รายงานวิจัยฉบับสมบูรณ์

โครงการ ผลของระดับสารผสมยูเรีย-แคลเซียมในอาหารก้อนคุณภาพสูง ต่อการกินได้ การย่อยได้ นิเวศวิทยารูเมน และรูปแบบกระบวนการหมักในโคเนื้อพื้นเมืองไทย

โดย อ.ดร.อนุสรณ์ เชิดทอง

กรกฎาคม 2557

สัญญาเลขที่ MRG5580077

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อ.ดร.อนุสรณ์ เชิดทอง มหาวิทยาลัยขอนแก่น

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

<u>เอกสารแนบหมายเลข 2</u>

รูปแบบ Abstract (บทคัดย่อ)

Project Code: **(รหัสโครงการ)**	MRG5580077
Project Title: **(ชื่อโครงการ)**	Effects of urea-calcium mixture levels in high-quality feed block on feed intake, digestibility, rumen ecology and fermentation pattern in Thai-native beef cattle (ผลของระดับสารผสมยูเรีย-แคลเซียมในอาหารก้อนคุณภาพสูงต่อการกินได้ การย่อยได้ นิเวศวิทยารูเมน และรูปแบบกระบวนการหมักใน โคเนื้อพื้นเมืองไทย)
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Project Period: **(ระยะเวลาโครงการ)**	Two years (2 ปี)

Abstract

The objectives of this study was to develop feed blocks (FB) with urea-calcium mixture levels and to investigate the effects of different levels of urea-calcium mixture in FB on feed intake, nutrient digestibility and rumen fermentation in Thai-native beef cattle fed with rice straw as a basal. Two experiments were evaluated including *in vitro* gas production technique and *in vivo* experiment with swamp buffalo. Firstly, **Exp I** aimed to determine the effect of urea-calcium sulphate mixture (U-cas) levels in FB on ruminal digestibility, fermentation and gas kinetics in rumen fluid of swamp buffalo by using *in vitro* techniques. The treatments were 7 levels of U-cas incorporated in FB at 0, 30, 60, 90, 120, 150 and 180 g/kg and the experimental design was a Completely randomized design. Gas production rate constants for the insoluble fraction, potential extent of gas and cumulative gas were linearly increased when increasing level of U-cas in FB. The *in vitro* DM digestibility, *in vitro* OM digestibility, true digestibility and microbial mass were altered by treatments and were greatest at 180 g/kg of U-cas supplementation. Concentration of propionate was linearly increased when increasing levels of U-cas and was highest with U-cas supplementation at 180 g/kg. The NH_3–N concentration was highest when urea was added in the FB while NH_3–N concentration tended to be reduced with increasing level of U-cas. The findings from **Ex I** suggest supplementation of 180 g/kg U-cas in FB improves kinetics of gas production, rumen fermentation, digestibility and microbial mass as well as control the rate of N degradation in the rumen of swamp buffalo. Lastly, **Exp II** evaluated the effect of U-cas level in FB on feed intake, apparent digestibility of nutrients, rumen fermentation, population of ruminal microorganisms, predominant cellulolytic bacteria, microbial protein synthesis, N utilization, blood biochemistry and hematology parameters. Four Thai male native beef cattle, initial body weight (BW) 100$\pm$3.0 kg and fed rice straw were randomly assigned in a 4×4 Latin square design to receive four dietary treatments with inclusion of U-cas in FB at 0, 120, 150 and 180 g/kg DM. The present results revealed that rice straw intake was increased with the increasing level of U-cas inclusion in the FB. Total intakes of DM and energy (ME, MJ/d) were the highest with U-cas inclusion at 180 g/kg DM fed group, followed by 150, 120 and 0 g/kg DM, respectively. Apparent digestibility of nutrients other than ADF was enhanced with the increasing level of U-cas supplementation. Rumen pH and temperature were not changed by U-cas levels inclusion. The concentration of ruminal NH_3-N at 4 h post feeding was decreased with the increasing level of U-cas supplementation (P<0.05). Inclusion of U-cas at 180 g/kg DM in the FB could increased the propionic acid concentration in the rumen at 4 h post feeding which resulted in lower ratio of acetic: propionic acid and acetic plus butyric: propionic acid (P<0.05). Population of rumen bacterial increased quadratically (P<0.05), whereas fungal population was linearly greatest (P<0.05) with FB inclusion of U-cas at 180 g/kg DM (7.2 x 10^{11} cell/ml and 2.4 x 10^{4} cell/ml, respectively). An effect of hour after feeding (P<0.05) was observed, and there was no interaction of diet × hour. For 180 g/kg DM of U-cas in FB, rumen bacteria and fungal population increased at 4 h after feeding. Inclusion of U-cas in FB was linearly greatest (P<0.05) concentration means of total bacteria, whereas quadratic effects (P<0.05) were observed on *F. succinogenes* population with increasing U-cas concentration (8.2 x 10^{11} and 6.3 x 10^{9} copies/ml of rumen content, respectively). Microbial crude protein yield (MCP) and efficiency of microbial N synthesis (EMNS) were linearly increased when U-cas was included in FB at 180 g/kg DM (P<0.05). Supplementation at 180 g/kg DM reduced total N excretion (4.1 g/d), compared to other treatments, while N retention and ratio of N retention to N intake were increased up to 6.9 g/d and 14.9%, respectively. Blood biochemistry and hematological parameters were not different among treatments except concentration of plasma urea N, plasma glucose and total blood protein were improved especially with U-cas supplementation at 180 g/kg DM. Inclusion of U-cas at 180 g/kg DM in the FB resulted in improved feeding value, rumen fermentation, major cellulolytic bacteria, N utilization and blood biochemistry in Thai native cattle fed on rice straw.

Keywords: Cattle, blood biochemistry, feed block, rumen fermentation, ruminal microorganism, slow release urea

บทคัดย่อ

วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อพัฒนาสูตรอาหารก้อนร่วมกับการใช้สารผสมยูเรีย-แคลเซี่ยมที่ระดับต่าง ๆ และทำการศึกษาผลของระดับสารผสมยูเรีย-แคลเซี่ยมในสูตรอาหารก้อนต่อการกินได้ การย่อยสลายได้ของโภชนะ และกระบวนการหมักในรูเมนของโคเนื้อพื้นเมืองไทยที่ได้รับฟางข้าวเป็นแหล่งอาหารหยาบหลัก โดยการวิจัยครั้งนี้แบ่งเป็น 2 การทดลองย่อย ซึ่งประกอบไปด้วยการศึกษาในห้องปฏิบัติการด้วยเทคนิคการผลิตแก๊ส และทำการทดลองในโคเนื้อพื้นเมืองไทย สำหรับ**การทดลองที่ 1** มีวัตถุประสงค์เพื่อศึกษาผลของระดับสารผสมยูเรีย-แคลเซี่ยมซัลเฟต (urea-calcium sulphate mixture; U-cas) ในสูตรอาหารก้อนต่อการย่อยสลายได้ กระบวนการหมัก และจลศาสตร์การผลิตแก๊สจากของของเหลวในรูเมนของกระบือปลักโดยเทคนิคการผลิตแก๊ส ปัจจัยการศึกษาประกอบด้วยระดับของ U-cas 7 ระดับ ในอาหารก้อน คือ 0, 30, 60, 90, 120, 150 และ 180 g/kg โดยวางแผนการทดลองแบบ Completely randomized design ผลการศึกษาพบว่าปริมาณแก๊ส ณ เวลาในการหมักบ่มเป็น 0 (ค่าจุดตัดแกน y) (ค่า a) ค่าศักยภาพการผลิตแก๊ส (a+b) และ ปริมาณแก๊สสะสมมีค่าเพิ่มขึ้นแบบเส้นตรงเมื่อมีการเพิ่มระดับของ U-cas ในอาหารก้อน การย่อยได้ของวัตถุแห้ง การย่อยได้ของอินทรียวัตถุ การย่อยสลายได้จริงและมวลของจุลินทรีย์ในหลอดทดลองมีความแตกต่างกัน และมีค่าสูงที่สุดเมื่ออาหารก้อนมี U-cas ที่ระดับ 180 g/kg เป็นองค์ประกอบ ค่าความเข้มข้นของโพรพิโอเนต มีค่าเพิ่มขึ้นแบบเส้นตรงตามการเพิ่มขึ้นของระดับ U-cas และมีค่าสูงที่สุดที่ระดับ 180 g/kg ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจน มีค่าสูงที่สุดในกลุ่มที่มีการใช้ยูเรียในอาหารก้อน ในขณะที่อาหารก้อนที่มีการเสริม U-cas ทำให้ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจน มีแนวโน้มที่ลดลงและมีค่าต่ำที่สุดเมื่อมีการใช้ U-cas ในระดับสูงที่สุด จากการทดลองที่ 1 สามารถสรุปได้ว่า การเสริม U-cas ที่ระดับ 180 g/kg ในอาหารก้อน สามารถปรับปรุงจลศาสตร์การผลิตแก๊ส กระบวนการหมักในรูเมน การย่อยสลายได้ และมวลจุลินทรีย์ นอกจากนี้ เมื่อเปรียบเทียบกับกลุ่มควบคุมยังพบว่าการเสริม U-cas สามารถควบคุมอัตราการปลดปล่อยไนโตรเจนในของเหลวในรูเมนของกระบือปลักได้ **การทดลองที่ 2** ทำการศึกษาผลของระดับ U-cas ในอาหารก้อน ต่อการกินได้ การย่อยสลายได้ของโภชนะ กระบวนการหมักในรูเมน ประชากรจุลินทรีย์ในรูเมน แบคทีเรียกลุ่มหลักที่ย่อยสลายเยื่อใย การใช้ประโยชน์ไนโตรเจน ค่าทางชีวเคมีและโลหิตวิทยาของเลือด โดยทำการศึกษาในโคเนื้อพื้นเมืองไทยเพศผู้จำนวน 4 ตัว น้ำหนักตัวเริ่มต้น 100±3.0 kg และให้ฟางข้าวเป็นอาหารหยาบหลัก ใช้แผนการทดลองแบบ 4 x 4 Latin Square เพื่อให้สัตว์ทุกตัวได้รับอาหารทั้ง 4 ปัจจัย ซึ่งประกอบไปด้วยระดับการเสริม U-cas ในอาหารก้อนที่ 0, 120, 150 และ 180 g/kg DM ผลการทดลองพบว่าการกินได้ของฟางมีค่าสูงขึ้น เมื่อมีการเพิ่มระดับ U-cas ในอาหารก้อน การกินได้วัตถุแห้งทั้งหมด (ME, MJ/d) มีค่าสูงที่สุดในกลุ่มที่ได้รับอาหารก้อนที่มี U-cas 180 g/kg และรองลงมาคือกลุ่มที่ได้รับ 150, 120 และ 0 g/kg ตามลำดับ การย่อยได้ของโภชนะมีค่าเพิ่มขึ้นเมื่อมีการ

