g/d	3.78[b]	18.03[a]	21.70[a]	28.78[a]	3.56	**	ns	*
g/kgW$^{0.75}$	0.07[b]	0.29[a]	0.35[a]	0.45[a]	0.05	**	ns	*

[a, b, c, d] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels
[1/]Nitrogen absorption = Nitrogen intake – Nitrogen feces
[2/]Nitrogen retention = Nitrogen intake – Nitrogen excretion

Linear effects of greater fecal DM (P<0.01) was observed with increased dietary CP (Table 4.7). These effects were likely related to the linear increase (P<0.01) in DM intake and the linear decrease (P<0.05) in DM digestibility described earlier (Table 4.3 and 4.4), as well as to the excretion of undigested dietary CP. There

was a significant difference in fecal N among diets, which can be explained that endogenous losses from digestive tracts may not be different in each animal (Kearl, 1982).

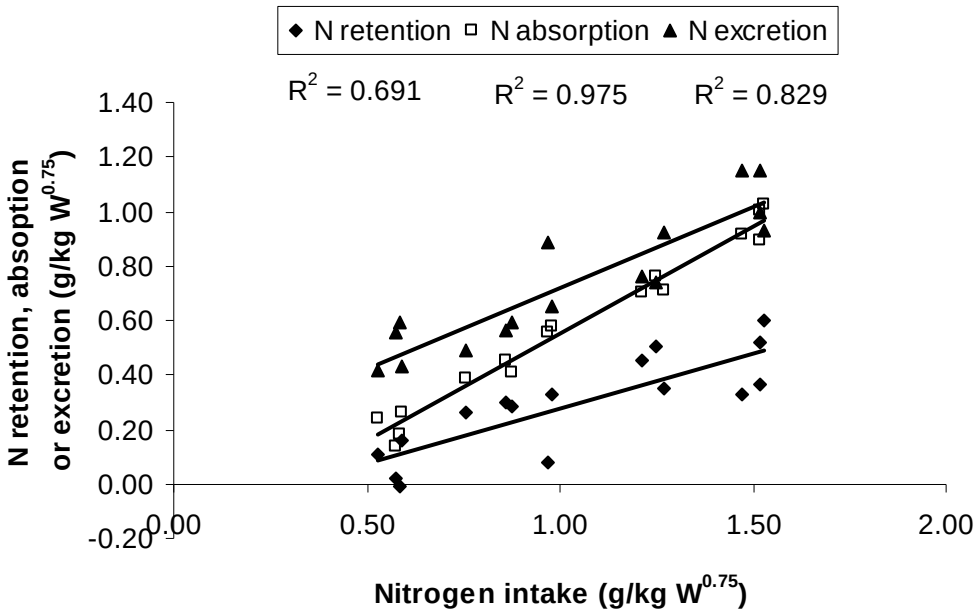

Figure 4.2 Relationship between nitrogen intake and nitrogen retention, absorption and excretion (g/kg $W^{0.75}$).

It has been demonstrated that N excretion through urine increased with increasing CP diet and N intake (Pimpa et al., 2009; Devant et al., 2000) and is also directly related to RDP ratio in dietary protein (Paengkoum et al., 2006a; Sultan et al., 2009). High RDP ratio in dietary protein increased urine N but decreased N balance (Javaid et al., 2008). Normally, nitrogen or protein degradability has a major effect on urinary N output because of excess soluble N in the rumen from diets with high RDP (Paengkoum et al., 2006a). In addition, the kind and amount of N consumed, energy level and intake, age, sex, and condition of the animal all profoundly influence nitrogen balance (Asplund, 1994). The imbalance of available N and N captured by

microbes results in ammonia being accumulated in rumen liquor and being absorbed into the blood, carried to the liver, converted to urea and their excreted via urine N (McDonald et al., 1995).

Purine derivatives excretion

The daily urinary PD excretions, creatinine excretion, PD:creatinene ratio and PDC index of buffaloes fed different level of dietary protein are presented in Table 4.9. The urinary PD excretion (mmol/d or μmol/kg $W^{0.75}$) responded significantly ($P<0.01$) to dietary protein, total urinary PD excretion of buffaloes increased with increasing protein content. Total urinary PD excretion (allantoin, uric acid, xanthine and hypoxanthine) concentration in this study ranged from 16.3 to 31.3 mmol/d, which the value was within ranges (12.8-47.0 mmol/d) as found previous for buffaloes (Wanapat and Rowlinson, n.d.; Pimpa et al., 2003). A significantly ($P<0.05$) increase in allantoin and total PD, and non-significant response in uric acid, xanthine and hypoxanthine excretion in urine were observed with respect increase in dietary protein content. The results of present study in agreement with the work of Paengkoum et al. (2006a) who demonstrated that PD excretion of goat increased with increasing of addition of urea 10 to 30 g/kg OPF. Daily urinary allantoin and uric acid excretion in the present study (10.5-25.3 and 1.5-3.8 mmol/d) were also within ranges (12.1-37.0 and 2.0-9.1 mmol/d) as found earlier for buffaloes (Wanapat and Rowlinson, n.d.; Pimpa et al., 2003).

Table 4.9 Effect of dietary protein on daily excretion of urinary urine derivatives (PD) and creatinine excretion of growing swamp buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Allantoin								
mmol/d	10.52[b]	14.83[b]	13.77[b]	25.26[a]	1.90	**	ns	ns
µmol/kg $W^{0.75}$	180[b]	240[b]	225[b]	405[a]	39.8	**	ns	ns
%	65.33[b]	77.13[a]	78.28[a]	80.61[a]	2.54	**	ns	ns
Uric acid								
mmol/d	3.00	2.09	1.50	3.82	0.75	ns	ns	ns
µmol/kg $W^{0.75}$	56.25	34.00	24.50	61.50	15.8	ns	ns	ns
%	11.34	10.81	8.53	12.06	2.81	ns	ns	ns
Hypoxanthine								
mmol/d	0.97	0.83	0.65	0.50	0.16	ns	ns	ns
µmol/kg $W^{0.75}$	18.00	10.75	8.25	13.00	3.54	ns	ns	ns
%	5.67[a]	3.46[b]	2.92[b]	2.71[b]	0.62	*	ns	ns
Xanthine								
mmol/d	1.80	1.61	1.74	1.40	0.22	ns	ns	ns
µmol/kg $W^{0.75}$	3.00	27.50	27.50	25.00	2.88	ns	ns	ns
%	11.66[a]	8.59[ab]	10.28[a]	4.62[b]	1.58	*	ns	ns
Total PD								
mmol/d	16.28[b]	19.18[b]	17.51[b]	31.32[a]	2.59	**	ns	ns
µmol/kg $W^{0.75}$	287[c]	312[b]	287[b]	503[a]	55.4	*	ns	ns

Table 4.9 Effect of dietary protein on daily excretion of urinary purine derivatives (PD) and creatinine excretion of growing swamp buffaloes (Cont.).

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Creatinine								
mmol/d	30.08	25.63	19.68	32.56	4.23	ns	ns	ns
μmol/kg $W^{0.75}$	552	416	322	519	86.7	ns	ns	ns
A:C	0.39^{b}	0.59^{ab}	0.71^{a}	0.78^{a}	0.06	**	ns	ns
PD:Creatinine	0.58^{b}	0.76^{ab}	0.90^{a}	0.97^{a}	0.07	*	ns	ns
PDC index	34.86^{b}	47.34^{ab}	55.97^{a}	61.42^{a}	4.98	*	ns	ns

[a, b, c] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; A:C = Allantoin:Creatinine; PD = Total purine derivatives

Urinary excretion of creatinine (μmol/kg $W^{0.75}$) was not significantly differ (P<0.05) among various diets. However, the ratio of A:C, PD:C and PDC index were affected by increasing CP in the diet. This study found that increasing CP content, increased feed intake (Table 4.3). This result is in disagreement with the finding in swamp buffaloes (Pimpa et al., 2007) and in murrah buffaloes (Dipu et al., 2006). However, Liu and McMeniman (2006) reported that creatinine excretion was significantly affected by diets, which were formulated by combining different amounts of forage. It has been reported that total daily creatinine excretion in urine is breed or species specific and more closely correlated with muscle mass than body

weight and creatinine excretion did not depend on dietary intake (Dipu et al., 2006; Lui and McMeniman, 2006).

In this current study, the proportion of allantoin, uric acid, hypoxanthine and xanthine excretion in urine of buffaloes ranged from 65.3-80.6, 8.2-12.1, 2.7-5.6 and 4.6-11.7%, respectively. These data findings are also within the ranges suggested by Chen and Gomes (1995) who noted that the proportions to be 60-80, 30-10 and 10-5% for allantoin, uric acids and xanthine plus hypoxanthine, respectively. The presence of hypoxanthine in buffalo urine was low probably owing to higher activity of xanthine oxidase in the intestine and plasma (Pimpa et al., 2003; 2007; Chen et al., 1996).

Microbial nitrogen supply and microbial nitrogen efficiency

Microbial N supply, microbial purine base flow and microbial N efficiency of growing swamp buffalo fed different levels of dietary protein are presented in Table 4.10. Microbial purine base flow (mmol/d) and microbial N supply (g N/d) and microbial efficiency in terms of microbial N g/kg DOMR and g/kg DMI, OMI and TDN increased linearly ($P<0.01$), but in terms of microbial N g/g CPI did not differ significantly ($P>0.05$) with increasing protein intake. This data is in agreement with the work of Paengkoum et al. (2006a) who reported that microbial N synthesis increased with increasing urea from 10 to 30 g/kg OPF.

This finding is also similar to the results of Kim et al. (2009) who found that microbial N supply was significantly higher, with increasing dietary protein concentration (9 vs. 11% of DM) and RDP fraction (52 vs. 80% of CP). Similarly, Olmos Colmenero and Broderick (2006a) demonstrated that the omasal flow of total bacterial non-NH_3-N showed linear increase from 425 to 480 g/d when dietary CP increased from 13.5 to 19.4% of DM, resulting in a linear increase in microbial

efficiency. Also similar to the work from Reynal and Broderick (2005) who showed that microbial non-NH_3-N omasal flow increased from 348 to 470 g/d when RDP levels increased from 10.6 to 13.2%. In contrast, Devant et al. (2000) found that microbial protein synthesis was not affected by protein intake and degradability but increased with age.

Table 4.10 Effect of dietary protein on microbial purine flow, microbial nitrogen supply at the duodenum and microbial nitrogen efficiency of growing swamp buffaloes.

Items[1]	**Crude protein for maintenance (M)**				**SEM**	**Contrast**		
	1.0M	**1.4M**	**1.8M**	**2.2M**		**L**	**Q**	**C**
PB flow	37.99^{c}	56.44^{b}	42.99^{b}	155.26^{a}	24.6	*	ns	ns
Microbial N supply								
g N/d	27.60^{b}	41.03^{b}	31.25^{b}	112.87^{a}	17.9	*	ns	ns
g N/kg DOMR	17.70^{b}	20.38^{b}	15.90^{b}	54.23^{a}	8.34	*	ns	ns
g N/kg OMI	8.07^{b}	9.23^{b}	6.57^{b}	22.39^{a}	3.72	*	ns	ns
g N/kg DMI	7.19^{b}	8.26^{b}	5.81^{b}	19.76^{a}	3.29	*	ns	ns
g N/kg CPI	0.14	0.12	0.06	0.19	0.04	ns	ns	ns
g N/kg TDNI	36.04^{c}	48.92^{bc}	60.50^{b}	85.26^{a}	5.24	*	ns	ns

[a, b, c] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; DOMR = Digestible OM fermented in the rumen; OMI = Organic matter intake; DMI = Dry matter intake; CPI = Crude protein intake; TDNI = Total digestible nutrient intake.

However, microbial protein production may be improved by balancing the overall daily ratio of ruminally available energy and N intake in the diet (Chumpawadee et al., 2006). It is noted that in high-energy and low-protein diets, available N for microbial growth may limit microbial protein synthesis (Devant et al., 2001). Microbial yield in the rumen depends on many factors, such as the availability of carbohydrates and N in rumen (Devant et al, 2001), ruminal pH, physiological effects, sources and levels of N components (Sannes et al., 2002) and stabilizing ruminal fermentation (McDonald et al., 1995).

Microbial populations in rumen

Total counts of rumen microbes are presented in Table 4.11. These results clearly show that total bacterial populations increased linearly ($P<0.05$) with increasing crude protein levels in the diet. Cellolytic bacterial populations had a quadratic effect ($P<0.05$) with increasing dietary protein concentration. Increasing dietary protein content did not affect ($P>0.05$) total protozoal, fungal zoospores and proteolytic bacterial populations in the rumen of buffaloes. These data are in accordance with reports by Chikunya et al. (1996) who noted that total viable bacterial populations increased with increasing nitrogen concentration in the diet (18.0 vs. 23.5 g N/kg DM), but prozoal and cellulolytic bacterial populations were not affected.

Chanjula et al. (2004) found that the number of fungal zoospores increased, but total bacterial counts and groups of bacteria, including total viable, cellulolytic, amylolytic and proteolytic bacteria were not changed by increasing protein intake. Granum et al. (2007) demonstrated that fungal zoospores and bacterial counts were higher in buffalo fed high dietary protein by supplementation of cassava hay, whereas, protozoal counts was unaffected by dietary protein intake. But these results are in

disagreement with Olmos Colmenero and Broderick (2006c) who found that total bacterial nonammonia N flow was not affected with increasing dietary protein content from 15.6 to 17.6% CP.

Moreover, Brito et al. (2007) also showed that microbial nonammonia N flow was similar among the true protein supplements but lower in cows fed urea. Wanapat et al. (2009) summarized that fungal zoospores and groups of bacteria, including total viable, cellulolytic, amylolytic and proteolytic bacteria, were not changed by different urea levels (15 vs. 30 g urea/kg concentrate). The results of Javiad et al (2008) found that total bacterial and protozoal counts in buffalo bulls were increased with increasing levels of rumen degradable protein (RDP) (50 to 82% CP). While, the work from Wanapat et al. (2009) found that protozoal counts were affected by increasing dietary energy concentration, but were not affected by increasing urea levels in the diets.

Normally, digestion of proteins results in the production of peptides, ammonia and amino acids, and all of them may individually serve as sources of N for various microbes, and the total population achieves the highest growth rate on mixtures of all three sources (Hoover and Stokes, 1991). In fact, carbohydrates and protein are the major nutrient supporting microbial growth (Ørskov, 1992). Synchronization for rapid fermentation with more degradable starch and protein stimulates greater microbial protein synthesis (Herrera-Saldana et al., 1990). However, the number of total bacteria and protozoa population are usually not significantly different ($P<0.05$), but the number of fungal zoospores decreased with an increasing synchrony index level (0.39 to 0.74) (Chumpawadee et al., 2006).

Table 4.11 Effect of dietary protein on rumen microbe populations of growing swamp

buffaloes.

Items[1]	Crude protein for maintenance (M)				SEM	Contrast		
	1.0M	1.4M	1.8M	2.2M		L	Q	C
Protozoa (10^5 cells/ml)								
0 h post feeding	7.81	4.63	6.56	4.81	2.10	ns	ns	ns
4	9.25	4.75	8.63	7.38	3.17	ns	ns	ns
Fungal zoospores (10^7 cells/ml)								
0 h post feeding	1.72^{b}	3.35^{ab}	4.14^{ab}	6.54^{a}	0.97	**	ns	ns
4	3.36	3.63	4.51	3.35	0.56	ns	ns	ns
Bacteria (10^9 cells/ml)								
0 h post feeding	1.33	1.40	1.39	1.80	0.18	ns	ns	ns
4	1.01^{b}	1.09^{b}	1.23^{b}	1.69^{a}	0.13	*	ns	ns
Amylolytic bacteria (10^6 CFU/ml)								
0 h post feeding	2.06^{b}	12.81^{ab}	6.94^{b}	39.25^{a}	9.61	*	ns	ns
4	6.19	4.19	19.88	18.44	6.91	ns	ns	ns
Cellulolytic bacteria (10^8 CFU/ml)								
0 h post feeding	0.98^{b}	1.20^{b}	3.15^{a}	1.27^{b}	0.53	ns	ns	*
4	0.68^{b}	0.90^{ab}	1.75^{a}	0.73^{b}	0.27	ns	*	ns
Proteolytic bacteria (10^6 CFU/ml)								
0 h post feeding	3.81	3.19	2.69	5.92	1.83	ns	ns	ns
4	3.38	3.19	4.13	5.00	1.20	ns	ns	ns

[a, b] Values on the same row under each main effect with different superscript differ significantly (P<0.05); * = Significantly different (P<0.05); ** = Significantly different (P<0.01); ns = Not significantly different (P>0.05)
[1]SEM = Standard Error of Means; L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; CFU = Colony forming unit

Nitrogen requirement

The nitrogen requirement of growing swamp buffaloes fed different levels of dietary protein are showed in Figure 4.3. The values of average daily gain (ADG) (g ADG/kg $W^{0.75}$) and N intake (g N/kg $W^{0.75}$) regressed linearly for the determination of dietary nitrogen requirement for growth and maintenance. The regression equation between N intake and ADG of buffalo was nitrogen intake = 0.0725ADG + 0.8663 (R^2 = 0.577, P<0.001, n = 16). Based on this equation the N requirement for maintenance and growth can be estimated, the N intake at which ADG equal to zero was N requirement for maintenance, and ADG index as a slop was N requirement for growth. Consequently, nitrogen requirements for maintenance of growing swamp buffaloes were 0.866 g N/kg $W^{0.75}$ or equivalent to 5.41 g CP/kg $W^{0.75}$/d and the nitrogen requirement for growth of growing swamp buffaloes were 0.073 g N/g ADG or equivalent to 0.46 g CP/g ADG/d.

Based on these results the protein requirement for maintenance of buffaloes was slightly higher than that recommended by Kearl (1982) for domestic buffaloes (5.24 g CP/kg $W^{0.75}$), but was slightly lower than as recommended by NRC (1996) for beef cattle (5.67 g CP/kg $W^{0.75}$). Therefore, the CP requirement for growth of buffaloes was 24% lower than that recommended by Kearl (1982) for domestic buffaloes (0.65 g CP/g ADG), and was similar to that suggested by NRC (1996) for beef cattle (0.46 g CP/g ADG). However, Basra et al. (2003b) found that the protein requirement for growth of *Nili-ravi* buffalo male calves (9 to 12 months of age) was the same as NRC described for cattle. The present data show that the CP requirement for maintenance of growing male swamp buffalo is lower than in *Nili-ravi* buffalo heifers (5.89 to 9.38 g CP/kg $W^{0.75}$), but the CP requirement for growth is within the same range (0.24 to 0.48 g CP/g ADG) of reported by Paul and Patil (2007) who

demonstrated that the requirement of CP of *Nili-ravi* buffalo heifers in the 125 to 400 body weight ranges. Differences of breeds, sex, feed quality, growth rate, body conditions, body size and climatic conditions will affect protein requirements of animals (Ørskov, 1992).

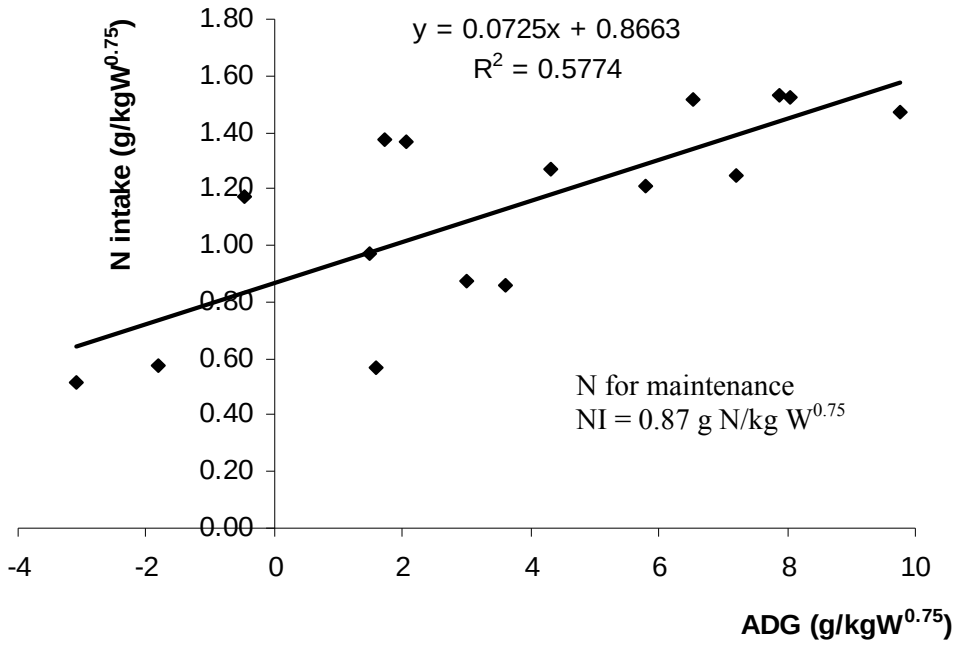

Figure 4.3 Relationship between ADG and N intake (g/kg $W^{0.75}$) in growing male swamp buffaloes.

4.6 Conclusions

Based on this study, it can be concluded that increasing dietary protein significantly increased ($P<0.05$) growth rate, nutrient intake and digestibility, N utilization, N balance, urinary PD excretion, microbial N supply and rumen microbial counts in growing male Thai swamp buffaloes. The protein requirement for maintenance and growth of growing male Thai swamp buffaloes are 5.41 g CP/kg $W^{0.75}$ and 0.46 g CP/g ADG, respectively.

CHAPTER V

EXPERIMENT III

EFFECT OF DIETARY CRUDE PROTEIN AND ENERGY ON PERFORMANCE OF GROWING SWAMP BUFFALOES

5.1 Abstract

This experiment was conducted to investigate the effect of dietary crude protein and energy on rumen fermentation, nutrient digestibility, animal performance, and the protein and energy requirements for the maintenance and growth of growing swamp buffaloes. Twenty-four growing male (bulls) swamp buffaloes with average initial weight 205 ± 45.6 kg, and between 12 to 36 months old, were used in a Randomized Complete Block Design with 2 x 3 factorial arrangement. The factors were two levels of dietary crude protein were 6 and 12% of DM, and three levels of dietary energy (metabolizable energy, ME) were 1.0, 1.4 and 1.8 time of energy requirement for maintenance (M). The results showed that either increasing the levels of protein or energy significantly ($P<0.01$) increased growth rate, nutrient intake and digestibility, total volatile fatty acids (TVFA) concentration, nitrogen balance, urinary purine derivative (PD) excretion and microbial nitrogen supply to the duodenum of growing Thai swamp buffaloes. Significant ($P<0.05$) interactions were found between protein and energy for crude protein intake and digestibility, and concentration

of TVFAs, N balance, urinary PD excretion, microbial nitrogen supply and microbial protein synthesis efficiency of growing Thai swamp buffalo. Increasing energy concentration in the diet also improved the efficiency of N utilization. The relationship between average daily gain (ADG) (g/kg $W^{0.75}$) and N intake (NI, g/kg $W^{0.75}$) in male swamp buffaloes was NI = 0.098ADG + 0.499 (R^2 = 0.744; n = 24; P<0.001). Based on this study the relationship between ADG (g/kg $W^{0.75}$) and metabolizable energy intake (MEI, kcal/kg $W^{0.75}$) was MEI = 5.225ADG + 132.72 (R^2 = 0.366; n = 24; P< 0.01). From these study it can be concluded that the protein requirements for maintenance and growth of growing male swamp buffaloes are 3.12 g CP/kg $W^{0.75}$/d and 0.61 g CP/g ADG, and the metabolizable energy requirements for the maintenance and growth of growing Thai male swamp buffaloes are 132.72 kcal/kg $W^{0.75}$/d and 5.23 kcal/g ADG.

5.2 Introduction

Swamp buffaloes can utilize agricultural crops residues and farm by-products, such as rice straw, sugarcane, cassava, sweet potato and maize stover, that are abundantly available (Wanapat et al., 1994). Seasonal fluctuations have a great impact on feed resource quantity and quality (Wanapat, 1999). Normally, agricultural crop-residues or farm by-products contain high levels of lingo-cellulosic materials, low levels of fermentable carbohydrate and low levels of good quality protein (Wanapat and Rowlinson, n.d.). However, seasonal feed resources are important for swamp buffaloes to support the efficient production under prevailing small-holder farming systems.

In order to meet the nutrient demands of growing buffaloes, the flow of nutrient from rumen fermentation should be maximized before supplementing the diet with bypass sources of protein and energy (Stokes, Hoover, Miller and Blauweikel, 1991). Wanapat et al. (2009) demonstrated that corn cob and urea at 15 g/kg could be efficiently utilized in the rumen and thus, could provide good fermentation end-products for the host swamp buffaloes.

Energy and protein are the nutritional factors that most often limit microbial growth and animal production. It has been well recognized that essential fermentation vats that are capable of supplying end-products, particularly volatile fatty acids (VFAs) and microbial proteins, are important sources of energy and protein for the ruminant host (Wanapat and Rowlinson, n.d.). Clark et al. (1992) suggested that ruminal fermentation and the flow of microbial and dietary protein to the small intestine are affected by feed intake and by the amount and source of energy and protein in the diet. Synchronization for rapid fermentation with more degradable starch and protein was found to stimulate greater microbial protein synthesis, nutrient and amino acid flow to the small intestine and improve animal production (Herrera-Saldana et al., 1990; Mabjeesh, Arieli, Bruckental, Zamwell and Tagari, 1997; Clark et al., 1992). Similarly, the work of Stokes et al. (1991) suggested that nonstructural carbohydrate greater than 24% of DM and ruminally degradable protein greater than 9% of DM enhanced microbial protein flow from the rumen of lactating Holstein cows.

A system to monitor the nutritional status of buffaloes would be beneficial to reduce losses and maximize efficiency of nutrient utilization (Mahmoudzadeh et al., 2007). The nutrient needs of growing swamp buffaloes probably differ from cattle

breeds found in temperate countries because of differences in genetic make-up, mature body size, growth rate, composition of body tissue, quality of feed and climatic conditions (Kearl, 1982; Paul and Patil, 2007). Mahmoudzadeh et al. (2007) indicated that the optimum fattening performance of 15 months old buffalo males may be obtained by providing around 10.4 MJ/kg of dietary metabolizable energy and about 10.2% of crude protein. The CP requirement of *Nili-ravi* buffaloes male calves were found to be 20% lower that those suggested by NRC (2001) for cattle, while the energy requirements were 20% higher than those suggested by NRC Basra et al. (2003c). Energy and protein requirement for maintenance and growth are of fundamental importance in the correct feeding of any animals. Because there are no definite guidelines on the energy and protein requirements for swamp buffaloes there was a need to undertake the following study.

5.3 Objective

The objectives of this experiment were to investigate the effect of dietary protein and energy levels on rumen fermentation, microbial population, nitrogen metabolism and to estimate the energy and protein requirements of growing male swamp buffaloes.

5.4 Materials and Methods

Experimental location

The experimental location was described in similar manner as that showed in Chapter III.

Animals, dietary treatment and experimental design

Twenty-four growing male (bulls) swamp buffaloes, with average initial weight of 205 ± 45.6 kg, and approximately 12-36 months of age, were allocated to a Randomized Complete Block Design (RCBD) with a 2 x 3 factorial arrangement of treatments with 4 replications. The factors were two levels of dietary crude protein (6 and 12% of DM), and three levels of dietary energy (Metabolizable energy, ME) were 1.0, 1.4 and 1.8 times of energy requirement for maintenance (M) (Kreal, 1982, and calculated from 1 kg TDN = 3.62 Mcal). The animals were divided into 4 groups (six animals per group) according to body weight and were assigned to treatments randomly within groups to evaluate the response to the treatment combinations of dietary protein and energy levels. The treatments combination were total mixed rations with varying amount of crude protein and energy concentrations as follows :

T1 : 6% CP, 1.0M,

T2 : 6% CP, 1.4M,

T3 : 6% CP, 1.8M,

T4 : 12% CP, 1.0M,

T5 : 12% CP, 1.4M and

T6 : 12% CP, 1.8M.

All animals were kept in well ventilated shed and concrete floor with individual feeding, mineral blocked and watering arrangement. The animals were

dewormed for endo and ecto parasites with ivormed before commencing the experimentation. The animals were weighed monthly, before feeding and watering (fasted overnight; shrunk body weight) to record live-weight changes for feed formulated during the experiment. All animals were fed rice straw as roughage, and with cassava pulp, ground corn and soybean meal as energy and protein sources according to the respective treatments. The ingredients and chemical composition of dietary treatment are presented in Table 5.1.

Experimental period

The experiment was from January 2009 to April 2009. The experimental period and collection period were described in similar manner as that showed in Chapter IV.

During the experiment, the average temperature ranged from 19.8 to 32.6°C.

Data collection and sampling procedures

Feed offered, refusal, faeces, urine, rumen fluid and blood samples were collected, analyzed and measured in similar manner as that described in the previous Chapter (Chapter III).

Weight gain of animals were calculated in similar manner as that described in previous Chapter (Chapter IV).

Data analysis and calculations

The nitrogen requirement for growth were estimated in similar manner as that described in Chapter IV.

The supply of microbial N and microbial purine absorption were calculated in similar manner as that described in Chapter III.

Statistical analysis

All data were analyzed as a 2 x 3 factorial arrangement in RCBD with 4 replications using the general linear model (GLM) procedure of the Statistical Analysis System Institute (SAS, 1996). The model contained effects of protein concentration, energy concentration and the interaction between these factors. For each analyzed variable, if the interaction of protein and energy was detected or was statistically significant differed ($P<0.01$), the data of treatment combination was analyzed as RCBD using the general linear model (GLM) procedure of the SAS (1996). Duncan's New Multiple Range Test and Orthogonal Contrast Analysis (Steel and Torie, 1980) were used to compare treatment means. Unless otherwise noted, high significance was declared at $P<0.01$, significance was declared at $P\leq0.05$, and non-significance was declared at $P>0.05$.

The model of this experiment was :

$$Y_{ijk} = \mu + B_i + P_j + E_k + (PE)_{jk} + \varepsilon_{ijk}$$

Where;

Y_{ijk} = The criteria under study, response of buffalo in block i of protein level j and energy level k,

μ = Over all sample mean,

B_i = Effect of block i,

P_j = Effect of protein level j,

E_k = Effect of energy level k,

$(PE)_{jk}$ = Interaction of protein level j and energy level k and

ε_{ijk} = Protein j and energy k error (random error)

5.5 Results and Discussions

Chemical composition and ingredient diets

The experimental feed ingredients are presented in Table 5.1 and their chemical compositions are reported in Table 5.2. NDF, ADF, ADL and ash contents in the diet treatments decreased slightly with increasing levels of energy concentration, while organic matter (OM) content increased slightly with increasing level of energy concentration. The crude protein (CP) content in the formulated concentrated mixtures was almost identical with the estimated values (6 and 12% of DM). Total digestible nutrient (TDN) were 57, 61 and 63% of DM, and Metabolizable energy (ME) were 2.07, 2.20 and 2.25 Mcal/kg DM, when diets were formulated at 1.0, 1.4 and 1.8 time of energy requirement for maintenance levels, respectively.

Body weight and average daily gain

The average daily gain (ADG) and body weight of buffaloes are presented in Table 5.3. There were significant differences among the groups with respect to growth during the 90 d of the experimental feeding preriod. The ADG, average body weight and final body weight of animals were higher ($P<0.01$) with increased protein content and energy content in the diets. No significant interaction effect of protein and energy was observed on the body weight and ADG of animals. The ADG range of all treatments was 0.01 to 0.62 kg/d. The growth rate increased with increased availability of protein and energy levels in the diets (Lohakare et al., 2006). The observations were in in agreement with Basra et al. (2003a; 2003c) who concluded that the *Nili–ravi* buffalo calves fed ration medium protein–high energy gained maximum weight, whereas calves fed ration low protein–low energy gained minimum weight. Similarly, results from Rouzbehan et al. (1996) reported that growth

performance of growing steers was significantly improved by molassed sugar beet pulp supplementation with white fish meal. When dietary energy sources are sufficient, it is most likely that growth rates of animal respond linearly with CP or degradable intake protein (DIP) levels (Cooper, Milton, Klopfenstein and Jordon, 2002).

Table 5.1 Feed ingredient of the experimental diets (%DM basis)

Items[1]	**6% CP**			**12 % CP**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**
Feed ingredient (kg)						
Rice straw	76.17	64.74	58.53	73.54	63.55	60.33
Cassava pulp	0	10.30	10.47	0.66	6.51	7.99
Ground corn	19.62	20.93	27.93	6.96	12.94	15.71
Soybean meal	4.07	3.91	2.99	18.71	16.60	15.65
Urea	0	0	0	0	0.30	0.20
Premix[2]	0.13	0.12	0.08	0.13	0.10	0.08
Total	100	100	100	100	100	100

[1]M = Maintenance; 1.0; 1.4; 1.8 (M) = time of metabolizable energy requirement for maintenance

[2]Premix contained (per 5 kilogram) : vitamin A, 20 mIU; vitamin D3, 2 mIU; vitamin E, 20 IU; Mn, 80 g; Zn, 50 g; Fe, 120 g; Cu, 10 g; Se, 0.25 g; Co, 1 g; I, 2.5 g

Table 5.2 Feed chemical compositions of the experimental diets.

Items[1]	**6% CP**			**12 % CP**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**
Chemical compositions						
DM	89.43	89.63	89.74	89.34	89.56	89.60
	% of DM					
OM	87.18	88.66	89.72	86.77	88.24	88.75
CP	5.91	5.86	5.82	12.09	12.31	11.98
NDF	65.24	58.82	56.19	61.56	56.54	54.90
ADF	42.25	38.15	35.36	41.33	37.32	35.90
ADL	23.39	21.48	19.95	23.17	21.16	20.35
TDN[2]	57.25	61.13	63.63	57.27	60.68	62.03
ME (Mcal/kg DM)[3]	2.07	2.21	2.30	2.07	2.20	2.25

[1]DM = Dry matter; CP = Crude protein; OM = Organic matter; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; TDN = Total digestible nutrient; M = Maintenance; 1.0; 1.4; 1.8(M) = time of metabolizable energy requirement for maintenance
[2]Calculated from NRC (2001); TDN = tdNFC + tdCP + (tdFA x2.25) + tdNDF – 7
[3]ME = Metabolizable energy; calculated from Kearl (1982); 1 kg TDN = 3.62 Mcal

In present study, the results showed that ADG was 0.16 and 0.48 kg/d when the buffaloes were fed with dietary protein levels of 6 and 12% of DM, respectively. The ADG increased linearly ($P<0.01$) with increasing protein content in the diets. This result similar to that reported by Barsa et al. (2003c) who recorded that the weight gain of *Nili–ravi* buffalo calves increased when protein levels increased and that ADG increased as CP level in feed increased (Hwangbo et al., 2009). McDonald

et al. (1995) suggested that protein is an essential nutrient for animal growth and plays an important role in muscle growth and animal development.