เพิ่มระดับของ U-cas ยกเว้นการย่อยได้ของ ADF ที่ไม่มีความแตกต่างกัน ค่าความเป็นกรดด่าง และค่าอุณหภูมิในรูเมน ไม่มีความแตกต่างกันทางสถิติ ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจน ที่ชั่วโมงที่ 4 หลังการให้อาหารมีค่าลดลงเมื่อมีการเพิ่มระดับ U-cas สูงขึ้น (P<0.05) การเสริม U-cas ที่ระดับ 180 g/kg ในอาหารก้อนส่งผลทำให้ค่าความเข้มข้นของโพรพิโอเนตในรูเมนหลังจากชั่วโมงที่ 4 ของการให้อาหารมีค่าเพิ่มขึ้น ในขณะที่สัดส่วนของอะซิเตตต่อโพรพิโอเนต และสัดส่วนของอะซิเตตรวมกับบิวทิเรตต่อโพรพิโอเนตมีค่าต่ำลง (P<0.05) ประชากรของแบคทีเรียในรูเมนมีค่าเพิ่มสูงขึ้นแบบกำลังสอง (P<0.05) ในขณะที่ประชากรของเชื้อรามีค่าสูงที่สุดเมื่อสัตว์ได้รับอาหารก้อนที่มี U-cas ที่ระดับสูงขึ้น (7.2×10^{11} cell/ml และ 2.4×10^{4} cell/ml ตามลำดับ) ผลของชั่วโมงหลังจากการให้อาหาร ทำให้ประชากรของจุลินทรีย์มีความแตกต่างกัน (P<0.05) แต่ไม่พบอิทธิพลร่วมระหว่างอาหาร x ชั่วโมงการให้อาหาร ในสัตว์ที่ได้รับการเสริม U-cas ที่ 180 g/kg ในอาหารก้อนหลังจาก 4 ชั่วโมง ส่งผลทำให้ประชากรแบคทีเรียและเชื้อรามีค่าสูงที่สุด นอกจากนี้การเสริม U-cas ในอาหารก้อน ทำให้ค่าเฉลี่ยของประชากรแบคทีเรียรวมมีค่าเพิ่มขึ้นแบบเส้นตรง (P<0.05) ในขณะแบคทีเรียกลุ่ม *F. succinogenes* มีค่าเพิ่มขึ้นแบบกำลังสองเมื่อมีการเพิ่มระดับของ U-cas ในอาหารก้อน (8.2×10^{11} และ 6.3×10^{9} copies/ml ตามลำดับ) ผลผลิตจุลินทรีย์โปรตีน และประสิทธิภาพการสังเคราะห์จุลินทรีย์ไนโตรเจน มีค่าเพิ่มขึ้นแบบเส้นตรงเมื่อมีการเสริม U-cas ในสูตรอาหารก้อนที่ระดับ 180 g/kg นอกจากนี้ เมื่อเปรียบเทียบกับกลุ่มควบคุมพบว่า การเสริม U-cas ที่ระดับ 180 g/kg ส่งผลทำให้ค่าการขับออกของไนโตรเจนลดลง (4.1 g/d) ในขณะที่ค่าการไหลเวียนของไนโตรเจนและสัดส่วนของการไหลเวียนไนโตรเจนต่อปริมาณไนโตรเจนที่ได้รับมีค่าเพิ่มสูงขึ้น (6.9 g/d และ 14.9% ตามลำดับ) ค่าพารามิเตอร์ทางชีวเคมีและโลหิตวิทยาของเลือดพบว่าทุกค่าไม่มีความแตกต่างกันทางสถิติระหว่างกลุ่มทดลอง ยกเว้นค่าความเข้มข้นของยูเรีย-ไนโตรเจน กลูโคส และโปรตีนรวมในเลือดพบว่าการเสริม U-cas ที่ระดับ 180 g/kg ในอาหารก้อนจะส่งผลดีต่อค่าพารามิเตอร์ดังกล่าว ดังนั้น จากการวิจัยครั้งนี้ สามารถสรุปได้ว่าการเสริม U-cas ในอาหารก้อนที่ระดับ 180 g/kg สามารถปรับปรุงประสิทธิภาพการกินได้ของอาหาร กระบวนการหมักในรูเมน แบคทีเรียกลุ่มหลักที่ย่อยสลายเยื่อใย การใช้ประโยชน์ของไนโตรเจน และค่าพารามิเตอร์ทางชีวเคมีของเลือดในโคเนื้อพื้นเมืองไทยที่ได้รับฟางข้าวเป็นอาหารหลัก

คำสำคัญ: โคเนื้อ, ชีวเคมีของเลือด, อาหารก้อน, กระบวนการหมักในรูเมน, จุลินทรีย์ในรูเมน, ยูเรียปลดปล่อยช้า

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Introduction to the research problem and its significance

Dietary protein plays an important role in the nutrition of ruminants, since besides providing amino acids; it is also a source of nitrogen for the synthesis of microbial protein (Nocek and Tamminga, 1991). Therefore, it is considered the most important nutrient and also the most expensive, which must be efficiently used. Strategies to reduce the feed cost without interfering negatively in production have been constantly researched. The substitution of traditional feeds in the diets of ruminants is common as economic condition changes (∅rskov, 1999). Soybean meal (SBM) has long been used as a prominent source of crude protein for ruminants, however, with its increasing price, the use results in higher cost of production. Thus, use of urea as a protein replacement is attractive in ruminant diets because of its low cost compared with true protein feeds such as SBM (Wanapat, 2009; Xin et al., 2010; Cherdthong et al., 2011a,b,c).

Since the early demonstration by Krebs (1937) of its potential value when fed to ruminants, urea has become widely used as a substitute for preformed protein in ruminant diets. The presently accepted mechanism of urea action in ruminant nutrition is the hydrolysis of urea by rumen urease to ammonia plus carbon dioxide, carbohydrate fermentation to volatile fatty acids, amination of keto acids to give amino acids, incorporation of the amino acids into microbial protein, and digestion of the microbial cells in the small intestine with subsequent absorption of the resulting amino acids (Nocek and Russell, 1988; Nocek and Tamminga, 1991; Calsamiglia et al., 2008). However, amount of urea can be used in diets is limited due to their rapid hydrolysis to NH_3 in the rumen by microbial enzymes (Golombeski et al., 2006; Highstreet et al., 2010). This rapid breakdown to NH_3 can occur at a much faster rate than NH_3 utilization by the rumen bacteria, resulting in accumulation and escape of NH_3 from the rumen. The net result is that a potentially large part of the nitrogen from NPN sources is excreted in the urine and can contribute to environmental pollution (Broderick et al., 2009; Huntington et al., 2009; Inostroza et al., 2010).

An alternate solution could be to modify urea to control its rate of release so that NH_3 release more closely parallels carbohydrate digestion (Pinos-Rodríguez et al., 2010). Slow-release urea compounds, which have been fed to ruminants, include biuret, starea, urea phosphate, coatings based on oil, formaldehyde treated urea and polymer-coated urea (Taylor-Edwards et al., 2009). These compounds have not been as advantageous as urea because a substantial part of the NPN in them may leave the rumen without being converted to NH_3, reducing its incorporation into microbial protein (Tedeschi et al., 2000; Galo et al., 2003; Firkins et al., 2007). More recently, slow-release urea has been achieved by using urea bounding to substrates like calcium sulphate to control the release rate of NH_3 from urea. In an earlier *in vitro* and *in vivo* experiments, supplementation of urea-calcium sulphate mixture (UCM) products in the concentrate diets have been also demonstrated to reduce ruminal NH_3 concentrations, improve microbial population as well as enhance performance efficiency in ruminants as compared with feed grade urea (Cherdthong et al., 2011a,b,c). However, supplementation of concentrate diet is not suitable on practical use for smallholder farmers especially Thai-native beef cattle farmer. This could be due to; 1) high price of concentrate, 2) complicate to feeding, 3) spend more time to manage 4) feeding of concentrate are quite suitable for dairy cows or commercial sector etc. High-quality feed block (HQFB) is one of strategic alternative feed block and easier feeding to ruminant when compared with concentrate diet. HQFB have been report to be beneficial to ruminants, especially with rice straw and other low quality roughages-based diets. Supplementation with urea-molasses block (Wanapat, 2003) or HQFB (Wanapat and Khampa, 2006; Foiklang et al., 2011) have shown a beneficial effect on growth performance, feed intake, nutrient digestibility and rumen fermentation. However, supplementation of UCM levels in HQFB for ruminants have not been investigated particularly in practical Thai-native beef cattle feeding in the tropics in order to increase production efficiency.

Therefore, the objectives of this study are to develop HQFB with urea-calcium mixture levels and to investigate the effects of different levels of urea-calcium mixture in HQFB roughage on feed intake, nutrient digestibility and rumen fermentation in Thai-native beef cattle fed with rice straw as a basal.

Objectives:

1. To develop high-quality feed block with urea-calcium mixture as a new product.
2. To evaluate the effects of different levels of urea-calcium mixture in HQFB on ruminal ammonia concentration, nutrients digestibility, microbial population using the *in vitro* gas techniques.

3. To evaluate the effects of different levels of urea-calcium mixture in HQFB on rumen ecology, rumen microorganisms, microbial protein synthesis, and digestibility of nutrients of Thai-native beef cattle.

Literature review

Livestock production in Thailand plays a crucial role, which extends beyond the traditional use of supplying only meat and milk. Livestock are used for multiple purposes such as draft power, a means of transportation, capital, credit, meat, milk, social value, by-product uses, hides and as a source of organic fertilizer for seasonal cropping. Livestock have a significant capacity to utilize on-farm resources, especially the agricultural crop residues and by-products that are abundantly available.

Livestock/crop holdings have been in the hands of the rural resource-poor farmers for many decades and it is likely to hold true for many years to come. In general, the farmers traditionally practice rice cultivation (1-3 ha). It is therefore essential to account for and integrate the on-farm activities of livestock and to diversity their contribution to increase the farmers efficiency of production and their income.

The livestock population has been compiled by Department of Livestock Development (2010) and is presented in Table 1. Under the prevailing conditions and production systems, the populations of beef and dairy cattle are anticipated boost production and to provide for large domestic demand. The optimum production levels for swine and poultry have been reached recently and the rate of increase in population is anticipated to be minimal.

Table 1. Livestock population distributed in Thailand (head)

Year	Cattle	Buffalo	Cow	Goat	Sheep
1999	4,918,396	1,799,606	282,655	132,845	39,485
2000	5,208,541	1,702,223	307,867	144,227	37,312
2001	5,571,283	1,710,095	343,679	188,497	42,720
2002	5,908,625	1,617,358	358,440	177,944	39,326
2003	5,916,323	1,632,706	380,203	213,917	42,883
2004	6,668,332	1,494,238	408,350	250,076	47,811
2005	8,275,108	1,624,919	478,836	338,355	50,779
2006	8,036,057	1,351,851	412,804	324,150	51,151
2007	9,337,985	1,577,798	489,593	444,774	50,963
2008	9,582,030	1,359,807	469,937	374,029	43,738

Source: Department of Livestock Development (2010)

Beef cattle production in Thailand

Farmers have been urging government agencies and academics to help improve the cattle-raising system, set a standardised pricing system and create a pilot project to show them better farming methods. The population reported in 2008 was 9.58 as compared to 1.35 million for cattle and buffaloes, respectively (Table 1). Between 1999–2008, the cattle population dramatically increased (Office of Agricultural Economics, 2010). Two million, or 40 per cent of the nationwide total of local-breed cattle, were raised in the lower Northeast. Cattle breeds are usually native, and small numbers are cross bred with Brahman and naive or other breeds. Brahman was introduces for breeding improvement protein of Thai-native beef cattle because of their resistance and tolerance to disease and climate in Thailand with their sound performance. However, insufficiency feeding is one of main problems of Thai-native beef cattle raising which causes low production output. Farmer rise their cattle in the public pasture, non-crop planting land and rice field after harvested. Rice straw is the major component of cattle feeding during dry season. Traditional, few farmer invested on growing forage crop, but during the past decade back-yard pastures were increased gradually in the semi-intensive farm. Moreover fattening Thai-native beef cattle was produces in small quantities because of limitation of good beef markets. But it seem to be having a high potential on increasing Thai-native beef cattle fattening promotion in order to meet the gradual increasing requirement.