However, it has been reported that body weight gain did not differ significantly from different levels of dietary protein fed to crossbred calves (Lohakare et al., 2006), buffalo calves (Basra et al., 2003a; 2003b), lactating dairy cows (Promkot et al., 2005), Thai-indigenous yearling heifers (Chumpawadee et al., 2009). Also Devant et al. (2000) who demonstrated that increasing protein (14 vs. 17% of DM) and degradability (42 vs. 58% of CP) did not improve ADG in growing heifers. Source of protein affected weight gain of growing buffalo calves, when the calves fed with sunflower meal that replaced cottonseed meal (30:0 to 0:36%) decreased weight gain when sunflower meal levels increased (Yunus, Khan, Alam, Sultan and Riaz, 2004).

These current study results showed that ADG of buffaloes was 0.17, 0.30 and 0.49 kg/d when buffaloes were fed dietary energy 1.0, 1.4 and 1.8 times of energy for maintenance, respectively (Table 5.3). Growth rate increased linearly ($P<0.01$) with increasing energy content in the diets. This result is in agreement with the work of Singh et al. (2009) who found that growth rate of Bhadawari buffalo calves increased with increasing energy content in the diets. Similarly, the study of Basra et al. (2003a) who reported that growth rate increased with increasing energy levels in the diets. In contrast, Basra et al. (2003b) working with *Nili-ravi* buffalo calves, found that weight gain decreased when increasing levels of energy.

Table 5.3 Body weight (kg) and average daily gain (ADG, kg/d) of growing swamp buffaloes fed varying dietary protein and energy levels.

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Initial weight, kg	206	205	205	206	206	205	6.95	-	-	-
Average weight, kg	206^{B}	210^{B}	222^{B}	221^{A}	228^{A}	233^{A}	6.80	0.018	0.161	0.868
Final weight, kg	206^{bB}	215^{bB}	238^{aB}	236^{bA}	251^{bA}	261^{aA}	7.24	<0.001	0.005	0.636
Average daily gain (ADG), kg/d	0.01^{cB}	0.10^{bB}	0.37^{aB}	0.33^{cA}	0.51^{bA}	0.62^{aA}	0.04	<0.001	<0.001	0.274

[a-c]Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by squares means for energy levels effect.
[A-B]Distinct capital letters in the same row differ at P<0.05 by least squares means for protein levels effect.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean

However, Mahmoudzadeh et al. (2007), when feeding male buffalo calves (287 kg) with low, medium and high–energy diets (contained 90, 100 and 110% of NRC, 1996, recommendation), found that the medium energy diet caused higher daily gains than that of the other energy diets. The ADG could be affected by genetic resources, initial body weight, age, environment, nutritional management and bioavailability of energy or protein or other nutrient.

Nutrient intake

Table 5.4 shows the nutrient intake of buffaloes fed with different levels of protein and energy. Nutrient intake of growing buffaloes were affected ($P<0.01$) by increasing energy content in the diets. Nutrient intakes were greater ($P<0.01$) for growing buffaloes fed on high energy than on low energy diets. However, there was no significant interaction between energy and protein for nutrient intake, except for CP and cellulose intake. While, increasing protein content had no affect on most nutrient intake of buffaloes, except for CP, ADF and cellulose intake, which were increased with increasing protein content in the diet. The reason for increased feed intake with increasing dietary energy, was probably due to increased dietary energy concentration, which NDF, ADF, ADL and ash decreased (Table 5.2). Another reason for increased feed intake was probably because the ration was formulated in total mixed ration, with increasing CP and energy content, meaning that increasing amount of diets and also increased rate of fermentation and rate of passage in the rumen, so animals could be consume more.

DM intake of buffaloes fed 1.0M, 1.4M and 1.8M times of energy for maintenance were 58, 72 and 87 g/kg $W^{0.75}$, respectively. The results from this study were similar to those from Paengkoum et al. (2006b) who indicated that DM intake of

goats were greater for goats fed high energy than low energy diets. Similarly, Basra et al. (2003c) reported that the feed consumption of *Nili-ravi* buffalo calves increased with the increasing levels of metabolizable energy (ME) in the rations. It has been noted that the feed and DM intake is mostly affected by the energy concentration in the diet (Nair et al., 2004). Mahmoudzadeh et al. (2007) concluded that energy levels significantly affected the DMI of male buffalo calves, where the highest amount of intake was for the standard energy levels, but animals on the higher energy diets consumed lower DM. In contrast, other results show no effect of different energy levels on feed intake in Bhadawari buffalo calves (Singh et al., 2009) and in *Nili-ravi* buffalo calves (Basra et al., 2003a; 2003b). Khezri, Rezayazdi, Danesh, Mesgaran and Moradi-Sharbabk (2009) found that inclusion of sucrose in the diets did not significantly affect DM intake of dairy cows. In addition, increasing energy content by decreasing NDF content (36-28%) in diets did not also affect DM intake of lactating cows (Broderick, 2003).

Olmos Colmenero and Broderick (2006b) found that DM intake of lactating dairy cows were not affected by increasing levels of dietary CP (13.5-19.4% of DM), which was similar to the results in this study. Similarly, Hwangbo et al. (2009) reported that DM intake was not significantly different among diets when Korean black goats were fed with increasing protein levels from 14-20%. Other studies also found that dietary protein levels had no affect on DM intake in crossbred calves (Lohakare et al., 2006) and in Thai indigenous heifers (Chantiratikul et al., 2009). But the work of Broderick, Stevenson and Patton (2009) and Broderick (2003) found that higher dietary CP was associated with increased DM intake of lactating cows. And there were no differences between urea and soybean meal (SBM) supplementation on

DM intake of dairy cows, increased CP levels did affect DM intake (Sannes et al., 2002). Ipharraguerre and Clark (2005) showed that increasing protein (14.8-18.7% of DM) did not affect DM intake, but DM intake was higher for cows fed animal-marine protein blend (AMB) than for those fed solvent-extracted soybean meal (SBM). In addition, Fadel Elseed (2005) found that DM intake was not influenced by feeding frequency of protein supplement. These different feed intake results might be attributable to not only dietary CP or energy content, but also to several different factors such as feed palatability (Hwangbo et al., 2009), feed quality and rumen capacity.

The buffaloes fed high levels of protein and energy had the highest CPI (10.8 g/kg $W^{0.75}$), and the lowest (3.6 g/kg $W^{0.75}$) when fed low levels of protein and energy (Table 5.4). Basra et al. (2003c) also found that CP consumed increased with increasing levels of CP and metabolizable energy contents in the diet of *Nili-ravi* buffalo calves. Also Olmos Colmenero and Broderick (2006b), Devant et al. (2000) and Hwangbo et al. (2009) found that nitrogen (N) intake consistently increased with increasing in dietary CP. Ipharraguerre and Clark (2005) reported that CP intake increased with increasing CP levels, but was not significantly altered by different protein sources. In contrast, Broderick (2003) found that increasing dietary energy by reducing dietary NDF in the diets affected feed intake, but did not affect CP intake of lactating cows. Singh et al. (2009) also found no differences in CP intake of Bhadawari buffaloes fed low, medium and high levels of energy. CP and metabolizable energy (ME) intakes were also not affected by increasing level of CP and ME contents in the diet of *Nili-ravi* buffalo calves (Basra et al., 2003a; 2003c).

The TDN and OM intake were 33.8 and 44.7; 55.6, and 51.3; 64.2 and 78.2 g/kg $W^{0.75}$, respectively, when the buffaloes fed 1.0M, 1.4M and 1.8M time of energy for maintenance (Table 5.4). These results are similar to Singh et al. (2009) who found that intake of energy increased with increasing energy content in the diets fed to Bhadawari buffaloes. Similarly, the work of Basra et al. (2003b; 2003c) showed that on three levels of CP and ME (80, 100 and 120% of NRC (2001) fed to *Nili-ravi* buffalo calves, increased ME consumed with increasing ME content in the diets. These results also corroborate those of Promkot and Wanapat (2005) and Lohakare et al. (2009) who found that OM intake were not altered by level of dietary CP.

Other work showed that ADF intake was significantly altered by increasing (13.5 to 19.4% of DM) CP content, but NDF intake was not affected in lactating cows (Olmos Colmenero and Broderick, 2006b). Promkot and Wanapat (2005) reported that both NDF and ADF intake were not affected by increasing protein concentration in the diet. Moreover, ruminally degradable protein (RDP) and ruminally degradable nonstructural carbohydrates also did not affect feed intake (Mabjeesh et al., 1997) and dietary RDP also did not affect DM, TDN and CP intake of crossbred cattle (Kumar et al., 2005). The variation in feed intake could be related to growth rate, physiological stages, as well as nutritional management, nutritional requirement and environmental conditions for example, high growth rate or high producing animal, pregnancy or lactating animal, and also deficient diet animal were consumed more to meet their nutrient requirement. High temperature affected heat stress and also decreased feed intake of buffaloes (Marai and Haeeb, 2009).

Table 5.4 Daily nutrient intake of growing swamp buffaloes fed varying crude protein levels and energy levels.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Dry matter intake										
kg/d	3.27^{cB}	3.87^{bB}	4.91^{aB}	3.29^{cA}	4.37^{bA}	5.32^{aA}	0.13	0.010	<0.001	0.173
g/kgW$^{0.75}$	60.24^{c}	70.80^{b}	85.47^{a}	57.74^{c}	74.53^{b}	89.80^{a}	1.51	0.153	<0.001	0.073
%Body weight	1.60^{c}	1.87^{b}	2.23^{a}	1.51^{c}	1.93^{b}	2.31^{a}	0.04	0.642	<0.001	0.136
Crude protein intake										
g/d	193^{h}	214^{h}	282^{g}	398^{f}	538^{e}	638^{d}	20.63	<0.001	<0.001	0.006
g/kgW$^{0.75}$	3.56^{h}	3.96^{h}	4.94^{g}	6.98^{f}	9.14^{e}	10.77^{d}	0.19	<0.001	<0.001	<0.001
Total digestible nutrient intake										
kg/d	1.88^{c}	2.40^{b}	3.17^{a}	1.89^{c}	2.65^{b}	3.31^{a}	0.08	0.066	<0.001	0.377
g/kgW$^{0.75}$	34.62^{c}	44.11^{b}	55.34^{a}	33.14^{c}	45.33^{b}	55.96^{a}	0.82	0.863	<0.001	0.257
ME intake, Mcal/d	6.79^{c}	8.71^{b}	11.48^{a}	6.82^{c}	9.60^{b}	12.00^{a}	0.30	0.066	<0.001	0.378

Table 5.4 Daily nutrient intake of growing swamp buffaloes fed varying crude protein levels and energy levels (Cont.).

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Organic matter intake										
kg/d	2.85^{cB}	3.42^{bB}	4.41^{aB}	2.86^{cA}	3.86^{bA}	4.71^{aA}	0.12	0.019	<0.001	0.189
g/kg $W^{0.75}$	52.56^{c}	62.64^{b}	76.77^{a}	50.13^{c}	65.79^{b}	79.61^{a}	1.34	0.298	<0.001	0.098
NDF intake, kg/d	2.14^{c}	2.26^{b}	2.73^{a}	2.03^{c}	2.47^{b}	2.92^{a}	0.08	0.160	<0.001	0.113
ADF intake, kg/d	1.38^{c}	1.48^{b}	1.70^{a}	1.36^{c}	1.63^{b}	1.91^{a}	0.05	0.021	<0.001	0.113
Hemicellulose, kg/d	0.75^{c}	0.78^{b}	1.03^{a}	0.67^{c}	0.84^{b}	1.00^{a}	0.03	0.445	<0.001	0.062
Cellulose, kg/d	0.62^{gf}	0.61^{g}	0.69^{ef}	0.60^{g}	0.71^{e}	0.81^{d}	0.03	0.009	<0.001	0.042

$^{a-c}$Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
$^{d-g}$Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
$^{A-B}$Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ME = Metabolizable energy.

Nutrient digestibility

Apparent nutrient digestibility (% and g/kg $W^{0.75}$) through the total tract are presented in Table 5.5. Digestibility of nutrient were affected by increasing energy content in the diet, except for digestible cellulose which was not influenced by increasing energy content. Protein concentration in the diets did not affect digestible nutrient, except for CP digestibility which was greater ($P<0.01$) for buffaloes fed 12% CP (67.0%) than 6% CP (33.7%) diets. However, there were no significant interactions between protein and energy for above parameters. Only digestible CP based on g/kg $W^{0.75}$ detected a significant interaction between protein and energy. The buffaloes fed with low protein (6% CP) and low energy (1.0M) had the lowest (0.97 g/kg $W^{0.75}$) digestible CP, whereas the buffaloes fed with high protein (12% CP) and high energy (1.8M) had the highest (7.66 g/kg $W^{0.75}$) digestible CP. These findings are in agreement with those of Promkot and Wanapat (2005), Arieli, Sasson-Rath, Zamwel and Mabjeesh (2005) and Devant et al. (2000) who found that the concentration of protein in rations for dairy cows also did not affect nutrient digestibility, except for CP. And the data from Broderick (2003) were also similar with the amount (15. to 18.4% of DM) of CP in the diet for dairy cows, having no effect on DM and OM digestibility, but affecting digestibility of NDF and ADF. In contrast, other researchers found significantly higher digestibility of nutrient (DM, OM, CP, NFE and fiber) in animal fed different levels of protein diets (Olmos Colmenero and Broderick, 2006b; Lohakare et al., 2006; Broderick et al., 2009). It has been reported that protein supplementation also increased the digestibility of nutrient in adult male buffaloes fed wheat straw based diets (Mehra et al., 2006), and in sheep (Viswanathan and Fontenot, 2009). Olmos Colmenero and Broderick (2006c) also

found that apparent total tract digestibility of nutrient was not influenced by either dietary CP content and sources (ESBM vs. SSBM). Broderick (2003) suggested that the higher fiber digestibility at 18.4% CP might have occurred in response to the higher rumen degradable protein (RDP) intake. Decreasing RDP reduced apparent digestibility of OM, and starch and tended to reduce DM digestibility (Gressley and Armentano, 2007).

The digestibilities of DM, OM, TDN and CP based on % of DM intake or g/kg $W^{0.75}$, in growing buffaloes fed high energy diets were significantly higher ($P<0.01$) than those of buffalo fed low energy diets (Table 5.5). There was no change in the percent of apparent digestibility of NDF, ADF, hemicellulose and cellulose with increasing energy concentration, but NDF, ADF and hemicellulose digestible based on g/kg $W^{0.75}$ increased with increasing dietary energy. The results from this study were similar to those from Broderick (2003) who reported that digestibility of DM and OM increased linearly with increasing dietary energy by decreasing dietary NDF. Similarly, the data from Paengkoum et al. (2006b) demonstrated that the digestibility of DM, OM, CP and NDF increased with increasing dietary energy content. Results of the present study, however, disagreed with the work of Singh et al. (2009) who suggested that dietary energy content in the diets had no effect on nutrient digestibility. Khezri et al. (2009) found in lactating cows fed diets with increasing levels (0 to 7.5%) of sucrose replacing corn starch, that sucrose levels in the diet did not affect nutrient digestibility. Increasing rumen degradable organic matter (RDOM) from 50 to 56% had no influence on DM, OM, and CP digestibility, but NDF digestibility was higher in the low rumen degradable organic matter, whereas digestibility of non-structural carbohydrates (NSC) tended to be affected by RDOM

(Arieli et al., 2005). Increasing RDP and RDOM in dairy sheep did not affect DM digestibility (Landau et al., 2005). As discussed earlier, the greater total tract fiber fractions (NDF, ADF and hemicellulose) digestibility in term of g/kg $W^{0.75}$ with increasing dietary content were likely related to the higher level of them in the diet treatments, when the diets were calculated up to high energy or protein level in the diet treatment.

In addition, the response of fiber fractions digestion may have occurred because an increase in proportion of dietary fiber fractions came from diets, and may not represent an effect of energy content or ruminal ammonia on fiber digestion. However, the higher energy content in the diet, also means that larger NFC or NSC content may have stimulated greater microbial protein activity, because ruminal pH was always still at nearly 7 in all experimental diets. It has been found that apparent digestibility of CP in lactating cows was higher for diets containing high concentrations of ruminally degradable nonstructural carbohydrates (NSC), but OM digestibility was similar (Mabjeesh et al., 1997).

The digestibility of nutrient was significantly higher in buffaloes than sheep (Bartocci and Terramoccia, 2006). However, the variations of digestible nutrient may be due to feed quality, levels of feeding and rumen conditions for example ruminal pH, rumen microbe, it was found that reduced pH decreases digestion of proteins, cellulose, hemicelluloes and pectins but has less effect on starch digestion (Hoover and Stokes, 1991). The suggested mechanism underlying this effect is that higher protein increased microbial fermentation in the rumen, which would improve digestion of DM or OM (Promkot and Wanapat, 2005).

Table 5.5 Nutrient digestibility (%) of growing swamp buffaloes fed varying dietary protein and energy levels.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	PxE
Nutrient digestibility (%)										
Dry matter	51.99^{b}	61.79^{a}	63.13^{a}	50.88^{b}	57.92^{a}	61.35^{a}	2.19	0.228	<0.001	0.809
Organic matter	58.56^{b}	66.84^{a}	66.93^{a}	57.98^{b}	63.24^{a}	66.75^{a}	2.08	0.406	0.002	0.675
Crude protein	27.21^{cB}	31.13^{bB}	42.77^{aB}	62.13^{cA}	67.73^{bA}	71.1^{aA}	1.91	<0.001	<0.001	0.107
TDN	80.91^{b}	86.78^{a}	87.11^{a}	83.89^{b}	85.70^{a}	86.67^{a}	0.89	0.808	0.003	0.518
NDF	44.66	49.36	50.53	40.59	43.94	46.64	3.02	0.090	0.166	0.962
ADF	33.29	40.59	43.16	31.46	35.08	37.50	3.27	0.125	0.075	0.805
Hemicellulose	65.37	65.70	62.73	59.01	60.86	64.03	3.03	0.201	0.909	0.428
Cellulose	19.24	20.88	27.40	18.95	22.74	21.62	4.39	0.701	0.485	0.676

Table 5.5 Nutrient digestibility (%) of growing swamp buffaloes fed varying dietary protein and energy levels (Cont.)

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Digestible Nutrient (g/kg W $^{0.75}$)										
Dry matter	31.30^{c}	43.76^{b}	53.97^{a}	29.40^{c}	43.14^{b}	55.09^{a}	1.80	0.756	<0.001	0.706
Organic matter	30.77^{c}	41.90^{b}	51.40^{a}	29.08^{c}	41.59^{b}	53.12^{a}	1.61	0.944	<0.001	0.579
Crude protein	0.97^{h}	1.23^{h}	2.11^{g}	4.34^{f}	6.21^{e}	7.66^{d}	0.23	<0.001	<0.001	0.001
TDN	28.71^{c}	38.28^{b}	48.20^{a}	27.80^{c}	38.84^{b}	48.50^{a}	0.83	0.984	<0.001	0.649
NDF	17.47^{c}	20.25^{b}	23.95^{a}	14.43^{c}	18.45^{b}	23.01^{a}	1.26	0.081	<0.001	0.712
ADF	8.41^{c}	10.85^{b}	12.73^{a}	7.51^{c}	9.75^{b}	12.14^{a}	0.92	0.270	0.001	0.964
Hemicellulose	9.06^{cA}	9.41^{bA}	11.22^{aA}	6.92^{cB}	8.70^{bB}	10.87^{aB}	0.41	0.006	<0.001	0.101
Cellulose	2.17	2.31	3.24	2.01	2.78	2.95	0.53	0.995	0.203	0.756

[a-c]Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
[d-h]Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
[A-B]Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1] M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; TDN = Total digestible nutrient

Ruminal pH, NH_3-N and blood urea nitrogen

The effects of varying dietary CP and energy levels on ruminal pH and ruminal ammonia nitrogen (NH_3-N) concentration at 0 and 4 h post-feeding are presented in Table 5.6. There was a considerable decrease ($P<0.01$) in ruminal pH with increases in dietary energy concentration from 1.0M to 1.8M. Ruminal pH also decreased 4h after feeding. Increasing protein levels in the diet did not affect ruminal pH, and there was no significant ($P>0.055$) interaction detected between protein and energy for ruminal pH.

These results agree with the work of Paengkoum et al. (2006b) who found that ruminal pH in goats was not affected by energy content. In another study, replacing corn starch with sucrose in TMR did not affect mean ruminal pH (Khezri et al., 2009). Richardson et al. (2003) and Hosamani et al. (1998) also found that there were no energy effects detected for ruminal pH. Research has found that sugar beet pulp diets tended to lower rumen pH more than hay diets (Chikunya et al., 1996). These results however are different to those of Viswanathan and Fontenot (2009) who found that supplementation of different protein diets (6.5 to 10.1% CP) fed to sheep, did not alter ruminal pH. Similar observations on lactating cows fed with protein concentration from 13.5 to 19.4% of DM, found that the ruminal pH was also not affected by increasing protein content (Olmos Colmenero and Broderick, 2006b). Devant et al. (2000; 2001) demonstrated that neither protein concentration nor source of supplemented SBM and type of protein source had an effect on average ruminal pH.

In disagreement with these results, rumen degradable protein (RDP) affected ruminal pH, increased RDP levels resulting in linear decreases in ruminal pH (Javaid et al., 2008). However, Gressley and Armentano (2007) stated that ruminal pH did not

influence by dietary RDP. Generally, ruminal pH was affected by many factors such as concentrate diets, dietary starch, soluble carbohydrate and RDP. However, this study found that the ruminal pH stayed within the normal range (6.8 to 7.2). The optimum level of ruminal pH in buffalo for digestion of nutrient, particularly fiber and protein should be between 6.2-7.0 (Pimpa and Wanapat, 1999).

Ruminal ammonia nitrogen (NH_3-N) concentrations at 0 and 4 h post-feeding and their means were significantly ($P<0.01$) affected by protein, and energy and their interactions (Table 5.6). The average ruminal NH_3-N concentrations increased with increasing protein concentrations, whereas increasing energy content in the diets, decreased ruminal NH_3-N concentration. The average ruminal NH_3-N concentration was significantly ($P<0.01$) higher (24.7 mg%) in buffaloes on high protein and low energy content (12% CP and 1.0M), and was lowest (12.6 mg%) in buffaloes on low protein and high energy content diets (6% CP and 1.8M). Buffaloes fed low energy content diets had higher ruminal NH_3-N concentrations than the buffaloes fed with high levels of energy at the same dietary protein levels.

Ruminal NH_3-N was linearly and positively correlated with CP content or CP intake (Figure 5.1). This agrees with the work of Promkot and Wanapat (2005). These other studies have also found that increasing CP content (Oh et al., 2008), RDP fraction (Davidson et al., 2003), source of protein (Reynal et al., 2007) and protein content and RDP level (Kim et al, 2009) in diets increased ruminal NH_3-N concentration. Chikunya et al. (1996), also found that ruminal NH_3-N concentrations were affected by protein concentration or source and energy content, and they declined when casein replaced urea in diets. Nisa et al. (2006) also found that in lambs, ruminal pH and ruminal NH_3-N concentration decreased when lambs were fed

increasing corn steep liquor (CSL) replacing for urea. In contrast, Gressley and Armentano (2007) found that increasing dietary RDP strongly increased ruminal NH_3-N concentration.

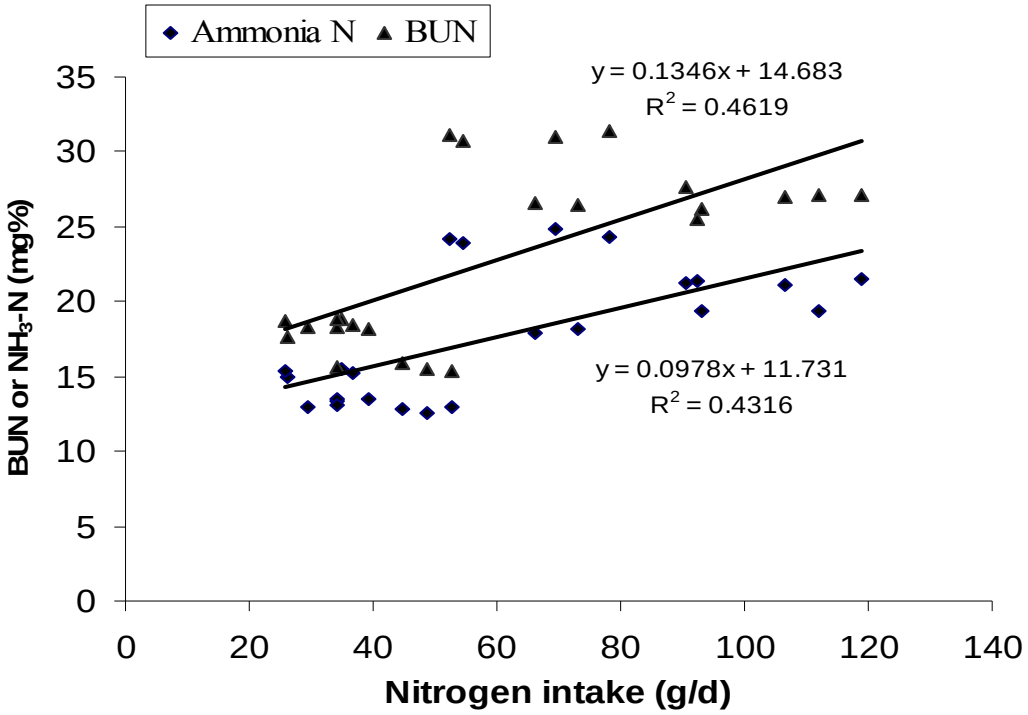

Figure 5.1 Relationship between nitrogen intake (g/d) and blood urea nitrogen (BUN) or ammonia nitrogen (NH_3-N) (mg%) in growing swamp buffalo.

Table 5.6 Effect of varying dietary crude protein and energy levels on ruminal pH and ruminal ammonia nitrogen of growing swamp buffaloes.

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Ruminal pH										
0 h post feeding	7.18	7.20	7.13	7.23	7.08	7.03	0.05	0.178	0.077	0.206
4 h	6.90^{a}	6.90^{ab}	6.73^{b}	7.10^{a}	6.75^{ab}	6.80^{b}	0.09	0.566	0.040	0.159
Ruminal NH_3-N (mg%)										
0 h post feeding	14.89^{g}	12.90^{h}	13.07^{h}	23.92^{d}	17.90^{f}	20.04^{e}	0.21	<0.001	<0.001	<0.001
4 h	15.63^{g}	13.73^{h}	12.61^{i}	24.68^{d}	19.57^{f}	22.30^{e}	0.32	<0.001	<0.001	<0.001

$^{a-c}$Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
$^{d-h}$Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
[1]M = Maintenance level; 1.0, 1.4 and1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean

Ruminal NH_3-N concentration accumulates in the rumen when the release of energy is not coupled with the release of NH_3-N in the early phase after feeding (Paengkoum et al., 2006b; Nisa et al., 2006; Javaid et al., 2008). Data have been reported in murrah buffaloes fed wheat straw with and without urea molasses mineral block (UMMB) and plus concentrate, that ruminal NH_3-N and serum urea concentration increased with increasing UMMB, whereas ruminal NH_3-N and serum urea concentrations were reduced when the buffaloes were fed UMMB plus concentrate diets (Hosamani et al., 1998). This reduction was probably due to more availability of energy from concentrate for microbial protein synthesis.

The concentration of blood urea nitrogen (BUN) at 0 and 4 h post-feeding are given in Table 5.7. Either protein or energy concentration affected BUN concentration, and there was a significant ($P<0.01$) interaction between protein and energy for this parameter. The concentration of BUN increased linearly with increasing protein content in the diets. The average BUN concentration was highest (32.7 mg%) in buffaloes fed on high levels of protein and energy, and the buffaloes fed on low level of protein and high energy level had the lowest (16.0 mg%) BUN concentration. However, the concentrations of BUN in buffaloes fed high levels of protein and energy were not significantly different from buffaloes fed high protein and low energy levels. These results show that ruminal NH_3-N and BUN concentrations increased with increasing energy or protein content, or nitrogen intake (Figure 5.1). This is supported by the studies of Promkot and Wanapat (2005) and Chantiratikul et al. (2009) who found BUN was highly correlated with ruminal ammonia absorbed from the rumen. This result agrees with the work of Promkot and Wanapat (2005) who summarized that correlations between ruminal NH_3-N and BUN are strongly

positive. Richardson et al. (2003) showed that plasma NH_3 levels were greater in lambs consuming barley compared with those consuming sugar beet-based diets. In contrast, energy source and urea levels did not affect on BUN concentration in buffaloes (Wanapat et al., 2009). Both, BUN and ruminal NH_3-N concentration were not affected by rumen degradable carbohydrate, adding sucrose (Sannes et al., 2002), and increasing rumen degradable OM (Arieli et al., 2005). But it has been found in other studies that BUN concentrations increased with increasing RDP concentration (Broderick and Reynal, 2009; Gressley and Armentano, 2007; Javaid et al., 2008; Kim et al., 2009; Sultan et al., 2009). High concentrations and fluctuation of ruminal ammonia and blood urea have been observed in animal received lower synchrony index diets (Chumpawadee et al., 2006).

Volatile fatty acid (VFA) concentration and molar proportions of VFA

Total volatile fatty acids (VFA) concentrations and molar proportions (% of total of VFA) at 0 and 4 h post-feeding are shown in Table 5.8. Ruminal total VFA, molar proportions (% of total of VFA) and the ratio of acetate to propionate were all affected by either protein or energy content, except for propionic acid which was not affected by protein content. However, there were significant ($P<0.01$) interactions between protein and energy for all parameters. Ruminal total VFAs of buffaloes were significantly higher ($P<0.01$) with increasing protein and energy concentration. Means of the total VFAs of buffaloes fed high content of protein and energy (12% CP and 1.8M) were highest (100.1 mmol/L), but lowest (68.2 mmol/L) when the buffaloes were fed with low content of protein and energy (6%CP and 1.0M). Increasing ruminal VFA concentrations occurred with diets containing high levels of protein and

energy. This was caused by increased DM intake and digestibility of nutrient (Table 5.3 and 5.4).

These results are consistent with other studies on increasing protein content and supplementation (Viswanathan and Fontenot, 2009), protein degradability (Nisa et al., 2006), and supplementation of fish meal (Rouzbeban et al., 1996) in the diets which increased total VFA concentration. However, other researchers have found that total VFAs were not affected by increasing CP (13.5 to 19.4% of DM) (Olmos Colmenero and Broderick, 2006b), protein concentration and degradability (Devant et al, 2000), CP content and protein source (Kim et al., 2009; Tiwari, Chandramoni, Jadhao, Gowda and Khan, 2001), and RDP source from urea (Broderick and Reynal, 2009). But Ivan, Mahadevan and Dayrell (1996) indicated that soybean meal showed higher total VFAs concentrations than protected soybean meal, and can reduce nitrogen source in the rumen for microbial protein synthesis.

Paengkoum et al. (2006b) also reported that either dietary energy or urea level influenced ruminal fluid total VFAs concentrations. Similarly, total VFAs concentrations were higher in animals fed sugar beet pulp diets than hay dies (Chikunya et al., 1996), and Stokes et al. (1991) reported that concentrations of total VFAs decreased as ruminal availability of carbohydrates and proteins decreased. However, it was found that total VFAs concentrations were not affected by replacing starch for sucrose (Khezri et al., 2009), 3.2% sucrose (Sannes et al., 2002), adding 200 g starch or 500 g sucrose (Oh et al., 1999), and synchrony index (Chumpawadee et al., 2006; Richardson et al. (2003). Generally, total VFAs concentrations related to the forage to concentrate ratio (Moorby et al., 2006) and high concentrate diets tended to increase total VFAs (Valadares, Broderick, Valadares Filho and Clayton, 1999).

The proportions of acetate and propionate (% of total of VFA) were significantly higher ($P<0.01$) as energy contents increased, whereas the proportion of butyrate declined with increasing the levels of either protein or energy (Table 5.8). The proportion of acetate was highest (63.8 mol/100mol) in buffaloes fed high protein and medium energy (12% CP and 1.4M) diets, and was lowest (58.7 mol/100mol) in buffaloes fed diets low in protein and energy (6% CP and 1.0M). Whereas, the proportion of propionate was highest (27.8 mol/100mol) in buffaloes fed high levels of both protein and energy. Molar proportions of acetate, propionate and butyrate were within the normal range for buffaloes (Wanapat et al., 2009).

Results of the present study agree with the work of Paengkoum et al. (2006b) who summarized that ruminal fluid proportions of VFAs increased with increasing energy content and urea. Nisa et al. (2006) found that replacing source of protein degradability increased the molar proportion of acetate and propionate. In contrast, it has been reported that molar proportions of VFA were not affected by increasing CP levels (13.5 to 19.4% of DM) (Olmos Colmenero and Broderick, 2006b), protein concentration and source of supplementation (Rouzbehan et al., 1996; Kim et al., 2009), protein content and degradability (Devant et al., 2000; 2001), dietary RDP with or without inulin infusion (Gressley and Armentano, 2007) and RDP source from urea (Broderick and Reynal, 2009). Moreover, replacing corn starch for sucrose (Khezri et al., 2009), and adding 200 g starch or 500 g sucrose (Oh et al., 1999) did not affect the molar proportion of most of the individual VFA. Wanapat et al. (2009) also found that molar proportions of individual VFAs in buffalo bulls were not affected by energy source and urea levels. Sannes et al. (2002) also demonstrated that rumen degradable carbohydrate and nitrogen levels (17.0 to 19.6% CP) did not affect total VFAs and

molar proportions of individual VFA. Jatana et al. (2000) found that energy and protein source affected both acetate and propionate molar proportions, and the molar proportions of acetate was also affected by protein source (fish meal vs. soybean meal), and the molar proportion of propionate was affected by energy source (paper pulp vs. corn flour). Stokes et al. (1991) reported that the trend for change in individual molar proportions of VFA reflects the carbohydrate compositions of the diets, and propionate increased but acetate decreased with increasing carbohydrate in the diets.