Use of urea as NPN source for ruminant feed

The substitution of traditional feeds in the diets of ruminants is common as economic condition changes (∅rskov, 1999; Devendra, 2007). Soybean meal (SBM) has long been used as a prominent source of crude protein for ruminants, however, with its increasing price, the use results in ultimately higher cost of production (Chalupa, 2007). Therefore, the use of urea as a protein (non-protein N, NPN) replacement is attractive in ruminant diets because of its low cost compared with other protein feeds such as SBM with high rumen degradability (Wanapat, 2009; Xin et al., 2010).

The sources of NH_3-N in the rumen include proteins, peptides and amino acids (see preceding section), and other soluble-N materials (Robinson et a., 2004; Robinson, 2010). Urea, uric acid and nitrate are rapidly converted to NH_3-N in the rumen. Nucleic acids in rumen fluid are probably also degraded extensively to NH_3-N. Figure 1 indicates possible sources of the NH_3-N pool.

The NH_3-N pool is a focus for studies of metabolism of N in the rumen, and much knowledge has been gained from measuring fluxes of N through this pool. NH_3-N is lost from rumen fluid by:

- Incorporation into microbial cells that pass out of the rumen.
- Absorption through the rumen wall.
- Passing out of the rumen in fluid.

The NH_3-N pool in the rumen is relatively small and turns over rapidly. The amount of NH_3-N entering the pool varies over a wide range according to quantity and degradability of protein in the diet and with the extent and method of supplementation of urea. Concentrations of NH_3-N in the pool can be expected to change rapidly even when animals have continuous access to food.

The amount of NH_3-N that flows out of the rumen in fluid is relatively small, and it follows that NH_3-N produced in the rumen that is not incorporated into micro-organisms is absorbed mainly through the wall of the reticulo-rumen.

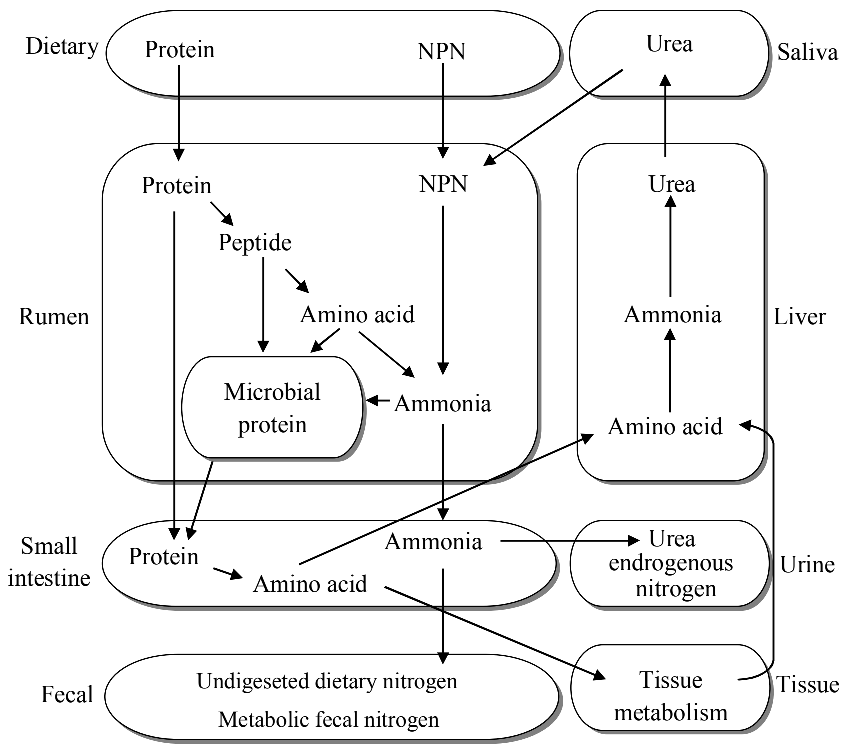

Figure 1 A model of the metabolism of nitrogen in the rumen (Leng and Nolan, 1984; Modified by Cherdthong and Wanapat, 2010)

To maintain a high level of NH_3-N in rumen fluid over 24 hours on low-protein diets requires urea to be taken in continuously. This can be ensured by spraying urea on the basal feed or by providing a urea block or liquid mixture which is licked at regular intervals. Urea given in a single meal is unlikely to maintain rumen NH_3-N levels above the minimum required for efficient fermentation for more than a few hours per day.

Disadvantages of urea utilization for ruminants

The mechanism of urea action in ruminant nutrition is the hydrolysis of urea by rumen urease to NH_3 plus carbon dioxide, carbohydrate fermentation to volatile fatty acids, amination of keto acids to give amino acids, incorporation of the amino acids into microbial protein, and digestion of the microbial cells in the small intestine with subsequent absorption of the resulting amino acids (Nocek and Tamminga, 1991; Calsamiglia et al., 2008). However, the amount of urea can be used in diets is rather limited due to their rapid hydrolysis to NH_3-N in the rumen by microbial enzymes (Golombeski et al., 2006; Highstreet et al., 2010). This rapid breakdown to NH_3-N can occur at a much faster rate than NH_3-N utilization by the rumen bacteria, resulting in accumulation and escape of NH_3-N from the rumen (Reynolds and Kristensen, 2008). The net result is that a potentially large part of the N from NPN sources is excreted in the urine and can contribute to environmental pollution (Broderick et al., 2009; Inostroza et al., 2010).

The topic of efficiency of protein use by ruminants has gained attention by environmentalists and government regulators in many parts of the world (Robinson, 2010). Animal feeding practices that reduce the amount of urea in urine have the potential to decrease NH_3 emissions to the environment since urine urea is rapidly converted to NH_3 in fecal/urine slurries due to the action of fecal and environmental ureases. Current dairy research in California, and other parts of the USA and Europe, is focused on decreasing the amount of dietary protein that appears in urine, while maximizing production of milk and its components (Highstreet et al., 2010). Increasing public concern has been focused on ruminant production systems as a major nonpoint source of pollution, which has spurred research aimed to reduce N excretion (Wanapat et al., 2009). Nutrient losses may affect ground and surface water quality; in addition, NH_3 and nitrous oxide emissions can affect air quality, and the latter has been implicated as a significant contributor to global warming, having a 310× more harmful mass-specific effect than CO_2 as a global warming agent (Marini and Van Amburgh, 2005). Therefore, ruminant production systems should support nature conservation, and the environmental load should be low.

Alternative urea product as slow-release ammonia

Nutritional models for feeding protein to dairy cattle have evolved from basic CP to more complex systems based on rumen-degradable and undegradable protein (NRC, 2001). The basic structure of all of the models is similar with N inputs provided by dietary, recycled, and endogenous N. Dietary protein is divided into rumen-degradable and undegradable protein with RDP composed of non-protein and true protein N. True protein is degraded to peptides and AA and eventually deaminated into NH_3-N or incorporated into microbial protein. Non-protein N is composed of N present in DNA, RNA, NH_3, urea, AA, and small peptides with the N from peptides, AA, and NH_3 being used for microbial growth (Bach et al., 2005).

Dietary urea has been used for decades as an effective and inexpensive source of N for ruminal microbial utilization (Taylor-Edwards et al., 2009). The amount of urea can be used in diets is rather limited due to their rapid hydrolysis to NH_3-N in the rumen by microbial enzymes (Golombeski et al., 2006; Highstreet et al., 2010). This rapid breakdown to NH_3-N can occur at a much faster rate than NH_3-N utilization by the rumen bacteria, resulting in accumulation and escape of NH_3-N from the rumen. Moreover, the majority of ruminal NH_3-N rapidly enters the blood and can cause adverse affects ranging from depressed feed intake and animal performance, to death from NH_3 toxicity (Huntington et al., 2006; Huntington et al., 2009). Reynolds and Kristensen (2008) dosed cattle with 0.125, 0.25, and 0.5 g urea/kg BW and measured rapid accumulation, and subsequent dissipation, of NH_3 from blood. There was essentially a linear response to dose, with the highest level causing acute NH_3 toxicity. Increased blood NH_3 levels were detected as early as 5 min after dosing, and maximal concentrations were reached 30 min after dosing in hepatic portal blood, and 60 min after dosing in jugular blood. Concentrations had not returned to pre-dose levels by 300 min. Hepatic portal concentrations of NH_3 were 10 times jugular concentrations.

One of the major functions of the liver is to remove potentially toxic NH_3 from circulation, use it for synthesis of nitrogenous compounds needed for metabolism, or convert it to urea as a nontoxic end product of N metabolism. Reynolds and Kristensen (2008) found that the liver of dairy cows was able to remove NH_3 added to portal blood until the supply reached 182 mg/min but, at higher infusion rates, peripheral blood concentrations increased at the same rate as concentrations in the portal vein. Clearly, rapid hydrolysis of dietary urea can exceed the liver's capacity to remove it. Furthermore, Huntington et al. (2006) pointed out that NH_3 can be absorbed directly into systemic circulation from the PDV, thereby "leaking" past the liver.

A potential way to minimize excess NH_3 reaching the liver is to increase microbial utilization of NH_3-N by modulating its appearance in the rumen. Therefore, an alternate solution could be to modify urea to control its rate of release so that NH_3-N release more closely parallels to carbohydrate digestion (Pinos-Rodríguez et al., 2010). A slow-release urea compound should be useful to reduce toxicity and might enhance acceptability of supplements and utilization of urea. The slow-release urea compounds, which have been fed to ruminants, include isobutylidene diurea, acetylurea, biuret, tung- and linseed-oil-coated urea, formaldehyde treated urea, urea with $CaCl_2$ and $CaSO_4$ (Tamminga, 1992; Galo et al., 2003; Tedeschi et al., 2002; Huntington et al., 2006; Golomeski et al., 2006; Taylor-Edwards et al., 2009; Highstreet et al., 2010; Inostroza et al., 2010; Pinos-Rodríguez et al., 2010; Xin et al., 2010). Other approaches have been investigated in an attempt to match NH_3-N release and energy availability, e.g., combinations of urea and starches, urea and cassava chip, urea and cellulose, urea-molasses plus formaldehyde (Puga et al., 2001; Chanjula et a., 2003; Galina et al., 2003).

The present invention provides a slow-release NH_3-N feed supplement that enables the use of higher level of a non-protein N source in ruminant feed than has heretofore been used. The feed supplement of the present invention is formulated to provide for substantially slower release of NH_3-N from a non-protein N source, e.g., urea, during anaerobic digestion, thus allowing the use of higher levels of NPN sources in ruminant while avoiding the risk of NH_3-N accumulation, escape of NH_3-N from the rumen, NH_3 toxicity as well as N loss.

In general, slow-release urea (SRU) product used in many studies was manufactured by several companies such as a slow-release coated urea product from Optigen® 1200, Alltech Inc. Nicholasville, KY, SA; coated urea from CPG Nutrients, Inc. Syracuse, NY; slow-release urea product from Agri-Nutrients Technology Group, Petersburg, VA) etc. Several studies have been conducted to investigate the influence of feeding slow- release urea products on rumen fermentation and performance efficiency in ruminants, but variable results from either *in vitro* and/ or *in vivo* experiments.

Supplementation of slow-release urea products in ruminants

Effect of slow-release urea on feed intake and digestibility

Digestion balances and feed intake have been a common means of diet evaluation, to the extent that digestibility values are now as much attributes of a feed or diet as compositional values are (Van Soest, 1994). Several studies have been conducted to investigate the influence of feeding slow-release urea on feed intake and nutrient digestibility (Table 2). Previous study from Puga et al. (2001) found that the forage to controlled-release urea (CRU) ratios at 70: 30 were significantly increased dry matter intake above the level of the control diet (100% forage: CRU).