The ratio of acetate to propionate (A:P) ranged from 2.2 to 2.7 and was highest in buffaloes fed diets with high protein and medium energy (12% CP and 1.4M). This may indicate that, the diets contained high energy and increased the intake of fiber fractions (Table 5.4). It was also noted that the amount of propionate produced were remarkably high and resulted in a suitable ratio of acetate to propionate ratio (2.2 to 2.6) (Wanapat et al., 2009). In the present study, the ratio of rougphage to concentrate was proximately 60:40 (Table 5.1), and this could explain why the molar proportions of VFA were within the normal range in this experiment. Animals fed with high concentrate usually produce a greater proportion of propionate due to a declined ratio of A:P (Devant et al., 2000; 2001). Increasing concentrate diet reduces the acetate proportion, but increases propionate proportion (Valadares et al., 1999).

Table 5.7 Effect of varying dietary crude protein and energy levels on blood urea nitrogen (BUN) in growing swamp buffaloes.

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
BUN (mg %)										
0 h post feeding	16.86^{g}	17.99^{f}	15.21^{h}	29.43^{d}	26.10^{e}	26.07^{e}	0.36	<0.001	<0.001	<0.001
4 h	19.88^{f}	18.84^{f}	16.02^{g}	32.68^{d}	27.04^{e}	27.49^{e}	0.37	<0.001	<0.001	<0.001

$^{d-h}$Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean

Table 5.8 Effect of varying dietary crude protein and energy levels on total volatile fatty acids of growing swamp buffaloes.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Total VFAs (mmol/l)										
0 h post feeding	62.72^{i}	68.60^{h}	88.41^{e}	75.82^{g}	82.51^{f}	94.44^{d}	1.22	<0.001	<0.001	0.010
4 h	68.18^{h}	78.51^{g}	85.21^{f}	69.15^{h}	90.03^{e}	100.05^{d}	0.70	<0.001	<0.001	<0.001
Acetate (mol/100mol)										
0 h post feeding	57.76^{f}	58.84^{f}	62.43^{de}	63.04^{d}	62.54^{de}	61.10^{e}	0.45	<0.001	0.022	<0.001
4 h	59.68^{gh}	61.56^{e}	60.26^{fg}	58.67^{h}	63.87^{d}	60.88^{ef}	0.36	0.044	<0.001	0.001
Propionate (mol/100mol)										
0 h post feeding	25.06^{e}	24.94^{e}	25.31^{e}	22.77^{f}	24.35^{e}	27.10^{d}	0.40	0.283	<0.001	<0.001
4 h	24.12^{g}	24.22^{g}	26.27^{e}	25.32^{f}	22.86^{h}	27.84^{d}	0.27	0.049	<0.001	<0.001

Table 5.8 Effect of varying dietary crude protein and energy levels on total volatile fatty acids of growing swamp buffaloes (Cont.).

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Butyrate (mol /100 mol)										
0 h post feeding	17.19^{d}	16.23^{e}	12.27^{hg}	14.19^{f}	13.12^{g}	11.80^{h}	0.29	<0.001	<0.001	<0.001
4 h	16.19^{d}	14.22^{e}	13.47^{f}	16.00^{d}	13.27^{f}	11.27^{g}	0.22	<0.001	<0.001	0.001
Acetate : Propionate	2.39^{f}	2.45^{ef}	2.38^{f}	2.53^{e}	2.68^{d}	2.22^{g}	0.03	0.012	<0.001	<0.001

[a-c] Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
[d-h] Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
[A-B] Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1] M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean

Nitrogen balance

The amount of feces, urine volume, fecal nitrogen and urinary nitrogen are shown in Table 5.9. Both of protein or energy affected the amount of feces, but did not influence urine volume. But urinary nitrogen excretion increased ($P<0.01$) with increasing protein content, and fecal nitrogen excretion was affected by increasing the levels of either protein or energy in the diets. These results agree with the work of Paengkoum et al. (2006b) who indicated that nitrogen excretion (faeces and urine) in goats fed both low and high energy with 5% urea was higher than 3 and 4% urea in the diet. Broderick (2003) and Lohakare et al., (2006) found that increasing dietary protein levels increased excretion of fecal and urinary nitrogen. Increasing protein levels (15.1 to 18.4% of DM) also influenced higher urine volume and increased the amount of feces (Broderick, 2003). The increased amount of feces with increasing dietary protein and energy content, was probably due to increases DM intake (Table 5.4). These findings, however, are in contrast to those of Broderick (2003) on lactating cows, who found that increasing energy levels by reducing NDF did not affect urine volume, but fecal DM declined.

Nitrogen balance including intake, excretion, absorption and retention of buffaloes fed with protein and different levels of energy are presented in Table 5.10. There were significant effects ($P<0.01$) of energy, and protein and their interaction on nitrogen balance of animals. Nitrogen intake, excretion, nitrogen retention and nitrogen absorption of buffaloes fed with high levels of protein and energy (12% CP and 1.8M) were highest (1.62, 1.10, 0.62 and 1.22 g/kg $w^{0.75}$), but were lowest (0.57, 0.59, -0.02 and 0.16 g/kg $w^{0.75}$) when the buffaloes fed low level of protein and energy (6% CP and 1.0M). This study finding, nitrogen excretion of buffaloes fed

with 12% CP was higher than those fed 6% CP in the diets (1.07 vs. 0.57 g/kg $w^{0.75}$). Intake, retention and absorption of nitrogen were significantly higher ($P<0.01$) with increasing both protein and energy content.

As a result, increasing nitrogen intake positively increased N excretion, retention and absorption (Figure 5.2), because of insufficient supply of energy. These results are also confirmed by other studies (Labussiere, Dubois, Milgen, Bertrand and Noblet, 2008; Khan and Iqbal et al., 2006; Olmos Colmenero and Broderick, 2006a; 2006b; Broderick et al., 2009). In addition, Wessels and Titgemeyer (1997) demonstrated that both solvent-extracted or expeller-processed soybean meal affected nitrogen intake, excretion and retention when increasing CP from 13.5 to 17.2% CP. Supplementation of protein sources (groundnut cake, soybean meal, linseed meal and mustard cake) were also found to affect nitrogen balance (Mehra et al., 2006). Fadel Elseed (2005) demonstrated that animals supplemented with cotton seed meal two times per day had higher nitrogen retention. Nitrogen balance in goats fed low and high levels of energy with urea were unaffected by increasing 3 to 5% urea in both energy levels, but nitrogen absorption and retention were reduced (Paengkoum et al., 2006b).

Others have similarly found that nitrogen intake and retention in buffaloes were significantly higher with supplementation of urea molasses mineral block (UMMB) at 400 to 600 g, whereas supplementation at 800 g, nitrogen intake and retention decreased (Verma et al. 1998). The previous observations in Murrah buffaloes fed UMMB with and without concentrate (700 g and 1400 g) found improved nitrogen intake and nitrogen balance (Hosamani et al., 1998). In addition, Singh et al. (2009) suggested that poor efficiency of protein utilization in calves fed

on low energy diet could be explained by the finding that urinary nitrogen losses were highest in calves fed on low energy diet. Source of carbohydrate (barley, corn, wheat and oats) in the dairy cows diets was found to influence fecal nitrogen, but did not affect urinary nitrogen, nitrogen intake and retention in dairy cows (Gozho and Mutsvangwa, 2008).

Previous results from Jatana et al. (2000) reported that protein source (fish meal vs. soybean meal) and energy source (paper pulp vs. corn flour) did not affect nitrogen intake, excretion and balance in sheep fed guinea grass as a basal diet. Arieli et al. (2005) noted that nitrogen intake in goats were higher when fed high protein with high rumen degradable organic matter (RDOM) diets than when fed low protein with high RDOM, however, nitrogen balance was higher due to increasing protein content.

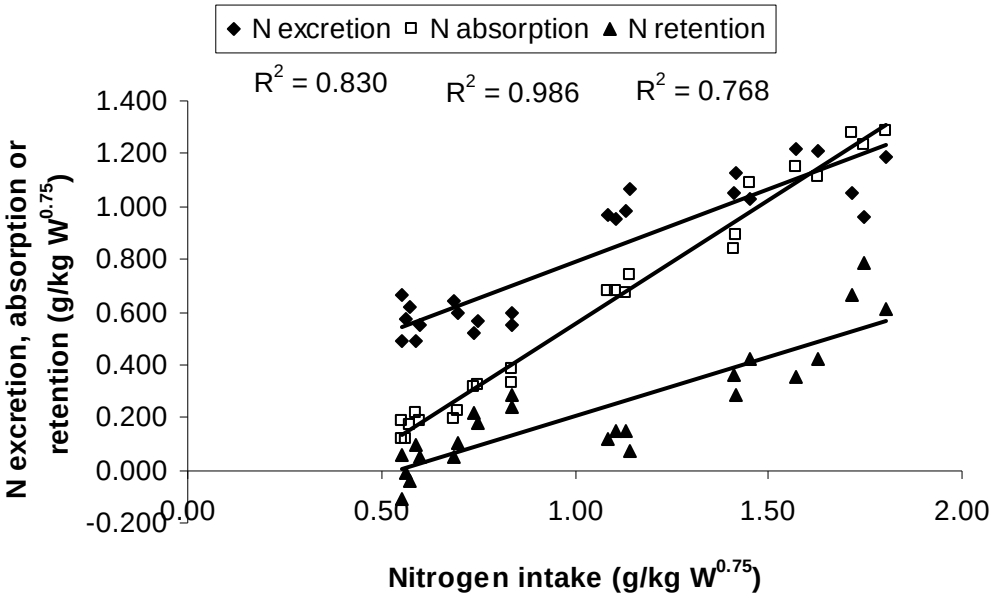

Figure 5.2 Relationship between nitrogen intake and nitrogen excretion, absorption and retention (g per kg $W^{0.75}$) in growth swamp buffalo.

Table 5.9 Urinary nitrogen and faecal nitrogen of growing swamp buffaloes fed varying dietary protein and energy levels.

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Feces , kg DM	1.65^{Bb}	1.57^{Bb}	1.93^{Ba}	1.71^{Ab}	1.90^{Ab}	2.18^{Aa}	0.11	0.032	0.008	0.487
Urine volume, L	6.07	3.11	4.27	8.20	5.71	4.37	1.73	0.273	0.215	0.750
Urine nitrogen excretion										
g/d	9.32^{B}	7.15^{B}	5.96^{B}	33.02^{A}	38.33^{A}	36.21^{A}	3.36	<0.001	0.859	0.494
g/kg $W^{0.75}$	0.17^{B}	0.13^{B}	0.11^{B}	0.57^{A}	0.64^{A}	0.61^{A}	0.04	<0.001	0.775	0.331
Feces nitrogen excretion										
g/d	22.22^{Bb}	23.52^{Bab}	25.80^{Ba}	23.91^{Ab}	26.70^{Aab}	29.35^{Aa}	1.19	0.011	0.006	0.714
g/kg $W^{0.75}$	0.41^{b}	0.44^{ab}	0.45^{a}	0.42^{b}	0.47^{ab}	0.50^{a}	0.02	0.097	0.036	0.666

[a-c] Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
[A-B] Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1] M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean

Table 5.10 Nitrogen balance of growing swamp buffaloes fed with varying protein and energy levels.

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Nitrogen intake										
g/d	30.96^{h}	34.23^{h}	45.11^{g}	63.67^{f}	86.05^{e}	102.00^{d}	3.30	<0.001	<0.001	0.006
g/kg W$^{0.75}$	0.57^{h}	0.64^{h}	0.79^{g}	1.12^{f}	1.46^{e}	1.62^{d}	0.03	<0.001	<0.001	<0.001
Total Nitrogen excretion										
g/d	31.54^{B}	30.66^{B}	31.76^{B}	56.93^{A}	65.04^{A}	65.56^{A}	3.57	<0.001	0.439	0.394
g/kg W$^{0.75}$	0.59^{B}	0.57^{B}	0.56^{B}	0.99^{A}	1.11^{A}	1.10^{A}	0.04	<0.001	0.472	0.198
Nitrogen absorption[1/]										
g/d	8.73^{g}	10.72^{g}	19.31^{g}	39.76^{f}	59.35^{e}	72.66^{d}	3.53	<0.001	<0.001	0.016
g/kg W $^{0.75}$	0.16^{h}	0.20^{h}	0.34^{g}	0.69^{f}	0.99^{e}	1.22^{d}	0.04	<0.001	<0.001	0.001

Table 5.10 Nitrogen balance of growing swamp buffaloes fed varying protein and energy levels (Cont.).

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Nitrogen retention[2/]										
g/d	-0.59^{g}	3.57^{g}	13.35^{f}	6.75^{gf}	21.01^{e}	36.44^{d}	2.35	<0.001	<0.001	0.014
g/kg $W^{0.75}$	-0.02^{h}	0.07^{gh}	0.23^{ef}	0.12^{fg}	0.35^{e}	0.62^{d}	0.04	<0.001	<0.001	0.029

[d-h]Distinct lowercase letters in the same row differ at P<0.05 by least squares means.
[A-B]Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean
[1/]Nitrogen absorption = Nitrogen intake – Nitrogen feces
[2/]Nitrogen retention = Nitrogen intake – Nitrogen excretion

Purine derivatives excretion

The excretion of urinary purine derivatives (PD) of buffaloes fed dietary protein levels with different dietary energy levels in terms of mmol/d, mmol/kg $W^{0.75}$/d and % are given in Table 5.11. Dietary treatments had a strong ($P<0.01$) influence on the urinary allantoin excretion. The excretion of allantoin and total PD increased with both increasing protein and energy content, however, the values of the proportion of PD was highest in buffaloes fed with high concentration of protein and energy, and was lowest in buffaloes fed with low concentration of protein and energy. There were no significant effects ($P<0.01$) of interaction between protein and energy on urinary PD excretion. Total urinary PD excretion concentration in this study ranged from 14.0 to 33.0 mmol/d, which the value was within the range (11.1 to 47.0 mmol/d) found previously for buffaloes (Wanapat and Rowlinson, n.d.; Chen et al., 1996).

These results corroborate those of Paengkoum et al. (2006b) who found that allantoin and total PD concentration increased with increasing energy supplementation, but decreased with increasing urea supplementation. Others have similarly found that different concentrations of energy and protein in the diets affect allantoin and uric acid (De Boever et al., 1998). However, Jetana et al. (2000) demonstrated that neither protein nor energy supplements did not affect urinary allantoin and Sannes et al. (2002) also reported that rumen degradable carbohydrate source (sucrose or ground corn) with or without urea and SBM did not affect total PD in lactating cows.

Table 5.11 Effect of varying crude protein and energy levels on urinary purine derivatives (PD) and creatinine excretion of growing swamp buffaloes.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Allantoin										
mmol/d	9.58^{Bc}	11.97^{Bb}	16.13^{Ba}	13.89^{Ac}	21.34^{Ab}	27.16^{Aa}	1.63	<0.001	<0.001	ns
mmol/kg $W^{0.75}$	0.17^{Bc}	0.21^{Bb}	0.28^{Ba}	0.24^{Ac}	0.36^{Ab}	0.45^{Aa}	0.03	<0.001	<0.001	ns
%	67.21^{Bb}	70.69^{Bb}	75.15^{Ba}	68.63^{Ab}	79.55^{Ab}	81.64^{Aa}	1.66	<0.001	<0.001	ns
Uric acids										
mmol/d	1.67^{B}	1.89^{B}	2.36^{B}	3.25^{A}	2.23^{A}	3.25^{A}	0.45	0.021	ns	ns
µmol/kg $W^{0.75}$	31.00^{B}	35.50^{Ba}	40.50^{B}	54.25^{A}	39.00^{A}	54.50^{A}	7.23	0.036	ns	ns
%	12.47	12.07	10.77	15.63	8.49	9.87	1.57	ns	ns	ns

Table 5.11 Effect of varying crude protein and energy levels on urinary purine derivatives (PD) and creatinine excretion of growing swamp buffaloes (Cont.).

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Hypoxanthine										
mmol/d	0.95	1.04	1.04	1.05	1.03	0.99	0.11	ns	ns	ns
µmol/kg W $^{0.75}$	18.00	19.25	18.25	18.075	18.00	17.50	2.07	ns	ns	ns
%	6.95^{Aa}	6.76^{Aa}	4.98^{Ab}	5.49^{Ba}	4.10^{Ba}	3.33^{Bb}	0.52	<0.001	0.004	ns
Xanthine										
mmol/d	1.82	1.71	1.82	1.83	2.07	1.57	0.19	ns	ns	ns
µmol/kg W $^{0.75}$	34.00	31.00	32.50	33.75	35.75	27.25	3.56	ns	ns	ns
%	13.37^{Aa}	10.48^{Ab}	9.10^{Ab}	10.25^{Ba}	7.84^{Bb}	5.16^{Bb}	1.78	0.004	0.004	Ns

Table 5.11 Effect of varying crude protein and energy levels on urinary purine derivatives (PD) and creatinine excretion of growing swamp buffaloes (Cont.).

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Total Purine derivative										
mmol/d	14.01^{Bc}	16.61^{Bb}	21.34^{Ba}	20.01^{Ac}	26.67^{Ab}	32.97^{Aa}	1.81	<0.001	<0.001	ns
mmol/kg W $^{0.75}$	0.26^{Bc}	0.30^{Bb}	0.37^{Ba}	0.35^{Ac}	0.46^{Ab}	0.55^{Aa}	0.03	<0.001	<0.001	ns
Creatinine										
mmol/d	29.60^{B}	31.83^{B}	27.32^{B}	36.88^{A}	41.29^{A}	38.19^{A}	2.91	0.002	ns	ns
mmol/kg W $^{0.75}$	0.53^{B}	0.57^{B}	0.48^{B}	0.62^{A}	0.70^{A}	0.63^{A}	0.05	0.007	ns	ns
A:Creatinine	0.33^{Bb}	0.37^{Bb}	0.59^{Ba}	0.39^{Ab}	0.52^{Ab}	0.72^{Aa}	0.05	0.009	<0.001	ns
PD:Creatinine	0.49^{b}	0.53^{b}	0.78^{a}	0.57^{b}	0.65^{b}	0.89^{a}	0.06	ns	<0.001	ns
PDC index	26.14^{Bb}	28.88^{Bb}	45.35^{Ba}	31.99^{Ab}	38.07^{Ab}	52.29^{Aa}	3.36	0.017	<0.001	ns

$^{a-c}$Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
$^{A-B}$Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein; E = Energy; SEM = Standard error of mean; PD = Purine derivative; A = Allantoin

Purine derivatives excretion usually increase with increasing proportion of concentrate in the diet (Moorby et al., 2006; Gonda, Emanuelson and Murphy, 1996; Valadares et al., 1999). Richardson et al. (2003) summarized that allantoin increased more in lambs fed barley than those fed sugar beet pulp, but uric acid and xanthine plus hypoxanthine were not affected. Several studies also have shown that source of carbohydrates in the diet influenced allantoin and PD, and increased more with barley than with corn, wheat and oats (Gozho and Mutsvangwa, 2008; Martin-Orue et al., 2000). When barley gain replaced fish meal in the diets, allantoin excretion in ewe urine declined (Martin-Orue, Dapoza, Balcells and Castrillo, 1996). In other studies neither protein concentration nor degradability had an effect on urinary PD excretion (Devant et al., 2000; Gressley and Armentano, 2007; Olmos Colmenero and Broderick, 2006c).

The differences between studies in the daily PD excretion are probably due to the differences in the digestible organic matter intake and ammonia nitrogen or available soluble carbohydrates in the rumen. It is well know that urinary excretion of PD have a positive correlation since an increase in rumen microbial synthesis normally implies an increase in energy and protein supply to the host animal and therefore an improvement in animal performance.

Based on this experiment, allantoin, uric acid, xanthine and hypoxanthine concentrations ranged from 67.2 to 81.6%, 8.5 to 12.5%, 5.2 to 13.4% and 3.3 to 6.9%, respectively. These findings are also similar to values reported in buffaloes in other studies, except for hypoxanthine and xanthine (Chen et al., 1992; Pimpa et al., 2003; Dipu et al., 2006). Normally, hypoxanthine and xanthine excretion in buffalo urine were low, it is possibly that the presence of xanthine oxidase (OX) in the

intestine and plasma. Belenguer et al. (2002) found that OX activities in cows were higher than those detected in goat and sheep and OX activities in cows were lower than those detected in buffaloes (Chen et al., 1996). The high values of hypoxanthine and xanthine found in this experiment are probably that under the experiment conditions, animals may be stress, and were affected OX activities. However, OX activities and OX present in plasma were not determined in this experiment.

Creatinine excretion

Creatinine excretion in urine, PD:C ratio, PDC index and allantoin:creatinine ratio are shown in Table 5.11. The urinary output of creatinine was significantly higher ($P<0.01$) with increasing protein content in the diet, and the values ranged from 0.48 to 0.70 mmol/kg$W^{0.75}$, which were within the range (0.50 to 0.73 mmol/kg$W^{0.75}$) for buffaloes reported by Pimpa et al. (2003) and Dipu et al., (2006). Gonda et al. (1996) stated that urinary creatinine excretion appears to be affected by increasing concentrate in the diet. But other researchers found that creatinine was not affected by feed intake (George et al., 2006), level of concentrate diet (Moorby et al., 2006; Valadares et al., 1999), type of dietary roughage (Chizzotti et al., 2007b) and source of carbohydrates (Matin-Orue et al., 2000).

The ratio of PD:C and anllantoin:creatinine, and PDC index were affected by either dietary protein or energy content, except for PD:C which was not affected by increasing protein in the diet. These results are similar to those from George et al. (2006) and Dipu et al. (2006) who found that PD:C and A:C were reduced with decreasing levels of feed intake. It has been suggested that the excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of intake (Chen et al., 1992). Moreover, the use of creatinine as an internal marker for

urinary excretion relies on the assumption that the creatinine excretion through urine is not affected by diet or the physiological status of the animal, but is excreted in proportion to body weight (Chizzotti et al., 2007b; George et al., 2006).

Microbial protein supply and efficiency

Microbial purine base (PB) flow, microbial nitrogen (MN) supply and microbial nitrogen synthesis efficiency of buffaloes fed with varying dietary protein and energy are presented in Table 5.12. There were significant effects ($P<0.01$) of energy and protein on the concentration of PB and microbial nitrogen flow. The flow of PB and microbial nitrogen to the duodenum of buffaloes increased with increasing either protein or energy concentration in the diet. The values of PB and microbial nitrogen flow ranged from 26.4 to 175.8 mmol/d and 19.2 to 127.8 g of N/d, respectively. Buffaloes fed with high concentrations of protein and energy (12% CP and 1.8M) had highest flow of PB and MN. The lowest flow of PB and MN was found in the buffaloes fed with low protein and energy content (6% CP and 1.0M) in the diet.

The efficiency of microbial nitrogen supply to the duodenum in terms of g of N/kg of DOMR, DMI, CPI and TDNI were influenced by increasing protein content, except for efficiency of microbial synthesis (g N/g CPI) which was not affected by protein content. The microbial synthesis efficiency (g N/kg DOMR and TDNI) were not affected by energy content (Table 5.12). The more efficient utilization of the dietary nitrogen was probably due to an enhanced microbial protein synthesis as a result of a higher availability of ruminally fermentable carbohydrates (Table 5.12). At the same level of protein content, microbial protein synthesis increased with increasing energy content.

Table 5.12 Microbial purine base (PB), microbial N supply and microbial N synthesis efficiency of growing swamp buffaloes fed varying dietary protein and energy levels.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
PB flow	26.41^{Bc}	46.80^{Bb}	82.52^{Ba}	71.76^{Ac}	124.80^{Ab}	175.77^{Aa}	15.84	<0.001	<0.001	ns
Microbial N supply										
g N/d	19.20^{Bc}	34.02^{Bb}	59.99^{Ba}	52.17^{Ac}	90.73^{Ab}	127.79^{Aa}	11.52	<0.001	<0.001	ns
g N/kg DOMR	16.95^{B}	21.90^{B}	30.71^{B}	49.01^{A}	58.23^{A}	59.67^{A}	8.01	<0.001	ns	ns
g N/kg DMI	5.65^{Bb}	8.49^{Bab}	12.02^{Ba}	15.35^{Ab}	20.96^{Aab}	23.52^{Aa}	2.63	<0.001	<0.001	ns
g N/g CPI	0.10^{b}	0.15^{ab}	0.21^{a}	0.13^{b}	0.17^{ab}	0.19^{a}	0.03	ns	<0.001	ns
g N/kg TDNI	9.88^{B}	13.71^{B}	18.57^{B}	26.68^{A}	34.47^{A}	37.75^{A}	4.30	<0.001	ns	ns

$^{a-c}$Distinct lowercase letters in the same row, within same CP level, differ at P<0.05 by least squares means for energy levels effect.
$^{A-B}$Distinct capital letters in the same row, differ at P<0.05 by least squares means for protein levels effect.
[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean; DOMR = Digestibility of organic matter fermented in rumen; DMI = Dry matter intake; CPI = Crude protein intake; TDNI = Total digestible nutrient intake; PB = Microbial purine base.

Results from the present study suggested that microbial nitrogen synthesis and efficiency responded positively and were related to increasing both protein and energy content in the diets and agreed with the data noted that microbial synthesis and efficiency responded positively to increasing energy supplementation, but decreased with increasing urea supplementation, which is reflected in decreased PD in the urine (Paengkoum et al., 2006b). Similarly, the work of Herrera-Saldana et al. (1990) who reported that microbial nitrogen synthesis was affected by energy source, but was not affected by protein source. The results of present study corroborated well with the observations of Chikunya et al. (1996) who suggested that microbial yield was highest with sugar beet pulp with casein diets compared to hay diets with or without urea and casein. Several studies have shown that protein and energy concentration affected microbial nitrogen yield (Sannes et al., 2002; Yu et al., 1999; De Boever et al., 1998). Others have similarly found that microbial nitrogen synthesis increased with increasing concentrate diet (Moorby et al., 2006; Valadares et al., 1999). Another study suggested that barley affected microbial nitrogen and PB flow more than corn (Matine-Orue et al., 2000), more than sugar beet pulp (Richardson et al.,2003), more than milo (Herrera-Saldana et al., 1990), and more than wheat and oats (Gozho and Mutsvangwa, 2008). This was probably due to less degradable starch sources in the barley diets compared to others which increased microbial nitrogen supply.

In contrast to previous reports, Jetana et al. (2000) found that neither energy nor protein concentration affected microbial nitrogen synthesis and PB flow. Mabjeesh et al. (1997) demonstrated that ruminally degradable protein affected microbial protein synthesis, but not ruminally degradable carbohydrates. Also, protein source did not affect PB and microbial nitrogen supply (Devant et al., 2001), but

increased microbial efficiency on true protein (Brito et al., 2007). And neither protein content nor degradability, significantly influenced microbial nitrogen and PB flow to the duodenum (Devant et al., 2000; Olmos Colmenero and Broderick, 2006c).

An enhanced microbial protein synthesis implies an enhanced efficiency in the utilization of the ammonia nitrogen and soluble carbohydrate by the rumen microorganisms (Gonda et al., 1996). The concurrent release of readily available energy from molasses and cassava waste, and ammonia from urea in the HE diets apparently produces better conditions for microbial growth in the rumen than on the low energy diets (Paengkoum et al., 2006b). Stokes et al. (1991) summarized that nonstructural carbohydrate greater than 24% and ruminally degradable protein greater than 9% of DM in diets will enhance microbial protein flow from the rumen.

Microbial counts

Rumen microbes of buffaloes fed dietary protein and energy levels are shown in Table 5.13. Rumen microbes were not affected by either protein nor energy content in the diet, and there were no significant ($P>0.055$) interaction between energy and protein for all parameters. The numbers of total bacteria ranged from 0.93 to 1.13 x 10^{11} cells/ml of rumen fluid. However, the populations of all rumen microbes were within the range reported in buffaloes, except for fungal zoospores which were higher than those reported by Wanapat (2001), Wanapat et al. (2009) and Wanapat and Rowlinson (n.d.).

These results agree with the work of Tiwari et al. (2001) who demonstrated that total protozoa counts in growing buffaloes were similar when fed diets supplemented with different protein sources. Also agree with the current results, increasing nonstructural carbohydrate (24 to 38%) with increasing degradable intake

protein (9.0 to 13.7%) in the diets did not influence protozoal numbers in the rumen (Stokes et al., 1991) and Chikunya et al. (1996) also noted that protozoal and cellulotytic bacterial counts were similar with the sugar beet pulp diet with or without urea and casein, compared to hay diet. Others have similarly found that fungal zoospores and the groups of bacteria (total viable, cellulolytic, proteolytic and amylolytic) were not changed by different energy sources and urea levels. Chumpawadee et al. (2006) pointed out that increasing synchrony index (0.39 to 0.74) were not affected total bacteria, fungal zoospore and protozoa populations. However, protozoal populations were higher in buffaloes fed cassava chips than corn cobs (Wanapat et al., 2009). Sinha and Ranganathan (1983) also indicated that the numbers of cellulolytic bacteria in buffalo's rumens vary with different feeds and time intervals after feeding, green fodders such as berseem and sorghum supported better growth of rumen bacteria, and cellulolytic bacteria, by comparison with a dry roughage like wheat straw. A similar study suggested that supplementation of cassava hay fed to buffaloes increased bacteria counts, but did not affect protozoa and fungi counts in the rumen (Granum et al., 2007). Chanjula et al. (2004) replaced cassava hay for urea treated-rice straw fed to buffaloes from 0 to 100%, and found that the numbers of protozoa decreased, while, fungal zoospores increased with increasing cassava hay up to 50%, but bacterial counts were not affected. Furthermore, it has been reported that the type of cellulolytic bacteria recovered from the rumen of adult buffaloes fed roughage were *Ruminococcus albus*, *R. flavefaciens, Bacteroides succinogenes* etc. (Sinha and Ranganathan, 1983). There was confirmed by Wara-au (2006) who found that there were three predominant cellulolytic bacteria species namely *F. succinogenes*, *R. albus* and *R. flavefaciens* by using specific primers.

Table 5.13 Effect of varying crude protein and energy levels on ruminal microbe populations of growing swamp buffaloes.

Items[1]	6% CP			12% CP			SEM	Effects		
	1.0M	1.4M	1.8M	1.0M	1.4M	1.8M		P	E	P x E
Bacteria (x 10^9 cells/ml)										
0 h post feeding	1.11	1.47	1.29	1.26	1.27	1.28	0.13	ns	ns	ns
4 h	1.21	1.36	1.10	1.21	1.46	1.48	0.15	ns	ns	ns
Protozoa (x 10^5 cells/ml)										
0 h post feeding	2.38	2.75	2.69	1.75	3.88	4.44	1.07	ns	ns	ns
4 h	3.69	3.17	3.00	1.44	2.81	5.00	1.03	ns	ns	ns
Fungal zoospores (x 10^7 cells/ml)										
0 h post feeding	2.90	3.47	3.79	2.13	5.03	4.42	0.81	ns	ns	ns
4 h	3.13	2.75	4.44	1.47	3.71	3.49	1.17	ns	ns	ns

Table 5.13 Effect of varying crude protein and energy levels on ruminal microbe populations of growing swamp buffaloes (Cont.).

Items[1]	**6% CP**			**12% CP**			**SEM**	**Effects**		
	1.0M	**1.4M**	**1.8M**	**1.0M**	**1.4M**	**1.8M**		**P**	**E**	**P x E**
Amylolytic bacteria (x 10^6 CFU/ml)										
0 h post feeding	1.63	6.83	5.88	11.06	8.25	2.69	3.92	ns	ns	ns
4 h	6.25	3.75	13.88	14.88	8.56	12.75	5.41	ns	ns	ns
Proteolytic bacteria (x 10^6 CFU/ml)										
0 h post feeding	5.50	4.58	2.50	13.31	12.50	4.50	5.94	ns	ns	ns
4 h	11.56	8.08	18.69	14.69	9.94	12.13	7.44	ns	ns	ns
Cellulolytic bacteria (x 10^7 CFU/ml)										
0 h post feeding	8.25	5.00	7.00	8.00	6.50	3.50	1.84	ns	ns	ns
4 h	6.75	6.33	6.25	7.50	7.25	11.50	3.05	ns	ns	ns

[1]M = Maintenance level; 1.0, 1.4 and 1.8 time of energy maintenance level; P = Crude protein and E = Energy; SEM = Standard error of mean; CFU = Colony forming unit

Nitrogen requirement

The nitrogen requirement of growing swamp buffaloes fed different levels of dietary protein and energy are showed in Figure 5.3. The values of average daily gain (ADG, g/kg $W^{0.75}$) and N intake (g N/kg $W^{0.75}$) regressed linearly for the determination of dietary nitrogen requirement for growth and maintenance. The regression equation between ADG and N intake of buffaloes is nitrogen intake = 0.0982ADG + 0.499 (R^2 = 0.744, P<0.01, n = 24). Based on this equation the N requirement for maintenance and growth of growing swamp buffaloes can be estimated with the N intake at which ADG equal to zero was 0.499 g N/kg $W^{0.75}$/d. As a result, the nitrogen requirements for maintenance of growing swamp buffaloes are 0.499 g N/kg $W^{0.75}$ or equivalent to 3.12 g CP/kg $W^{0.75}$/d and nitrogen requirement for growth of growing swamp buffaloes are 0.098 g N/g ADG or equivalent to 0.61 g CP/g ADG/d. Several studies have shown that the CP requirement for maintenance of Brahman cattle is 3.58 g/kg $W^{0.75}$ (Chaokaur et al., 2009a), and for growing Brahman cattle is 3.20 g/kg $W^{0.75}$ (Chaokaur et al., 2009b).