Table 2 Effects of slow-release urea products on dry matter intake and nutrient digestibility

Source	Type of SRU	Suppl., % diet	Animal	DMI kg/d	Digestibility, %			
					DM	CP	NDF	OM
Puga et al. (2001)	Urea	0	Sheep	5.9	58.6	-	67.8	57.6
	Control release	30*		8.2	64.8	-	74.0	63.2
Galina et al. (2003)	SRU	0	Beef	5.8	58.8	-	57.1	48.4
	SRU	1.8		8.2	68.7		75.1	59.7
Highstreet et al. (2010)	Urea	1.8	Cows	28.2	-	70.9	50.9	-
	Encapsulated urea	1.7		28.6	-	70.8	50.0	-
Xin et al. (2010)	Urea	0.6	Cows	20.2	46.3	43.5	13.9	46.7
	Polyurethane coated urea	0.6		22.8	51.0	44.6	18.5	51.2
Cherdthong et al. (2011a)	UCM**	6.7	Cows	12.2	66.4	65.8	60.6	73.2
Cherdthong et al. (2011b)	UCM	6.7	Beef	10.5	60.0	62.0	64.0	45.0

*Supplementation of 30% control release in forages.

**UCM=Urea-calcium mixture

The higher digestibility of the experiment diets was due to better activity of fiber fermentation in the rumen. It indicates that CRU improves nutrient imbalance for rumen bacteria by increasing availability of energy from simple carbohydrates such as molasses. Similarly, Galina et al. (2003) suggested that, supplementation of 1.8 kg dry matter of slow- release urea supplement (SRUS) with sugar cane tops (*Saccharum officinarum*) and maize (*Zea mays*) in 60 Zebu steers, while showing significantly ($P< 0.05$) better improved of digestibility. High fiber forages have been associated with more digestible feeds when NH_3 and urea were added to fibrous hay (Ørskov, 1999). In addition, another polymer-coated SRU (Optigen; CPG Nutrients, Syracuse, NY) has been demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo et al., 2003). These results were in agreement with the findings from Xin et al. (2010), who found that polyurethane coated urea were greater DMI and nutrients digestibilities than those in urea. Earlier experiment by Cherdthong et al. (2011a, b) reported that urea-calcium mixture were more efficiency than urea or urea–calcium chloride mixture products on digestibility both in cows and beef cattle (Table 2).

Effect of slow-release urea on rumen fermentation parameters

The development of products that slow the ruminal release of NH_3-N without limiting the extent of urea degradation in the rumen has been challenging (Males et al., 1979). Owens et al. (1980) reported that ruminal NH_3-N release was slower for slow release urea product than for uncoated urea, thereby increasing diet acceptability and improving rumen fermentation in ruminants. As reported that, supplementation of sugar cane tops (*Saccharum offcinarum*), corn stubble (Zea mays) and King grass (*Pennisetum purpureum*) (high fiber diets) with controlled- release urea supplement (CRUS) did improve fermentation in sheep (Puga et al., 2001) (Table 3). Adding 10, 20 or 30% CRUS showed improved NH_3-N and VFA production. This is strategies to improve the utilization of those feeds, suggesting providing supplements to correct the nutrient imbalances for rumen bacteria (Nocek and Russell, 1988). CRUS could have provided continuous NH_3-N for microbial growth, superior the minimum of 15-30 mg NH_3-N/100 ml rumen fluid for maximizing microbial growth previously suggested (Leng, 1991).

A recent study by Taylor-Edwards et al. (2009) who conducted the effects of slow-release urea (SRU) versus feed-grade urea on ruminal NH_3-N in beef steers. Multi-catheterized steers were used to determine effects of intraruminal dosing (5 kg of BW) SRU or urea on PDV nutrient flux and blood variables for 10 h after dosing. Intraruminal dosing of SRU prevented the rapid increase in ruminal NH_3-N concentrations that occurred with urea dosing. Urea undergoes rapid hydrolysis in the rumen to NH_3-N. Mean ruminal NH_3-N concentrations were 263% greater for steers dosed intraruminally with urea than steers dosed with SRU primarily because ruminal NH_3-N concentrations for urea treatment rose markedly within 0.5 h of dosing. This rapid rise in NH_3-N concentrations for urea treatment was substantial enough to increase ruminal pH by over 0.5 units within 0.5 h of dosing. Indeed, ruminal pH and ruminal NH_3-N concentrations were positively related, an effect that has been observed previously (Puga et al., 2001). Additionally, ruminal NH_3-N concentrations remained greater for steers dosed with urea than those dosed with SRU until 8 to 10 h after dosing. These results demonstrate that *in vivo* SRU does indeed have a slower release rate of NH_3-N than urea and can effectively modulate ruminal NH_3-N concentrations when substituted for urea (Huntington et al., 2006; Golomeski et al., 2006; Taylor-Edwards et al., 2009; Highstreet et al., 2010; Inostroza et al., 2010; Pinos-Rodríguez et al., 2010; Xin et al., 2010).

Table 3 Effects of slow-release urea products on rumen fermentation parameters

Source	Type of SRU	Suppl., % diet	Animal	NH_3-N, mg%	Total VFA, mM/L	VFA, %		
						C2	C3	C4
Galina et al. (2003)	SRU	0	Beef	6.8	-	78.2	14.4	7.4
	SRU	1.8		12.3	-	72.2	16.0	11.8
Golombeski et al. (2006)	Ruma Pro	0	Cows	5.4	54.0	62.9	21.2	11.4
	Ruma Pro	0.61		6.0	50.0	63.2	21.5	11.1
Taylor-Edwards et al. (2009)	Urea	1.6	Steers	14.1	99.7	62.7	19.7	14.0
	SRU	1.6		8.9	103.2	63.6	20.3	13.8
Pinos-Rodríguez et al., 2010	Optigen®	0.6	Steers	-	97.6	52.0	34.9	13.0
	Optigen®	1.1		-	94.8	52.3	35.2	12.5
Xin et al. (2010)	Urea	0.6	Cows	2.0	64.1	56.8	33.3	5.3
	PCU*	0.6		1.4	66.1	56.3	34.4	5.3
Cherdthong et al. (2011a)	UCM**	6.7	Cows	15.7	117.5	67.4	24.1	8.5
Cherdthong et al. (2011b)	UCM	6.7	Beef	14.5	119.2	70.4	22.3	7.3

*PCU=Polyurethane coated urea, **UCM=Urea-calcium mixture

Nitrogen utilization by rumen microorganisms can be reflected by ruminal NH_3-N concentration (Hungate, 1966). In the study by Xin et al. (2010), the NH_3-N concentrations of all the diets increased within 1 h, and then declined gradually. However, the polyurethane coated urea (PCU) diet resulted in the lowest concentrations of NH_3-N at all time points. During 8 h *in vitro* fermentation, the PCU diet decreased NH_3-N concentration by 8.2-20.6% as compared with the FGU diet. This agrees with the result of Prokop and Klopfenstein (1977), who found that slow-release urea (combination of urea and formaldehyde) could decrease ruminal NH_3-N concentration by 25.3% compared to urea. No significant differences were found between PCU and soybean mean (SBM) diets on ruminal NH_3-N release. A similar result was found in the report of Galo (2003), in which urea release from a polymer-coated urea was 83% as extensive as uncoated urea after 1 h incubation with distilled water. Other products, such as a urea-calcium combination, have had similar effects. Cass and Richardson (1994) made a comparison in an *in vitro* study and observed that a urea-calcium combination produced slower NH_3-N release rate than regular urea. Ammonia- N concentrations began to increase at 8 h for the FGU diet, which indicates that bacterial autolysis may occur. However, NH_3-N concentrations with PCU and SBM diets still declined. Based on this result, it could be inferred that slow-release urea diets prolong microbial utilization of additional N sources during ruminal fermentation. Therefore, the sychronization between ruminal NH_3-N release and carbohydrate availability might be improved, consequently resulting in greater microbial protein synthesis.

For more possibly reason, slow-release urea product reduced NH_3-N concentration through the inhibition of the hyper-ammonia-producing bacteria, a small group of ruminal bacteria that are responsible for the production of most of the NH_3-N (Chen and Russell, 1989). Ferme et al. (2004) also reported that the inhibition of major ammonia-producing bacteria (such as *Prevotella ruminantium* and *Prevotella bryantii*) resulted in a reduction in NH_3-N concentration in continuous culture fermenters of ruminal microbes. Continuous culture fermenters have low numbers of protozoa; however, *in vivo*, protozoa play a major role in protein degradation. The most important aspect of protozoa is their ability to engulf large molecules, protein, CHO, or even ruminal bacteria (Van Soest, 1994). In addition, protozoa play a role in regulating bacterial N turnover in the rumen, and they supply soluble protein to sustain microbial growth. Because protozoa are not able to use NH_3-N, a fraction of previously engulfed insoluble protein is later returned to the rumen fluid in the form of soluble protein

(Dijkstra, 1994). This is one of the main reasons why defaunation decreases NH_3-N concentration in the rumen (Eugene et al., 2004).

In some studies, Xin et al. (2010) who evaluated the effects of polyurethane coated urea on ruminal VFA concentration of Holstein dairy cows fed a steam-flaked corn-based diet. Three treatment diets with isonitrogenous contents (13.0% CP) were prepared: i) feedgrade urea (FGU) diet; ii) polyurethane coated urea (PCU) diet; and iii) isolated soy protein (ISP) diet. There were no significant differences in total VFA concentration among the three dietary treatments. Because ruminal VFAs are derived mainly from dietary carbohydrate fermentation (Firkins, 1996), the similar total ruminal VFA concentrations reflected no adverse fermentation by addition of FGU or PCU to the diet. Molar percentages of individual VFAs were significantly altered ($p<0.05$) by the dietary treatment. Urea-based diets resulted in a higher proportion of acetate and less propionate than the ISP diet, which caused a significantly higher ratio of acetate to propionate ($p<0.01$). The isobutyrate molar percentage on the ISP diet was several fold higher than the other two urea treatment diets. This observation is in agreement with the report that isobutyrate concentration increased linearly with increasing level of peptides in continuous culture (Jones et al., 1998). Isobutyrate is considered to be a product of valine catabolism during ruminal fermentation, so the lower concentration of isobutyrate with FGU or PCU diets is presumably a result of lower dietary valine content. The lower molar percentage of butyrate on PCU and FGU diets might be attributed to inter conversion between acetate and butyrate in the rumen (Sutton et al., 2003). Less acetate was used to produce butyrate with urea based supplementation in this study. The significance of valerate accumulation with the ISP diet in the present study was not clear, but the absolute values on all three diets were slightly higher than those noted by other researchers (Griswold et al., 2003) when urea was included in buffer solution in continuous culture.

Currently, Cherdthong et al. (2011a,b) found that supplementation of urea-calcium mixture at 6.7% of concentrate diet could improved NH_3-N release and VFA concentration in the rumen of dairy cows as well as beef cattle.

Effect of slow-release urea on rumen microbes and microbial protein synthesis

The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the small intestine, but also decreases N losses. Knowledge of the N compounds required for growth of ruminal bacteria is important in understanding the protein nutrition of ruminants and factors affecting ruminal fermentation, particularly fiber digestion. There is a long-held belief that cellulolytic ruminal bacteria use NH_3-N as their sole source of N. Some recently published results are not consistent with this conclusion, however. Bryant (1973), in summarizing the nutrient requirements of ruminal bacteria, concluded that cellulolytic bacteria used only NH_3 as an N source for growth. They were unable to grow on other N sources in the absence of NH_3 (Russell et al., 2009). The stimulation of cellulolytic species by precursors of various N sources also suggests a quantitative dependence on NH_3-N-release rate for optimum growth. Furthermore, there is experimental evidence that preformed slow-release NH_3-N stimulate microbial growth and increase fiber digestion.

Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein. The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by ruminal bacteria. Therefore, N metabolism in the rumen can be divided into 2 distinct events: protein degradation, which provides N sources for bacteria, and microbial protein synthesis (Russell and Sniffen, 1984). The NRC (2001) assumes that rumen-degradable protein (RDP) from NPN sources such as urea are as effective as RDP from true protein for microbial protein formation. Slow release urea that is more slowly hydrolyzed to NH_3-N than unprotected urea could potentially be used more efficiently by rumen microorganisms.

A recent study by Xin et al. (2010) who found that supplementation of feed grade urea (FGU) diet had the lowest microbial efficiency (11.3 g N/kg OMTD) and the isolated soy protein (ISP) diet (14.7 g N/kg OMTD) had the greatest ($p = 0.05$), with the polyurethane coated urea (PCU) diet (13.0 g N/kg OMTD) being intermediate. The higher microbial efficiency with the ISP diet might be explained by use of peptide or amino acid N to form true proteins to enhance microbial growth. However, according to NRC (2001), the microbial efficiency should be in the range of 12 to 54 g N/kg OMTD. The absolute values of microbial efficiency of all the diets in their study were slightly lower. This might reflect a limited N supply or lack of available N sources (peptide or amino acid) for ruminal microbial growth in the fermenters during incubation. Although all dietary treatments were under the same condition of limited N source which may constrain rumen microbial protein synthesis, the PCU diet had 15.6% greater microbial efficiency as compared to the FGU diet, which matched results of daily microbial N production. Moreover, the improving in rumen microbes when supplementation of urea-calcium mixture were also found in the currently study of Cherdthong et al. (2011a, b).

In contrast, Galo et al. (2003) reported that feeding polymer-coated urea (Optigen 1200 Controlled Release N; CPG Nutrients, Inc., Syracuse, NY) in dairy cows were not alter rumen microbial crude protein (MCP) production. NRC (2001) predicts MCP yields of 150 to 225 g MCP per kilogram of DOM with ruminal N balances of +20 and −20%, respectively. In a study by Timmermans et al. (2000), testing the effects of several dietary factors, MCP flow to the duodenum ranged from 765 to 1925 g/d, DMI ranged from 15.5 to 26 kg/d, and N intakes ranged from 428 to 832 g/d. Klusmeyer et al. (1990) fed cows two concentrations of N, 390 g/d (11% CP) and 500 g/d (14% CP) and found no changes in MCP flow from the rumen (2110 g MCP per day). Stokes et al. (1991) fed different levels of NSC and RDP to Holstein cows and found no differences in microbial efficiencies in terms of MCP/DOM; the average was 150 g MCP per kilogram of DOM. These authors did see a reduction (−700 g/d) in MCP flow from the rumen for cows eating a diet low in NSC (24%) and low in RDP (9%).

Effect of slow-release urea on milk production

Supplementation of slow-release urea to the diets of ruminants fed high levels of rapidly fermentable carbohydrates may improve the ability of microbial protein synthesis, these improving its efficiency of conversion into milk (Galo et al., 2003; Broderick et al., 2009) (Table 4). Previous study from Inostroza et al. (2010) who determine the effect of a controlled-release urea product (CRU; Optigen, Alltech Inc., Lexington, KY) on milk production in commercial Wisconsin dairy herd diets. Sixteen trial herds were randomly assigned to a treatment sequence, control to CRU to control, in a crossover design with two 30-d periods. The control diet for each herd was formulated by the herd nutritionist based on the level of milk production, and the CRU diets contained 114 g/d per cow of CRU, replacing an equivalent amount of supplemental CP, primarily from soybean meal. The results shown that milk yield was 0.5 kg/d per cow greater for CRU than for control. Similarly, Tikofsky and Harrison (2007) reported trends for increased milk yield when diets containing Optigen were fed to dairy cows. However, Galo et al. (2003) and dos Santos et al. (2008) reported that milk yield was unaffected when SBM was partially replaced by CRU and when uncoated prilled urea plus RUP sources were partially replaced by a polymer-coated prilled urea product, respectively. A greater yield of microbial N for CRU than for uncoated prilled urea in ruminal continuous culture has been reported (Chalupa, 2007; Tikofsky and Harrison, 2007; Harrison et al., 2008), which may partially explain their observed increase in milk yield. In addition, the filling of the diet formulation space created by the use of CRU with DM from either corn silage or corn grain may have improved the rumen-fermentable carbohydrate and energy status, thereby contributing to the response (NRC, 2001).

In some studies, Inostroza et al. (2010) reported that milk urea N (MUN) was greater for CRU than for control (13.2 vs. 12.4 mg/dL). These MUN values are within the normally expected range of 10 to 14 mg/dL (Wattiaux et al., 2005), and thus are probably not of consequence. An increase in MUN from 8.6 mg/dL for the control treatment to 9.8 mg/dL for the CRU treatment was reported by Broderick et al. (2009).

Table 4 Supplementation of slow-release urea product on milk production in dairy cows

Source	Type of SRU	Suppl., % diet	Animal	Milk, kg/d	Milk composition, %		
					Fat	Protein	Lactose
Galo et al. (2003)	Urea	0.3	Cows	35.6	3.8	3.1	-
	Optigen®	0.8		34.8	3.6	3.1	-
Golombeski et al. (2006)	Ruma Pro	0	Cows	26.1	4.2	3.7	4.8
	Ruma Pro	0.61		26.2	4.4	3.7	4.8
Inostroza et al. (2010)	Optigen®	0	Cows	35.4	3.7	3.0	-
	Optigen®	114*		35.9	3.7	3.0	-
Highstreet et al. (2010)	Urea	1.8	Cows	46.9	3.6	2.8	4.7
	Encapsulated urea	1.7		47.6	3.7	2.8	4.7
Xin et al. (2010)	Urea	0.6	Cows	32.5	3.7	2.9	5.1
	Polyurethane coated urea	0.6		34.5	4.0	3.2	5.0
Cherdthong et al. (2011a)	Urea-calcium mixture	6.7	Cows	13.4	4.2	3.3	4.7

*Fed 114 g of Optigen® per head per day.

Previous study from Xin et al. (2010) shown that *Butyrivibrio fibrisolvens* and *Ruminococus spp*. are two of the primary cellulose digesters with end product fermentation of succinate and acetate, respectively (Russell et a., 2009), reduced peak NH_3-N levels in cows fed the encapsulated urea diet may have shifted microbial species proportions in the rumen to change rumen volatile fatty acid (VFA) profiles and, if this resulted in increased acetate levels, it could have shifted fat synthesis from body to milk. In the absence of an increase in ruminal cellulose fermentation, suggested by similar whole tract aNDFom digestibility between treatments in cows at both stages of lactation, there is little likelihood that ruminal VFA production increased. This suggests that increased milk fat yield was due to a shift in the profile of VFA produced, perhaps due to a changed proportion of rumen cellulolytic microorganisms. Grummer et al. (1984) infused ammonium chloride to the rumen of dairy cows to increase the concentration of NH_3-N from 4.8 to 17.3 mg/dl. This also caused an increase in total VFA concentrations, as well as a decrease in the acetate to propionate ratio. Song and Kennelly (1989) infused ammonium chloride to the rumen to increase rumen NH_3-N concentrations and, while the total VFA concentration was not influenced by NH_3-Nconcentration, there were trends to decreased acetate and increased propionate proportions in rumen fluid with increasing NH_3 concentration, which resulted in a decreased acetate to propionate ratio. In a similarly designed study, Song and Kennelly (1990) infused varying levels of ammonium bicarbonate to the rumen of Holstein cows and also found no impact on ruminal degradation, but they did observe a proportional increase in mixed bacterial counts and total VFA concentrations. In addition, as the rumen NH_3-N levels increased, the acetate to propionate ratio decreased. Thus, under current study by Xin et al. (2010), found that increased milk fat synthesis in cows fed the encapsulated urea diet may have been due to lower rumen NH_3-N levels, at times of the day that they were the highest, that increased the acetate to propionate ratio in ruminally produced VFA. Recently, Cherdthong et al. (2011b) were also found that supplementation of urea-calcium mixture at 6.7% of concentrate diet enhanced milk yield and milk composition.

High-quality feed block (HQFB)

Supplementation of concentrate diet is not fashion on practical use for smallholder farmers especially Thai-native beef cattle farmer. This could be due to; 1) high price of concentrate, 2) complicate to feeding, 3) spend more time to manage 4) feeding of concentrate are quite suitable for dairy cows or commercial sector etc. High-quality feed block (HQFB) is one of strategic alternative feed block and easier feeding to ruminant when compared with concentrate diet. HQFB have been report to be beneficial to ruminants, especially with rice straw and other low quality roughages-based diets. Feed blocks using molasses and urea (NPN) have been used as strategic supplements for ruminants in the tropics. The urea-molasses block was reported to improve rumen efficiency (Krebs and Leng, 1984) and increase milk yield in lactating Murrah buffaloes receiving crop-residues and reduce the amount of concentrate supplement required (Kunju, 1986). High-quality feed blocks or pellets (HQFB/P) have been developed to contain local feed ingredients particularly those from different energy sources e.g. molasses, rice bran, cassava chip), NPN (urea), rumen by-pass protein (cottonseed meal, brewer's grain, chopped cassava hay) and essential minerals (S, Na, P). These have been used as strategic supplements, depending on amount and availability, as lick-block or as on-top supplementation (Wanapat et al., 1996, 1999).

Wanapat et al. (1996) found significant improvement in lactating Holstein Friesian crossbred cows receiving either urea-treated rice straw or grass. HQFB/P could enhance the utilization of basal roughage source and improve milk yield. HQFB was shown to reduce the need for high level concentrate supplementation in Holstein Friesian crossbreds in mid-lactation, therefore reducing feed costs (Wanapat et al., 1999). The work carried out in Vietnam by Vu et al. (1999) demonstrated the efficacy of supplementing with urea-molasses blocks (UMMB) in village-based dairy production. It was found that supplementing with UMMB or urea-treated rice straw in lactating cows, could significantly increase milk yield, milk fat (%) and most importantly improve reproductive efficiency in terms of length of estrus length, conception rate and calving interval. Similar results were obtained by Plaizier et al. (1999) in Tanzania. When dairy cows were supplemented with a urea-molasses block, milk yield and hay intake were significantly increased as was milk income. A participatory R&D was conducted involving 6 milking collection centers in the northeast of Thailand. Wanapat et al. (1999) reported that supplementation HQFB as a strategic supplement could be used efficiency as a means to increase milk yield and milk composition especially when cows are fed on low quality roughage with low level of concentrate. Feed intake of HQFB were ranged from 0.16 to 0.43 kg/d and tend to be highest when supplementation HQFB in dairy cow fed with high concentrate. It also increased roughage intake to help maintain normal fermentation and establish a more balanced rumen ecology, and most importantly it could provide a higher economical return to the farmers in the tropics where feeds are commonly scarce both in quantity and quality throughout the year.

Moreover, supplementation of malate level at 500 and 1,000 g and cassava hay in HQFB has been conducted by Khampa et al (2009) while the treatments were as follows: T1 = supplementation of high-quality feed block without cassava hay + malate at 500 g, T2 = supplementation of high-quality feed block without cassava hay + malate at 1,000 g, T3 = supplementation of high-quality feed block with cassava hay + malate at 500 g, T4 = supplementation of high-quality feed block with cassava hay + malate at 1,000 g, respectively. These results have revealed that combined use of cassava hay and malate at 1,000 g in high-quality feed block with concentrates containing high levels of cassava chip at 65% DM could highest improved rumen ecology and digestibility of nutrients in dairy heifers. Feed intake of those HQFB were 0.75 kg/d.

Earlier work by Foiklang et al. (2011) who investigated effect of various plant protein sources in HQFB on feed intake, rumen fermentation, and microbial population in swamp buffalo and were found that cassava hay, *P. calcaratus* hay, and mulberry hay are potential to be used as protein sources in HQFB especially cassava hay which can improve rumen fermentation efficiency by increasing total VFA and cellulolytic bacteria and remarkably decreased protozoal population. Feed intake of HQFB were ranged from 0.27 to 0.31 kg/d and tend to be highest when used cassava hay as protein sources in HQFB. Moreover, CP, NDF, and ADF digestibilities, ULRS, and nutrient intakes were significantly improved by cassava hay as protein sources in HQFB. HQFB are, therefore, recommended as lick-blocks for ruminants fed on low-quality roughages such as rice straw. Based on above studies, feed intake of HQFB were differed among experiments and the various ranged from 0.16 to 0.75 kg/d it could be due to the differences between feed resources and species of ruminants.

However, supplementation of UCM in HQFB for ruminants need to be investigated further in practical for Thai-native beef cattle.

Methodology

The two experiments were conducted and as follows;

Experiment I: Effects of different levels of urea-calcium mixture in HQFB on *in vitro* fermentation using a gas production technique

Experiment II: Effects of different levels of urea-calcium mixture in HQFB on rumen ecology, rumen microorganisms, microbial protein synthesis, and digestibility of nutrients of Thai-native beef cattle

Experiment I

Effects of different levels of urea-calcium mixture in HQFB on *in vitro* fermentation using a gas production technique

Materials and methods

Animals involved in this study were cared for according to the guidelines of the Khon Kaen University Animal Care and Use Committee. All standard procedures concerning animal care and management were taken throughout the entire period of the experiment.

Table 5 Ingredients and chemical compositions of high-quality feed block (HQFB) were used an *in vitro* experiment

	% of urea-calcium sulphate mixture (U-cas) in HQFB						
Items	0	3	6	9	12	15	18
Ingredients, %DM							
Rice bran	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Molasses	42.5	41.0	40.0	39.5	39.0	38.0	38.0
Urea	10.5	9.0	7.0	5.5	3.5	2.0	0.0
U-cas	0.0	3.0	6.0	9.0	12.0	15.0	18.0
Cement[a]	12.0	12.0	12.0	12.0	11.5	11.0	10.0
Sulfur	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Mineral premix	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Tallow	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition							
Dry matter, %	74.2	74.0	73.9	73.6	73.3	73.1	73.0
	-------------------------------% of DM--------------------------------------						
Crude protein	34.9	35.2	35.5	35.4	34.8	35.3	35.5
Crude ash	23.5	23.7	24.9	25.1	23.4	24.7	24.3
NDF	14.2	15.6	15.4	14.5	15.8	14.9	14.6
ADF	8.2	8.6	8.5	9.0	9.2	8.3	9.4

[a]Cement was the fine powdery form, provides calcium and was used as binder agent.

Diets and experimental design

Seven HQFB were formulated and the experimental design was a Completely randomized design (CRD). The dietary treatments were 7 levels of urea calcium sulphate mixtures (U-cas; 0, 3, 6, 9, 12, 15 and 18%) incorporated in HQFB. Rice straw and concentrate were used as substrate. U-cas products were prepared according to Cherdthong et al. (2011a) by, in brief, providing an aqueous solution (23 g $CaSO_4$ + 17 mL H_2O) of $CaSO_4$ at 50°C for 10 min and dissolving solid urea (60 g urea) in the aqueous $CaSO_4$, then heating and agitating the mixture at 50°C for 10 min prior to reducing the temperature of the solution to about 25°C. The proportions of ingredients in HQFB are reported in Table 5. All ingredients were mixed well together and then pressed into blocks of about 10 kg in a hydraulic compressive machine (Mineral Salt Block Hydraulic Press, Zhengzhou Rephale Machinery Company, He'nan, China) at 3 minute per block and left to sun-dry for 2 to 3 days to reduce moisture. The sample of HQFB, rice straw and concentrate were dried at 60ºC, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were chemically analysed (AOAC 1998) for dry matter (DM), crude ash and crude protein (CP). Acid detergent fiber (ADF) was determined according to an AOAC method (1998) and is expressed inclusive of residual ash. Neutral detergent fiber (NDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite. The proportions of ingredients in HQFB and nutrient contents of HQFB, concentrate and rice straw and chemical compositions of HQFB used in the *in vitro* gas production study are shown in Table 5.

Preparation of rumen inoculum

Two male, rumen-fistulated swamp buffaloes with an initial body weight of 350$\pm$ 50 kg were used as rumen fluid donors. Rumen fluid was collected from swamp buffaloes receiving concentrate (14% CP and 74% TDN) at 0.5% DM basis of BW in two equal portions, at 07.00 h and 16.00 h and rice straw ad *libitum*. The animals were kept in individual pens and clean fresh water and mineral blocks (Sirichok Company, Shupan Buri, Thailand) were offered as free choice. The mineral blocks contained mainly calcium, trace elements (Cu, Mn, Zn and Se) and few phosphorus and sodium. On day 20, 1000 mL rumen liquor were withdrawn from each animal before the morning meal using a 60-mL hand syringe. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory. The artificial saliva was prepared according to Menke and Steingass (1988), but the medium did not include a nitrogen source in the buffer. The artificial saliva and rumen fluid was mixed in a 2:1 ratio to prepare a mixed rumen inoculum. One hour before filling with 40 mL of the mixed rumen inoculums, the serum bottles with the respective substrates were pre-warmed in a water bath at 39 ºC.

In vitro *fermentation of substrates*

The 70:30 rice straw and concentrate ratio were used as substrates at 0.47 g with 0.03 g of respective HQFB and samples of 0.5 g were weighed into 50 mL serum bottles. For each treatment, five replications were prepared (five serum bottles per each U-cas treatment) and there were 35 sample bottles plus 5 blanks in total. The 40 bottles were incubated at various 13 incubation times. The amount of urea inclusion in HQFB and concentrate (substrates) were 14.0, 13.1, 11.9, 11.0, 9.8, 8.9 and 7.7 g/kg substrates for 0, 3, 6, 9 12, 15 and 18% of U-cas treatments, respectively. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39 ºC (96 h) for *in vitro* gas test. The bottles were gently shaken every 3 h. For each sampling time, five bottles containing only the rumen inoculums were included within each run and the mean gas production values of these bottles were used as blank. The blank values were subtracted from each measured value to give the net gas production. The 84 bottles [3 bottles/treatment x 7 treatments x 4 sampling times (0, 2, 4 and 6 h incubation)] were separately prepared for NH_3-N and volatile fatty acids (VFAs) analysis. Digestibility analysis was prepared with another set for 42 bottles [3 bottles/ treatment x 7 treatments x 2 sampling times (12 and 24 h incubation)].

Sample and analysis

During the incubation, data of gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer (American Sensor Technologies, Inc., New Jersey, USA) and a calibrated syringe (nSpire Health, Inc., Colorado, USA). To describe the dynamics of gas production over time the following Gompertz function (Schofield et al. 1994) was chosen:

$$GP = A \exp\left\{-\exp\left[\frac{1 + be\,(LAG - t)}{A}\right]\right\}$$

where *GP* is cumulative gas production (mL), *A* is the theoretical maximum of gas production, *b* is the maximum rate of gas production (mL/h) that occurs at the point of inflection of the curve, *LAG* is the lag time (h), which is defined as the time-axis intercept of a tangent line at the point of inflection, *t* is the incubation time (h) and *e* is the Euler constant. The parameters *A, b* and *LAG* were estimated by nonlinear regression analysis with weighted least squares means using the PROC NLIN (SAS 1998).

Inoculum ruminal fluid was sampled at 0, 2, 4 and 6 h post inoculations and then filtered through four layers of cheesecloth for NH_3-N and VFAs analysis. Samples were centrifuged at 16,000 *x g* for 15 min, and the supernatant was stored at -20ºC before NH_3-N analysis using the micro-Kjeldahl methods of AOAC (1998). The VFAs were analyzed using high pressure liquid chromatography (600E system with 484 UV detector attached with Nova-Pak C18 column, 3.9 mm × 300 mm, Waters; mobile phase: 10 mM H_2PO_4, pH 2.5) according to Samuel et al*.* (1997). *In vitro* digestibility was determined after termination of incubation at 12 and 24 h, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter digestibility (IVDMD). IVDMD (%) was calculated as follow: IVDMD = (((RS100– C) – (RB100 – C))/ WS) x 100, where RS100 is weight of the crucible and the residue after drying at 100ºC, RB100 is weight of the crucible and the chemical reagent residue after drying at 100ºC (blank), C is weight of the dried crucible and WS is weight of the sample (before incubation) on DM. The dried feed sample and residue left above was ashed at 550 ºC for 6 h and determination of *in vitro* organic matter digestibility (IVOMD) (Tilley & Terry 1963). At 48 h post inoculation a one bottle of each sample was determined *in vitro* true dry matter digestibility according to Van Soest et al. (1991). *In vitro* true dry matter digestibility (%) was calculated by the following equations: 100 – [(100-NDFD)*(NDF/100)], where NDF is neutral detergent fiber (% of DM) and NDFD is neutral detergent fiber digestibility (% of NDF).

The *in vitro* true dry matter digestibility was used to calculate microbial mass according to the method of Blümmel et al. (1997) and calculated as; Microbial mass (mg) = mg substrate truly degraded - (mL gas volume x 2.2).

Statistical analysis

All data from the experiment were statistically analyzed as a Completely randomized design using the GLM procedure of SAS (1998). Data were analyzed using the model:

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

where Y_{ij} is dependent variable; μ is the overall mean, M_i is effect of the level of U-cas (i=1-7), and ε_{ij} is the residual effect. Results are presented as mean values with the standard error of the means. Differences between mean control and U-cas supplementation group were determined by contrast. Differences among means with P<0.05 were accepted as representing statistically significant differences. Orthogonal polynomial contrast was used to examine their responses

Results and Discussion

Chemical composition of the diets

Table 6 showed the chemical compositions of HQFB, concentrate and rice straw. The concentrate diet and rice straw contained crude protein (CP) at 18.2 and 2.3% DM, respectively. While CP contents for HQFB products ranged from 34.8 to 35.5% and were similar to those reported by Wanapat et al. (1999) and Foiklang et al. (2011).

Cumulative gas and parameters of gas production

The cumulative gas production (96 h), and parameters of gas production estimated with the Gompertz function are presented in Table 7.The fermentation kinetics of feedstuffs can be determined from fermentative gas and the gas released from buffering of short chain fatty acids. Kinetics of gas production is dependent on the relative proportion of soluble, insoluble but degradable, and undegradable particles of the feed. In this experiment, maximum gas volume (*A*) were linearly increased with U-cas in HQFB (P<0.05) and was highest at 72.3 mL when supplementation 18% of U-cas in the HQFB while inclusion of only urea in HQFB was reduced in *A*. Similarly, the maximum rate of gas production (*b*) was highest (P<0.05) for 18% U-cas than other levels. The lag time (*LAG*) was not altered among the levels of U-cas (P>0.05).Under this study, improved performance of kinetics gas could be attributed by the slow release N source from U-cas. Thus, providing continuous NH_3-N for microbial protein synthesis and improving microbial activities in the rumen (Wanapat et al. 2009). These results were similar to our previous work reported by Cherdthong et al. (2011a), which supplemented U-cas with cassava chip as an energy source in concentrate diets, resulting in an increased gas production rate constant for the insoluble fraction and the potential extent of gas production value of the inoculums, as well as cumulative gas production.