These results indicate that the protein requirements for maintenance of buffaloes were lower than those suggested by Kearl (1982) for growing buffaloes (5.24 g CP/kg $W^{0.75}$), and were lower than those recommended by NRC (1996) for beef cattle (5.67 g CP/kg $W^{0.75}$). And the protein requirements for growth of buffaloes were lower than those indicated by Kearl (1982) for growing buffaloes (0.65 g CP/g ADG), and were higher than those suggested by NRC (1996) for beef cattle (0.46 g CP/g ADG). But the current results are in agreement with the work from (Basra et al. (2003c) and Tauqir et al. (2009a) who stated that the protein requirements of *Nili-ravi* buffalo calves were lower than those recommended by NRC (2001) for dairy cattle

calves. But the CP requirements for buffalo male calves were similar to Basra et al. (2003a), and were higher (Tauqir et al., 2009b) than those recommended by NRC (2001) for dairy cattle calves.

But further studies by Basra et al. (2003b) found that the protein requirements for growth of *Nili-ravi* buffalo male calves was the same as NRC (2001) described for cattle calves. These current results show that the CP requirement for maintenance of growing male swamp buffaloes were lower than those for *Nili-ravi* buffalo heifers (5.89 to 9.38 g CP/kg $W^{0.75}$), but the CP requirement for growth were higher than those for *Nili-ravi* buffalo heifers (0.24 to 0.48 g CP/g ADG/d) (125 to 400 body weight ranges) (Paul and Patil, 2007).

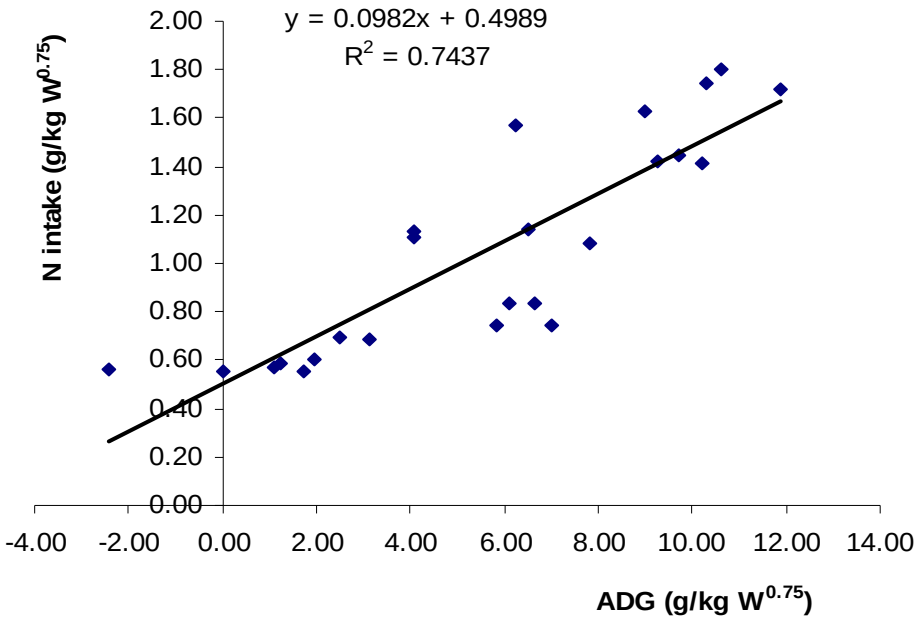

Figure 5.3 Relationship between average daily gain (ADG) and nitrogen intake (g/kg $W^{0.75}$) in growing swamp buffaloes.

Energy requirements

The energy requirements of growing swamp buffaloes fed different levels of dietary protein and energy are showed in Figure 5.4. The values of average daily gain (ADG, g/kg $W^{0.75}$) and metabolizable energy (ME) intake (kcal/kg $W^{0.75}$) were regressed linearly for the determination of dietary energy requirements for growth and maintenance. The regression equation between ADG and ME intake of buffaloes are ME intake = 5.225ADG + 132.72 (R^2 = 0.366, P<0.01, n = 24). Based on this equation can be used to estimate the ME requirements for maintenance and growth of growing swamp buffaloes with the ME intakes at ADG equal to zero were 132.72 kcal/kg $W^{0.75}$. Consequently, the ME requirements for maintenance of growing swamp buffaloes are 132.72 kcal or 558 kJ/kg $W^{0.75}$/d and metabolism energy requirement for growth of growing swamp buffaloes are 5.23 kcal or 21.97 kJ/g ADG/d.

These results do not agree with other studies where the ME requirements for maintenance of Brahman cattle were 454 to 486 kJ/kg $W^{0.75}$ (Chaokaur et al., 2009a; Nitipot et al., 2009; Chaokaur and Sommart, 2009), of growing Brahman cattle were 497 kJ/kg $W^{0.75}$ (Chaokaur et al., 2009b), and of Thai native cattle were 484 kJ/kg $W^{0.75}$ (Nitipot et al., 2009). Also the current results of ME requirement for maintenance of buffaloes were slightly higher than those suggested by Kearl (1982) for buffaloes (125.00 kcal/kg $W^{0.75}$), and slightly lower than those recommended by NRC (1996) for beef cattle (133.68 kcal/kg $W^{0.75}$). Whereas, the ME requirement for growth of buffaloes are lower than those indicated by Kearl (1982) for buffaloes (10 kcal/g ADG), and NRC (1996) for beef cattle (6.15 kcal/g ADG). Nitipot et al. (2009) who found that the ME requirements for growth of Barhman cattle and Thai native

cattle were 22.67 and 31.27 kJ/g ADG, respectively. Tauqir et al. (2009a) concluded that the ME requirements of *Nili-ravi* buffalo calves were lower than those described for cattle by NRC (2001).

Other studies have reported that the energy requirements of 8 months old buffalo calves (Singh et al., 2009) and 12-15 months old (Tauqir et al., 2009b) were similar to those recommended by NRC (2001) for cattle. Several other studies have shown that the energy requirements of buffalo male calves (Basra et al., 2003a; 2003c) were higher than those suggested by NRC (2001) for cattle. These present results shown that the ME requirement for maintenance of growing male swamp buffaloes were slightly higher than Paul and Patil (2007) found for *Nili-ravi* buffalo heifers (443 to 542 kJ/kg $W^{0.75}$), and for growth were slightly lower (26 to 53 kJ/g ADG/d). Mahmoudzadeh et al. (2007) reported that the optimum fattening performance of 15 month old buffalo male calves may obtained by providing around 10.42 MJ/kg of dietary ME.

5.6 Conclusions

From this study it can be concluded that increasing either dietary protein or energy significantly increased ($P<0.05$) growth rate, nutrient intake and digestibility, N utilization, N balance, urinary PD excretion and microbial N supply to the duodenum of growing Thai swamp buffaloes. The protein requirements for maintenance and growth of growing male Thai swamp buffaloes were 3.12 g CP/kg $W^{0.75}$/d and 0.61 g CP/g ADG. The metabolizable energy requirements for maintenance and growth of growing male Thai swamp buffaloes were 132.72 kcal/kg $W^{0.75}$/d and 5.23 kcal/g ADG.

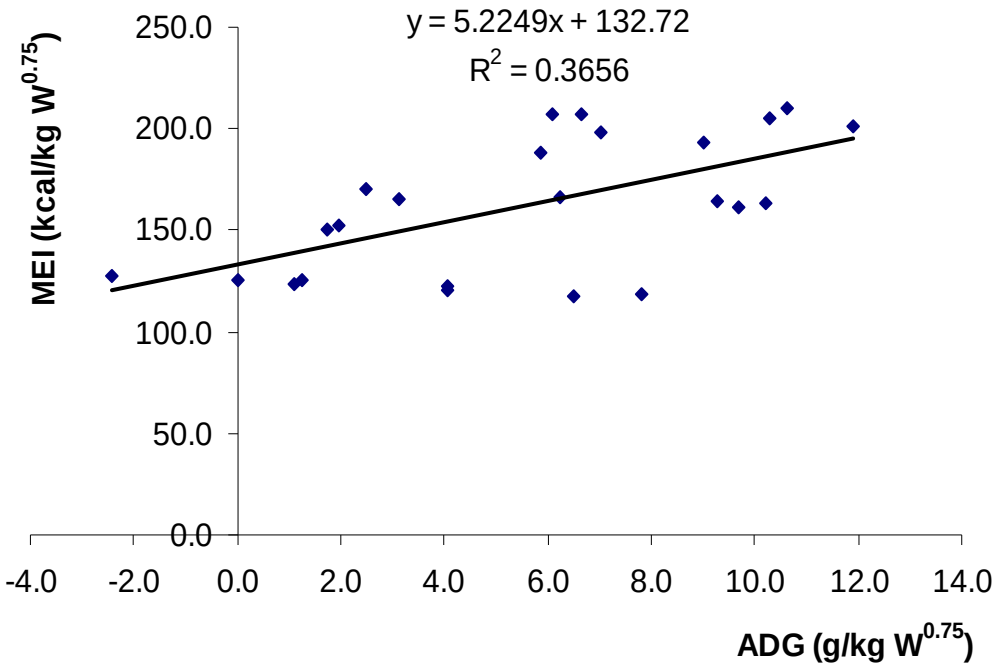

Figure 5.4 Relationship between average daily gain (ADG, g/kg $W^{0.75}$) and metabolizable energy intake (MEI, kcal/kg $W^{0.75}$) in growing swamp buffaloes.

CHAPTER VI

OVERALL DISCUSSION AND CONCLUSION

The purposes of the present study were to investigate the effects of dietary protein on nutrient intake and digestibility, rumen fermentation, rumen microbes, nitrogen metabolism and estimation of protein requirements for maintenance and growth of growing male swamp buffaloes.

Buffaloes are known to be more efficient in utilizing fiber component of the coarse roughages than cattle (Calabro et al., 2008; Wora-anu, 2006; Wanapat, 2001) and they thrive well on grazing native granges, crop residues and agricultural/and industrial by-products (Watnapat and Rowlinson, 2009; Mahmoudzaden et al., 2007). Nutrients intake and digestibility are higher for buffaloes compared to cattle (Granum et al., 2007; Wora-anu, 2006; Razdan, Sharma, Bhargava and Chawla, 1971). The reasons for the superior digestive capacity of buffalo over cattle have not been fully elucidated. However, it is most likely that much of the superiority may be explained by differences in the nature of the rumen microbial population (Wora-anu, 2006; Calabro et al., 2008), which would affect the type of fermentation (Wanapat and Rowlinson, n.d.), with fugal zoospores counts in particular significantly higher than those found in cattle fed similar diets or raised under similar conditions (Wanapat, 2001; Granum et al., 2007). Wora-anu, Wanapat, Wachirapakorn and Nontaso (2000) also found that ruminal cellulolytic, proteolytic and amylolytic bacteria of swamp buffaloes were higher than those found in cattle fed similar diets. Since rumen

microorganism impact on fermentation patterns and fermentation end-products, particularly volatile fatty acids and NH_3-N, the higher bacteria, lower protozoa and higher fungal zoospores in swamp buffaloes should result in greater fermentation efficiency and productivity than in cattle (Wanapat, 2001). Calabro et al. (2008) indicate that a greater proportion of the degraded OM is used for biomass production at the expense of VFAs in buffaloes when compared with cows, this may explain why diets containing the same energy but less protein can be fed to buffaloes than do cattle.

The major nutrients required for growth by rumen microbes are carbohydrates and proteins (Hoover and Stokes, 1991; Clark et al., 1992). Microbial activity is directed towards the generation of ATP for maintenance and growth of the microbial population, ATP is derived from fermentation of carbohydrate. Ammonia N or nitrogenous compounds in the rumen can supply the sole source of N for the rumen microorganisms (Ørskov, 1992). And in experiment II, there were increased rumen microbial populations with increasing protein and energy in the diets. Oh et al. (1999) demonstrated that production of microbial CP was greater when steers were fed on starch or sucrose with rapeseed meal than those without rapeseed meal. Ground soybean or urea enhanced rumen microbial population and rumen function in swamp buffaloes in Vietnam (Nguyen van Thu, n.d.). Although, microbial populations did not change in our studies with increasing levels of CP in the diet (experiment I and III), microbial populations in experiment III were higher than those compared with experiment I. In addition, in this study, fungal zoospores were higher than those previously reported in buffaloes (Wanapat, 2001; Wanapat and Rowlinson, n.d.; Chanjula et al., 2004). These higher microbial populations and fugal zoospores may

be due to the differences of type of roughage, concentrate, ratio of roughage and concentrate, available energy and protein, pH, NH_3-N and rumen conditions (McDonald et al., 1995; Ørskov, 1992). Sinha and Ranganathan (1983) found that the numbers of cellulolytic bacteria in buffalo rumen vary with different feeds and range from 3.3 x 10^6 to 1.9 x 10^8 count/ml, at 4 h after feeding. Wanapat (2001) demonstrated that in ruminants fed on low-quality roughage, critical rumen NH_3-N levels for microbial activities were found to be 5-20 mg/dl and this levels were within the ranges (10.6-20.3) found in experiments I and II. Ørskov (1992) suggested that no increase in microbial yield occurs as a result of increasing ammonia concentration in the rumen to more than 50 mg/L (5 mg/dl).

Microbial protein synthesis were estimated from microbial purine flow to the duodenum and significantly increased with increasing dietary protein content in the diets (found in all experiments). These results corroborate with those of Kim et al. (2009), Paengkoum et al. (2006a), Devant et al. (2001) and Sannes et al. (2002). However, Reynal et al. (2003) and Olmos Colmenero and Broderick, (2006a; 2006c) did not find any improvement in microbial protein synthesis by increasing the CP content of the diet. It is possible that microbial protein production in rumen depends on many other factors such as availability of nitrogen and carbohydrates, ruminal pH, source and levels of N component, stabilizing ruminal fermentation, physiological effect and balancing of ruminally available energy and protein intake (Ørskov, 1992; Chumpawadee et al., 2006; Hoover and Strokes, 1991; McDonald et al., 1995).

The rumen must act as an essential fermentation vat that is capable of supplying end-products, particularly volatile fatty acids (VFAs) and microbial proteins as a major energy and protein for the ruminant host (Wanapat and

Rowlinson, n.d.) and the production of microbial N and levels of VFAs in the rumen are enhanced by appropriate levels of non structural carbohydrate (NSC) and degradable intake protein (Strokes et al., 1991). Normally, the rate of VFAs production are influenced by carbohydrate fractions and degradability of carbohydrates (Chumpawadee et al. 2009). In experiments I, II and III, the concentration of VFAs did increase with increasing CP content in the diet, probably because energy was greater in the diet which lead to more microbial activity in the rumen and improved fiber and another nutrients digestibility. In experiment III, it appears that protein alone is not enough and that increased energy must also be supplied to interact with the protein. Herrera-Saldana et al. (1990) summarized that synchronization for rapid fermentation with the more degradable starch and protein stimulated greater microbial protein passage. Rumen fermentation and flow of microbial and dietary protein to the small intestine are affected by feed intake and by the amount and source of energy and protein in the diets (Clark et al., 1992), as also found in these studies.

In these present studies, increasing CP levels in the diets of growing buffaloes increased CP intake and digestibility, concentration of BUN and NH_3-N, and N balance. Ruminal NH_3-N and BUN concentration, and N balance were linearly and positively correlated with increasing CP content, which also agree with previous studies (Paengkoum et al., 2006a; Lohakare et al., 2006; Paengkoum and Tatsapong, 2009; Chantiratikul et al., 2009; Paengkoum and Yanee, 2009). Digestion of protein results in the production of peptides (ammonia and amino acid), which can accumulate in the rumen (Hoover and Stokes, 1991). And cause dietary protein degradation to be more rapid than synthesis. Ammonia will therefore accumulate in

the rumen liquid and the optimum concentration can be exceeded (experiment II and III) or an imbalanced of fermentable of energy and N available for microbial metabolism occurs (experiment I and III), and as a results BUN increase. When this happens, ammonia is list absorbed into the blood, then it is carried to the liver and converted to urea and excreted in urine (Paengkoum et al., 2006a; Chantiratikul et al., 2009), which that occurred in experiments I, II and III. Wanapat and Pimpa (1999) suggested that a ruminal NH_3-N concentration ranging from 13.6 to 17.6 mg/dl in swamp buffalo rumen was considered optimum for microbial growth and activity. However, in these studies, NH_3-N concentration ranged from 10.8 to 24.3 mg/dl which was within normal range in swamp buffaloes reported by Nguyen van Thu (n.d.) but was a wider range than those of Wanapat and Pimpa (1999). Wanapat (2001) suggested that high levels of rumen NH_3-N (15-30 mg/dl) improve intake and digestibility (also found in experiment II and III). In swamp buffaloes fed on rice straw, Wanapat and Pimpa (1999) also found that rumen NH_3-N levels of 13.6-34.4 mg/dl improved rumen fermentation by increasing digestibility and intake of rice straw.

The values of ruminal pH were not affected by dietary protein, because ruminal pH is partly regulated by the NH_3-N and VFAs concentration in the rumen (Stokes et al., 1991). The current results corroborate those of Promkot and Wanapat (2005), Chumpawadee et al. (2009) and Paengkoum and Yanee (2009) who suggested that dietary protein concentration did not affect ruminal pH. It is well established that reduced pH decreases digestion of proteins, cellulose, hemicellulose and pectins but has less effect on starch digestion (Stokes et al., 1991). The microbial activities would be seriously inhibited when ruminal pH declined below pH 6.2 (Ørskov, 1992).