In vitro *digestibility and microbial biomass*

As shown in Table 8, the IVDMD, IVOMD and true digestibility were altered by treatments (P<0.01) and were greatest at 18% of U-cas supplementation. Moreover, supplementation of 18% U-cas in HQFB resulted in the highest concentration of microbial biomass. This could possibly be that U-cas was more slowly hydrolyzed to NH_3 concentration than urea treatment, which was used more efficiently by rumen microorganisms, led to increase in *in vitro* digestibility (Galo et al. 2003; Cherdtong et al. 2011b). Furthermore, Cherdthong et al. (2011b) explained that the composition of the U–cas product contained sulfur to form $CaSO_4$ in which sulfur has long been recognized as an essential amino acids (methionine and cysteine) for ruminant microorganism growth. Thus, the continuous availability of N with sulfur for ruminal fermentation is important and could improve rumen microbial population as well as enhance *in vitro* digestibility. These results were in agreement with Cherdthong et al. (2011a), who reported that supplementation of urea calcium mixture product as a slow release NPN source in concentrate diet could improve digestibility and microbial mass in *in vitro* rumen fluid of cattle. Moreover, the digestibility of fiber and cellulolytic bacterial population (*Fibrobacter succinogenes*) were enhanced when dairy cows or beef cattle supplemented with U-cas (Cherdthong et al. 2011b).

In vitro *volatile fatty acids (VFAs) and NH_3-N*

The effect of levels of U-cas in HQFB on *in vitro* volatile fatty acids (VFAs) and NH_3-N production at 0, 2, 4 and 6 h of incubation is shown in Table 9. The mean values of total VFA, acetate and butyrate concentration were not different among treatments while propionate concentration and acetate to propionate concentration ratio were significantly different (P<0.05). Inclusion of U-cas in HQFB at 18% DM increased propionate concentration in the rumen fluid of swamp buffaloes. This could be higher values of IVDMD, IVOMD, *in vitro* true digestibilities in U-cas than urea fed group (Table 8). In addition, our previous study in dairy cows revealed that increasing propionate concentration could probably due to higher population of *F. succinogenes* in U-cas when compared with urea treatment (Cherdthong et al. 2011b). *F. succinogenes* is a major rumen cellulolytic species and produces succinate, formate, and CO_2 and the most of propionate in the rumen is produced by the decarboxylation of succinate to propionate and CO_2 (Wolin 1974).

NH_3–N concentration were rapidly increased in urea treatment while the concentrations of NH_3-N were quite stable throughout the sampling periods when supplementation of 18% U-cas in HQFB ($P<0.05$). This could be due to U-cas controling the rate of N degradation in the rumen and leading to a slow rate of NH_3-N released when compared with 0% of U-cas in HQFB. Similar to previous reports by Chanjula et al. (2003), Cherdthong et al. (2011a) who found that supplementation of urea as rapidly fermentable N source in the concentrate diet could increase the NH_3-N concentration in the rumen both *in vitro* and *in vivo* study. Cherdthong et al. (2011a) explained that slow NH_3-N formation in the rumen of U-cas is likely due to hydrogen bonding in U-cas between the sulphate from $CaSO_4$ and amino group in the urea compound. Sulphate anions are linked between layers of sulphate and chelated by urea groups. The urea molecules take part in hydrogen bonding as both donors and acceptors, as described by Gale et al. (2010). Water molecules are also included, and form an additional hydrogen bond with sulphate. One water molecule further forms hydrogen bonds to the urea CO group (Custelcean et al. 2007). In agreement with these observations, Cherdthong et al. (2011a,b) reported that supplementation of U-cas as a slow-release urea in concentrate diet reduces the rapidity of a NH_3 release in the rumen without affecting other ruminal fermentation parameters.

Conclusion

Based on the results of this experiment, it was confirmed that higher level of U-cas in HQFB does not adversely affect the *in vitro* fermentation. Supplementation of U-cas at 18% DM of HQFB improved *in vitro* kinetics of gas production, rumen fermentation, microbial mass and digestibility. Moreover, U-cas could control the rate of N degradation in the rumen and leading to a slow rate of NH_3-N released.

Table 6 Ingredient and chemical composition of concentrate and rice straw used in the experiment

Items	Concentrate	Rice straw
Ingredients, %DM		
Cassava chips	45.9	
Brewer's gain	13.7	
Rice bran	6.7	
Coconut meal	11.8	
Palm kernel meal	13.9	
Sulfur	0.5	
Mineral premix[a]	1.0	
Molasses	3.0	
Urea	3.0	
Salt	0.5	
Chemical composition		
Dry matter, %	93.1	97.0
	------------% of DM--------------	
Crude protein	18.2	2.3
Crude ash	8.7	13.3
Neutral detergent fiber	18.0	75.1
Acid detergent fiber	9.0	54.4

[a]Minerals premix (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

Table 7 The effect of levels of urea-calcium sulphate mixture (U-cas)in high-quality feed block (HQFB) on cumulative gas production (96 h), and parameters of gas production estimated with the Gompertz function

% of U-cas in HQFB	Parameters of Gompertz function[a]			Cumulative gas (mL) produced at 96 h
	A (mL)	*b* (mL/h)	*LAG* (h)	
0	63.2	1.8	2.9	65.7
3	66.1	2.1	2.6	68.4
6	66.3	2.0	3.0	68.4
9	67.6	2.1	2.8	69.7
12	70.3	2.2	3.2	73.4
15	70.1	2.2	3.0	73.0
18	72.3	2.4	3.1	75.7
SEM	0.4	0.1	0.9	1.1
Contrast				
Control *vs* U-cas	*	*	ns	*
Orthogonal polynomials				
Linear	*	*	ns	ns
Quadratic	ns	*	ns	*
Cubic	ns	ns	ns	ns

[a]*A* = the theoretical maximum of gas production of 0.5 g DM basis, *b* = the maximum rate of gas production, *LAG* = the lag time; SEM, standard error of the mean; *$p < 0.05$; ns,non- significant.

Table 8 The effect of levels of urea-calcium sulphate mixture (U-cas)in high-quality feed block (HQFB) on *in vitro* digestibility DM (IVDMD) and OM (IVOMD), *in vitro* true digestibility DM (IVTDMD) and microbial mass

% of U-cas in HQFB	*In vitro* digestibility, %				IVTDMD, %	Microbial mass, mg/0.5 g DM substrate
	IVDMD		IVOMD			
	12 h	24 h	12 h	24 h		
0	50.2	60.4	52.3	62.3	57.4	18.7
3	50.4	61.3	52.3	63.4	58.9	18.9
6	51.6	62.5	53.4	64.5	59.1	19.0
9	53.4	65.4	54.8	66.6	62.1	19.0
12	54.7	66.6	55.8	67.9	62.0	22.2
15	57.6	67.5	58.0	69.2	65.4	22.8
18	57.4	67.7	58.9	69.9	65.7	25.6
SEM	5.0	1.9	4.3	1.6	1.5	0.4
Contrast						
Control *vs* U-cas	ns	*	ns	*	**	*
Orthogonal polynomials						
Linear	ns	*	ns	*	**	*
Quadratic	ns	ns	ns	Ns	ns	*
Cubic	ns	ns	ns	Ns	ns	ns

IVDMD*, in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; Microbial mass (mg) mg substrate truly digested – (mL gas volume x 2.2) (Blümmel et al. 1997); SEM, standard error of the mean; **p* < 0.05; ***p* < 0.01; ns, non- significant.

Table 9 The effect of levels of urea-calcium sulphate mixture (U-cas)in high-quality feed block (HQFB) on *in vitro* volatile fatty acids (VFAs) and NH_3-N at different times of incubation

% of U-cas in HQFB	*In vitro* volatile fatty acids (VFA)						NH_3-N, mg/dl
	Incubation time, h	Total, mM	C2, %	C3, %	C4, %	C2:C3 ratio	
0	0	42.3	65.5	21.1	13.4	3.1	18.2
	2	45.7	68.4	19.4	12.2	3.5	24.4
	4	50.3	69.2	19.3	11.5	3.6	29.5
	6	52.3	70.9	18.0	11.1	3.9	27.7
	Mean	47.7	68.5	19.5	12.1	3.5	25.0
3	0	42.9	64.3	23.4	12.3	2.7	16.7
	2	47.7	65.6	22.3	12.1	2.9	21.2
	4	52.4	65.7	21.5	12.8	3.1	25.6
	6	55.2	66.2	20.8	13.0	3.2	24.1
	Mean	48.3	65.5	22.0	12.6	3.0	21.9
6	0	43.1	64.2	23.7	12.1	2.7	16.3
	2	47.9	66.7	20.8	12.5	3.2	22.8
	4	53.6	67.5	21.1	11.4	3.2	24.5
	6	55.6	66.7	22.4	10.9	3.0	23.4
	Mean	50.0	66.3	22.0	11.7	3.0	21.8
9	0	42.8	63.2	25.6	11.2	2.5	15.6
	2	48.7	66.8	21.1	12.1	3.2	20.5
	4	53.9	66.7	22.9	10.4	2.9	24.5
	6	55.7	68.6	20.5	10.9	3.3	22.0
	Mean	50.3	66.3	22.5	11.2	3.0	20.7
12	0	42.3	65.5	23.6	10.9	2.8	14.2
	2	48.9	65.7	23.1	11.2	2.8	18.9
	4	54.4	66.6	23.2	10.2	2.9	22.3
	6	56.8	68.5	21.7	9.8	3.2	21.1
	Mean	50.6	66.6	22.9	10.5	2.9	19.1
15	0	42.8	64.2	24.9	10.9	2.4	14.5
	2	48.8	64.4	24.2	11.4	2.7	18.1
	4	55.4	65.4	24.5	10.1	2.7	19.8
	6	56.9	65.6	24.2	10.2	2.7	17.2

Table 9 The effect of levels of urea-calcium sulphate mixture (U-cas)in high-quality feed block (HQFB) on *in vitro* volatile fatty acids (VFAs) and NH_3-N at different times of incubation (Cont.)

% of U-cas in HQFB	*In vitro* volatile fatty acids (VFA)						NH_3-N, mg/dl
	Incubation time, h	Total, mM	C2, %	C3, %	C4, %	C2:C3 ratio	
	Mean	51.0	64.7	24.3	10.7	2.6	17.4
18	0	43.0	63.7	26.6	9.7	2.4	13.3
	2	48.9	64.9	24.0	11.1	2.7	16.2
	4	56.8	65.5	24.7	9.8	2.7	18.1
	6	58.6	66.1	23.6	10.3	2.8	16.5
	Mean	51.8	65.1	24.7	10.2	2.6	16.0
SEM		5.5	2.7	0.9	3.3	0.3	1.5
Contrast							
Control *vs* U-cas		ns	ns	**	ns	*	*
Orthogonal polynomials							
Linear		ns	ns	**	ns	*	*
Quadratic		ns	ns	ns	ns	ns	ns
Cubic		ns	ns	ns	ns	*	ns

SEM, standard error of the mean; *$p < 0.05$; ns, non- significant; C2, acetate; C3, propionate; C4, butyrate.

Experiment II

Effects of different levels of urea-calcium mixture in HQFB on rumen ecology, rumen microorganisms, microbial protein synthesis, and digestibility of nutrients of Thai-native beef cattle

Materials and methods

Dietary treatments preparation

Rice straw and concentrate were obtained from the Ruminant Metabolism Center, Tropical Feed Resources Research and Development Center (TROFREC), Khon Kaen University, Thailand. Rice straw was a single-crop variety of *Oryza sativa indica*. The U-cas was prepared according to Cherdthong et al. (2011a) by, producing an aqueous solution of $CaSO_4$ (1.35 g/mL) and dissolved with 60 g urea in the aqueous $CaSO_4$ and then agitated the mixture at 50 °C for 10 min prior to reduce the temperature of the solution to about 25°C. All ingredients in the feed block (Table 11) were mixed together and then pressed into blocks of about 10 kg by hydraulic compression for 3 min per block then left to dry in the sun for 2 to 3 days or under open room with roof.

Animals, experimental design and feeding

Four, Thai native beef cattle with initial body weight (BW) of 100±3.0 kg were randomly assigned according to a 4×4 Latin square design to receive U-cas supplementation in feed blocks at 0, 120, 150 and 180 g/kg DM. A concentrate mixture (Table 10) was fed to animals at 5 g/kg of BW daily and offered in two equal meals per day at 7:00 and 16:00 hours. Rice straw was fed by allowing for refusals of 100 g/kg. All animals were kept in individual pens. Clean fresh water and feed blocks were available at all times. Individual intakes of rice straw, concentrate and feed blocks were recorded daily by weighing the offered and refused feeds. The experiment was conducted for 4 periods, lasting 21 days per each. The first 14 days were an adaptation period and last 7 days animals were moved to metabolism crates and fed the straw at 900 g/kg of the previous voluntary feed intake of straw. Concentrate was still offered at 5 g/kg of BW daily and feed blocks were available at all times during which animals were in metabolism crates.

Data collection and sampling procedures

Feed offered, refusals and fecal samples were collected during the last 7 days of each period at morning and afternoon feedings. The samples were firstly dried at 60°C and ground (1 mm screen using a Cyclotech Mill, Tecator, Sweden) and then analyzed using AOAC (1995) method for DM (ID 967.03), N (ID 984.13), EE (ID 954.02), ash (ID 942.05), and ADF (ID 973.18)**.** Neutral detergent fiber (aNDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite and results are expressed inclusive of residual ash. Metabolizable energy (ME) was calculated according to the equation described by Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×CP) + (3.9× EE) + (1.8×organic matter) ×*in vitro* organic matter digestibility (ivOMD))

where: CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM.

Digestible organic matter fermented in the rumen (DOMR) was calculated according to the equation described by ARC (1984) as:

DOMR (kg/d) = digestible organic matter intake (DOMI, kg/d) x 0.65

where: DOMI=[digestibility of organic matter (kg/kg DM) x organic matter intake (kg/d)]/100, 1 kg DOMI = 15.9 MJ ME/kg (Kearl, 1982).

Urine samples were analyzed for urinary N using the Kjeldahl procedure described by the AOAC (1995).

At the 21^{st} day of each period, jugular vein blood samples (10 ml) were collected at 0 h (before feeding) and 4 h after feeding for determination of hematological parameters and blood chemistry. All samples were taken using a 21-ga needle and the tubes containing 12 mg of EDTA as anticoagulant and plasma was separated by centrifugation at 500×*g* for 10 min at 4°C and stored at − 20°C until used.

Concentrations of albumin (Alb), plasma urea N (PUN), plasma glucose (PGlu), and non-esterified fatty acid (NEFA) were determined using a diagnostic kit (Albumin-HRII, L typeWako UN, Glucose-HRII Wako, and NEFA-HR; Tokyo, Japan). Plasma creatinine (PCre) was measured by the Roche Hitachi 912 Plus automatic analyzer (Indianapolis, IN). Total blood protein (BP) concentrations were determined by a refract meter (SPR-Ne; Atago Co., Tokyo, Japan). Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and γ-glutamyl transpeptidase (γ-GTP) were analyzed according to the standard methods established by Oguri et al. (2013).

The packed cell volume (PCV) was determined by microhaematocrit method (Igene and Iboh 2004). The hemoglobin (Hb) concentration was measured spectrophotometrically by the cyanmethemoglobin method using the SP6-500UV spectrophotometer (PYE, UNICAM, UK). The red blood cell (RBC) and white blood cell (WBC) counts were measured with the aid of Neubaur counter (haemocytometer) as reported by Oni et al. (2010). Differential leukocyte counts were analyzed by the ADVIA 120 hematology system (Tarrytown, NY). Platelets count was measured by the Roche Hitachi 912 Plus automatic analyzer (Indianapolis, IN). Mean corpuscular volume (MCV) were calculated from PCV, Hb and RBC values (Schalm et al. 1986).

At the end of each period, rumen fluid was collected at 0 and 4 h after feeding. Approximately 100 ml of rumen fluid was taken from middle part of the rumen by a stomach tube (12.7 mm i.d., 19 mm e.d.; Regular Plastic Stomach Tube, CDMV Inc., St-Hyacinthe, QC) connected to a vacuum pump (model DOA-P104-AA, GAST Manufacturing Inc., Benton Harbor, MI). Rumen fluid was immediately measured for pH and temperature using (Hanna Instruments HI 8424 microcomputer, Singapore) after withdrawal. Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided into 3 portions; first portion was used for NH_3-N analysis with 5 ml of 1 mol H_2SO_4 added to 45 ml of rumen fluid. The mixture was centrifuged at 16,000 × *g* for 15 min, and the supernatant was stored at − 20 °C before NH_3-N analysis using the Kjeltech Auto 1030 Analyzer. Volatile fatty acids (VFAs) were analyzed using high pressure liquid chromatography using the method of Samuel et al. (1997). A second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution. The total direct count of bacteria, protozoa, and fungal zoospores were made by the methods of Galyean (1989) based on the use of a hemocytometer (Boeco, Hamburg, Germany). The third was cultured for groups of bacteria using a roll-tube technique (Hungate 1969) for identifying bacteria groups (cellulolytic, proteolytic, amylolytic, and total viable count bacteria). Another portion was stored at − 20 °C for DNA extraction (Yu and Morrison 2004). Community DNA was extracted from 0.25 ml aliquots of each sample by the RBB+C method (Yu and Morrison, 2004), which was shown to substantially increase DNA yields. In total, 32 samples belonging to four treatments, four periods and two times of rumen fluid sampling (0, and 4 h post-feeding). The quality and quantity of these DNA samples were also determined by agarose gel electrophoresis and spectrophotometry. The primers used for the real-time PCR are as follows: primers for *Fibrobacter succinogenes*, Fs219f (5'GGT ATG GGA

TGA GCT TGC-3’) and Fs654r (5’-GCC TGC CCC TGA ACT ATC- 3’), were selected to allow amplification (446-bp product) of all 10 *F. succinogenes* strains deposited in GenBank. For *Ruminococcus albus* primers, Ra1281f (5’-CCC TAA AAG CAG TCT TAG TTC G-3’) and Ra1439r (5’ CCT CCT TGC GGT TAG AAC A- 3’) (175-bp product). *Ruminococcus flavefaciens* primers, Rf154f (5’-TCT GGA AAC GGA TGG TA-3’) and Rf425r (5’- CCT TTA AGA CAG GAG TTT ACA A-3’), were also selected to allow species–species amplification (295 bp) of all seven *R. flavefaciens* strains deposited in GenBank. All these primer sets were previously published by Koike and Kobayashi (2001).

Regular PCR conditions for *F. succinogenes* were as follows: 30 s at 94 °C for denaturing, 30 s at 60 °C for annealing and 30 s at 72 °C for extension (48 cycles), except for 9 min denaturation in the first cycle and 10 min extension in the last cycle. Amplification of 16S rRNA for the other two species was carried out similarly except an annealing temperature of 55 °C was used. Quantification of total bacteria population, primer and condition, was previously published by Kongmun et al. (2010). Four sample-derived standards were prepared from treatment pool set of community DNA. The regular PCR was used to generate sample-derived DNA standards for each real-time PCR assay. Then the PCR product was purified using a QIA quick PCR purification kit (QIAGEN, Inc., Valencia, CA) and quantified using a spectrophotometer. For each sample-derived standard, copy number concentration was calculated based on the length of the PCR product and the mass concentration. Tenfold serial dilution was made in Tri-EDTA prior to real-time PCR (Yu et al. 2005). In total, 4 real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the same as those of the regular PCR described above. Biotools QuantiMix EASY SYG KIT (B&M Labs, S. A., Spain) was used for real-time PCR amplification. All PCRs were performed in duplicate.

Statistical analysis

The rumen pH, temperature, excretion of urinary derivatives (PD), microbial crude protein and efficiency of microbial N synthesis data were analyzed using the MIXED procedure (SAS 1996) as a 4×4 Latin square design with 4 treatments, 4 animals and 4 periods, according to the following model: $Y_{ijk}=\mu+D_i+A_j+P_k+\gamma_{ijk}$, where: Y_{ijk}, observation from animal j, receiving diet i, in period k; μ, the overall mean, D_i, effect of the different level of U-cas (i=1, 2, 3, 4), A_j, the effect of animal (j=1, 2, 3, 4),

P_k, the effect of period (k=1, 2, 3, 4), and γ_{ijk} the residual effect. The LSMEANS option was used to generate individual diet means. Orthogonal polynomials for diet responses were determined by linear, quadratic, and cubic effects.

Ruminal microoganism measures were analyzed as repeated measures over time by using the MIXED procedure (SAS 1996), according to the following model: Y = $\mu + D_i + A_j + P_{ij} + H_k + (DH)_{jk} + \gamma_{ijk}$, where Y_{ijk}, observation from animal j, receiving diet i, in period k; μ, the overall mean, M_i, effect of the different level of U-cas (i=1, 2, 3, 4), A_j, the effect of animal (j=1, 2, 3, 4), P_k, the effect of period (k=1, 2, 3, 4), H_k is the effect of hour after feeding (k = 1 and 4); $(DH)_{jk}$ is the interaction of the different level of U-cas × hour after feeding, and γ_{ijk} the residual effect.

The best fitted covariance structure for bacteria, fungal zoospore, total bacteria, and *F. succinogenes* was the autoregressive. The unstructured covariance was used for ruminal protozoal concentration, whereas the antedependence structure was adopted for *R. flavefaciens* and *R. albus*. The LSMEANS option (SAS 1996) was used to generate individual diet means. Effects of diet, hour, and the interaction of diet × hour were defined by the **F**-test of ANOVA. The SLICE command (SAS 1996) was used to separate the significant interactions of diet × hour. Comparisons among diets within hour after feeding were performed by Tukey’s test. Orthogonal polynomials for diet responses were determined by linear, quadratic, and cubic effects.

Results and Discussion

Intake of rice straw, concentrate and feed blocks

Supplementation of feed blocks containing different levels of U-cas inclusion influenced on the intake of rice straw, total feed and energy in Thai native beef cattle (Table 12). Feed blocks intake by cattle in the present study was 0.3 kg/d and this was inicated that U-cas can replace urea in feed blocks for Thai native beef cattle without adverse intake effects. The feed blocks intakes in the present result were similar to Foiklang et al. (2011) who reported the range of fee block intake of swamp buffalo feed blocks ranged from 0.27 to 0.31 kg/d. Though the intake of feed blocks was not changed as a result of supplementation of U-cas; however, the feed blocks supplementation enhanced the intake of basal roughage significantly ($P<0.05$). Increased in DMI of rice straw by supplementation of U-cas in the feed blocks licks was due to the availability of limiting nutrients (sulfur from $CaSO_4$) and progressive change in rumen fermentation. Similar results to present study were also reported by Wanapat and Khampa (2006) and Foiklang et al. (2011) who found that supplementation of feed blocks could increase feed intake of urea-lime treated rice straw and total intakes while on change on feed blocks intakes were found among treatments. Moreover, steers consuming formulated molasses block; containing base ingredients of beet molasses, cane molasses, or concentrated separator by-product (Greenwood et al., 2000) and varying levels of urea and/or feed grade biuret as CP sources (Löest et al., 2001) increased intake of low-quality prairie hay. In contrast, Wu et al. (2005) revealed that the DM intake of roughages slightly decreased with urea-minerals lick block supplementation. Furthermore, it was reported that the use of cement as binding agent in feed block is necessary in order to solidify the blocks (Foiklang et al., 2011). In this study, supplementation of cement at 90-105 g/kg DM have not shown such adverse effects on intake of feed blocks and were similar to those previously reported by Hadjipanayiotou et al. (1993) who found that 100 g/kg of cement in the formula provided good blocks quality.

Apparent digestibility of nutrients (Table 12)

The improvement in nutrients digestibility in 180 g/kg of U-cas supplement in