Ruminal pH found in these studies ranged from 6.8 to 7.2 which was optimal for rumen fermentation, microbial activities and nutrients digestion (Wanapat and Pimpa (1999).

Dietary energy improved intake and digestibility of nutrients and protein utilization in experiments II and III. However, Singh et al. (2009) concluded that energy level did not affect nutrients intake and digestibility in buffalo calves, but did improve N utilization and growth rate. Herrera-Saldana et al. (1990) demonstrated that starch degradability affected utilization of nutrients in the rumen more than protein degradability which was also found in experiments II and III. Mehra, Chetal, Singh and Saxena (1978) concluded that the biological value of dietary protein in ruminants depends on precisely upon the following three factors: the dietary protein that evades degradation in the rumen, the quantity and quality of microbial protein synthesized from dietary and endogenous nitrogen, and the amount of net protein flowing into the abomasum.

The nutrition of growing male buffaloes is important as it plays a role in the onset of puberty in buffaloes raised for breeding and it influences the quantity and quality of the meat produced by the buffaloes (Barsa et al. 2003c). Dietary protein supply is one of the factors that greatly influence the productivity of animals. The duodenal flow of CP encompasses 3 major fractions: RUP, microbial crude protein (MCP), and endogenous protein (Lapierre et al., 2006). The contribution of each fraction to the total flow is directly related to the diet composition and DM intake and varies widely, with the MCP fraction usually supplying the majority of the proteins (Clark et al, 1992). Growth rate is affected by increasing protein content in the diet (Chumpawadee et al. (2009), Paengkoum and Tatsapong (2009) and Paengkoum and

Yanee (2009), as found in experiments II and III. Although, increasing protein content did not affect weight gain in experiment I this was probably explained that due to short time of the trial (nitrogen balance trial) and was not feeding trial.

The values of N balance and N intake regressed linearly for the determination of nitrogen requirement for maintenance, while the relationship between ADG and N intake can be used to estimate the nitrogen requirements for growth and maintenance of buffaloes. The CP requirements for maintenance were found in experiments I, II and III to be 4.63, 5.41 and 3.12 g CP/kg $W^{0.75}$, respectively. The CP requirements for growth found in experiments II and III to be 0.46 and 0.61 g CP/g ADG, respectively.

The relationship between nitrogen intake and balance (Figure 6.1) of the data derived from experiments I, II and III, found that the protein requirements for maintenance of growing swamp buffaloes are 4.64 g CP/kg $W^{0.75}$/d or approximately 6% of dietary crude protein. As a result, the value of protein requirements for maintenance estimation from experiment I are the same as the estimation from the data derived from experiment I, II and III. The protein requirements for maintenance and growth can be estimated from the linear regression of average daily gain and nitrogen intake. The regression equation between ADG and N intake of data derived from experiments II and III are shown in Figure 6.2. Based on this equation can be concluded that the protein requirements for maintenance and growth of growing swamp buffaloes are 4.35 g CP/kg $W^{0.75}$/d, and 0.50 g CP/g ADG/d, respectively.

Based on these studies the daily protein requirements for maintenance of growing buffaloes were 12.9% lower than those suggested by Kearl (1982) for growing buffaloes weighing 150-300 kg (5.20-5.42 g CP/kg $W^{0.75}$), and were 26.3% lower than those recommended by NRC (1996) and Wilkerson (1993) for growing

beef cattle (5.67 g CP/kg $W^{0.75}$). And the protein requirement for growth (g ADG/d) of growing buffaloes were 23% lower than those suggested by Kearl (1982) for growing buffalo (0.65 g CP), and were 8% higher than those reported by NRC (1996) for growing beef cattle (0.46 g CP).

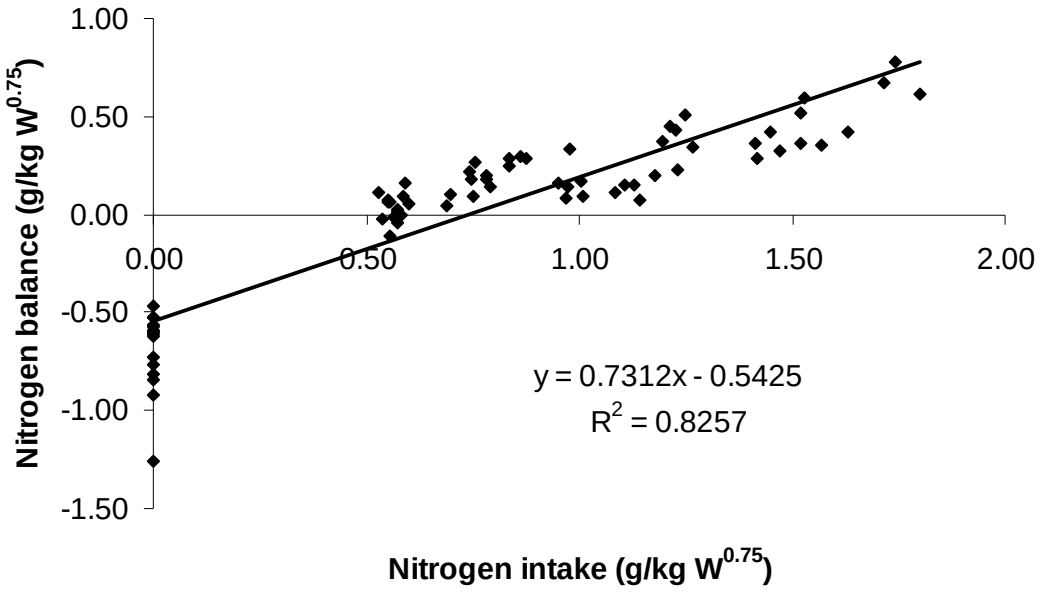

Figure 6.1 Relationship between nitrogen intake and nitrogen balance (g/kg $W^{0.75}$) in growing swamp buffaloes. The equation was N balance = 0.731N intake – 0.543 (R^2 = 0.826; P<0.001; n = 72).

The data from Tangjitwattanachai and Sommart (2009) found that the CP requirement for maintenance and growth of Thai native, Brahman and Brahman crossbred beef cattle were 5.03, 4.52 and 5.47 g CP/kg W0.75/d, and 0.38, 0.56 and 0.59 g CP/g ADG, respectively. The result from this study suggest that the CP requirements for maintenance of growing buffaloes were higher than those found in yearling Thai native cattle (4.36 g CP/kg $W^{0.75}$/d) (Sereethai et al., 2009), and in

Brahman cattle (3.94 g CP/kg $W^{0.75}$/d) (Chaokaur and Sommart, 2009). Lohakare et al. (2006) reported that crossbred calves can be satisfactory reared on a 25% lower protein level from those recommended by Kearl (1982) for developing countries.

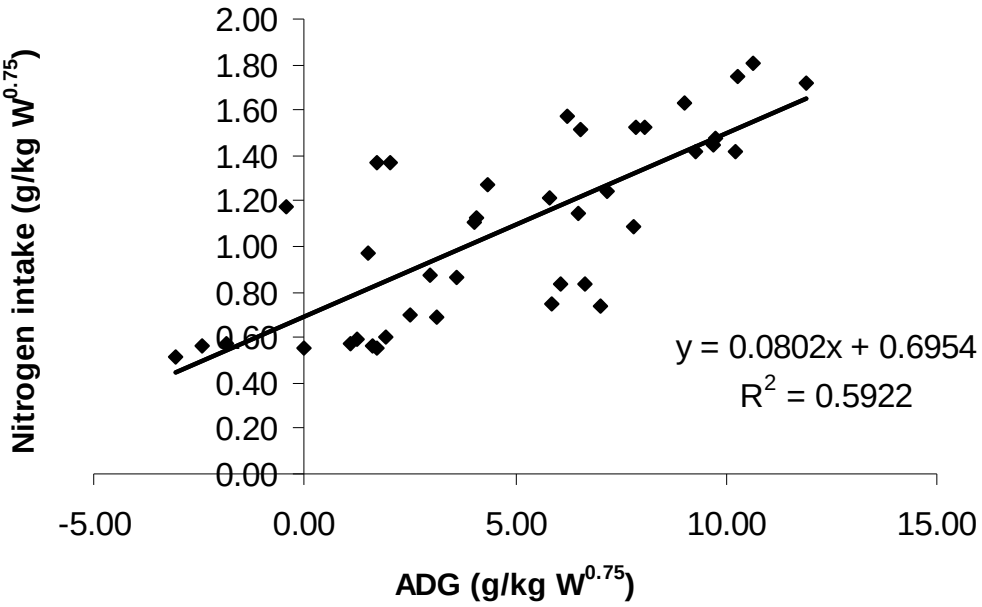

Figure 6.2 Relationship between nitrogen intake and average daily gain (ADG) (g/kg $W^{0.75}$) in growing swamp buffaloes. The equation was N intake = 0.0802ADG + 0.6954 (R^2 = 0.592, P < 0.001, n = 40)

The protein requirements of buffaloes based on metabolizable protein (MP) and digestible protein (DP) were calculated from the results in these studies, the MP requirements can be estimated by multiplying the CP requirements found in this study by 0.67 (NRC, 1996). The metabolizable protein requirements of growing Thai swamp buffaloes for maintenance and growth therefore are 3.10 g MP/kg $W^{0.75}$/d, and 0.34 g MP/g ADG/d. The DP requirements were estimated by the relationship between DP intake and N balance (Figure 6.3) of the data derived from experiments I,

II and III. From this equation the DP requirements calculated for maintenance of growing swamp buffaloes are 2.53 g DP/kg $W^{0.75}$/d. The regression equation between ADG and DP intake of the data derived from experiments II and III are shown in Figure 6.4. Based on this equation. it can be concluded that the DP requirements for growth of growing swamp buffaloes are 0.50 g DP/g ADG/d. In addition, DP requirements for maintenance and growth of growing swamp buffaloes were found to be similar to those of Kearl (1982) recommendation for growing buffalo (2.54 g DP/kg $W^{0.75}$ and 0.50 g DP/g ADG).

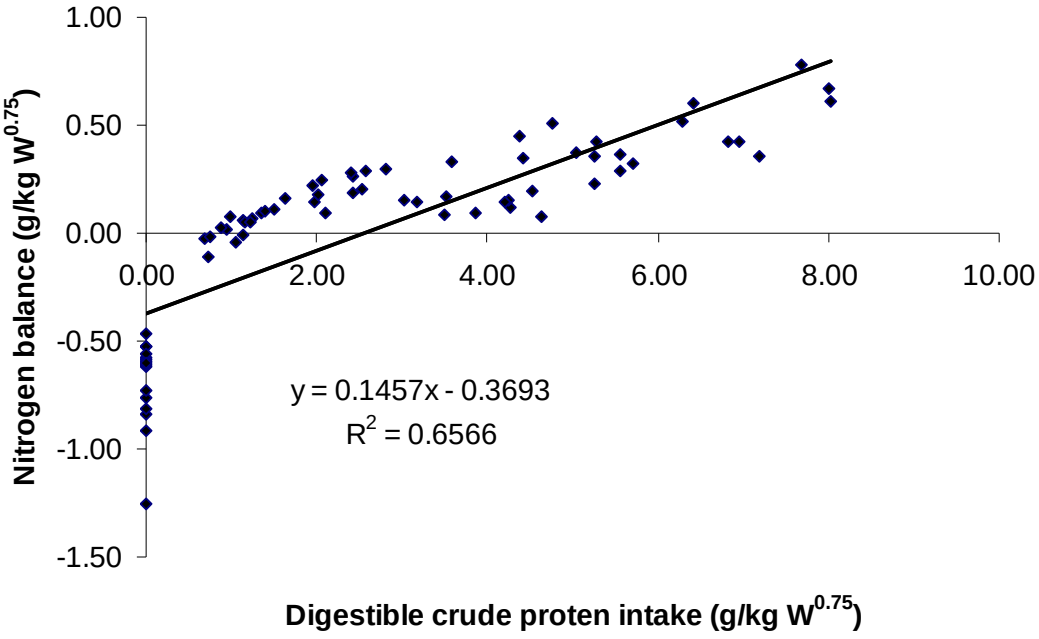

Figure 6.3 Relationship between digestible protein intake and nitrogen balance (g/kg $W^{0.75}$) in growing swamp buffaloes. The equation was N balance = 0.1457DCP intake – 0.3693 (R^2 = 0.657; P<0.001; n = 72).

The protein requirements of growing swamp buffaloes found in these studies, differ from those of *Nili-ravi* buffaloes and other cattle breeds, because of differences in genetic make-up, mature body size, growth rate, sex, age, quality of feeds and feed utilization efficiency (Paul and Patil, 2007). Chizzotti, Valadares Filho, Tedeschi, Chizzotti and Carstens (2007) summarized that there were no differences in net protein requirements for maintenance and growth for bulls, steers and heifers of Nellore x Red Angus crossbreds.

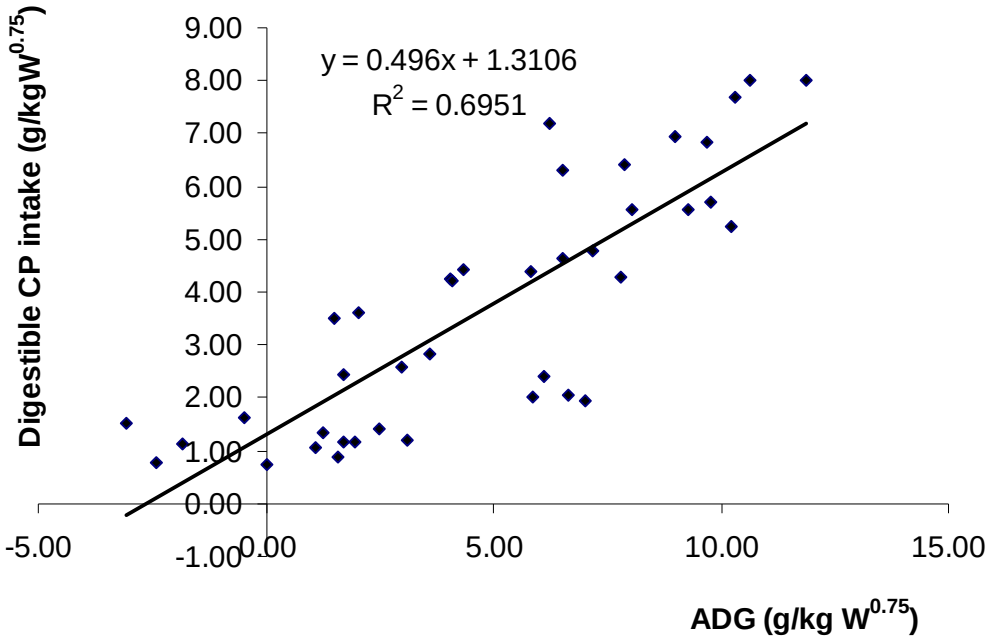

Figure 6.4 Relationship between digestible crude protein intake and average daily gain (ADG) (g/kg $W^{0.75}$) in growing swamp buffaloes. The equation was DP intake = 0.496ADG + 1.3106 (R^2 = 0.695, P < 0.001, n = 40)

In conclusion, based on the three experiments conducted in this thesis, it can be concluded that ruminal pH and NH_3-N concentration were within the optimal ranges for improvement of rumen fermentation and rumen ecology, improving rumen microbes, fiber digestion and VFAs concentration, pH of 6.8 to 7.2 and NH_3-N of 10.8 to 24.3 mg%. The protein requirements for maintenance and growth of growing male Thai swamp buffaloes are 4.64 g CP/kg $W^{0.75}$ and 0.50 g CP/g ADG, respectively.

However, further studies should be conducted for the validation of nutrients requirement (especially protein and energy) for different physiological stages of buffaloes. To optimize energy and protein efficiency and to reduce nitrogen wastage, diets need to be formulated to provide optimum fermentable energy source and nitrogen concentration for maximum rumen microbial yield and growth.

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BIOGRAPHY

The author, Pattaraporn Tatsapong, was born on May 7, 1974 in Udon Thani, Thailand. In 1997, she obtained her Bachelor's degree in Animal Science from Ubon Ratchathani University, Ubon Ratchathani, Thailand. From 1997 to 1998, she worked as a Researcher, under the Forage Research Project, Faculty of Agriculture, Ubon Ratchathani University.

In 1999, while continues to be employed by the Forage Research Project, she was awarded a scholarship from the Thailand Research Fund to study her Masters of Science (Animal Production technology) (M. Sc.) at Suranaree University of Technology, Nakhon Ratchasima, Thailand, where she graduated with a M. Sc. degree in 2001. She, then continued to work as a Researcher for the Forage Research Project, Faculty of Agriculture, Ubon Ratchathani University, for another 5 years. In 2005, she obtained her Bachelor of Business Administration (General Management) from Sukhothai Thammathirat Open University, Nonthaburi, Thailand.

In 2006, she was awarded a scholarship from the Office of the Higher Education Commission, Thailand, under the program Strategic Scholarships for Frontier Research Network, to study for her Doctor of Philosophy degree in Animal Production Technology. She enrolled at Suranaree University of Technology study Ruminant Nutrition in the School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand.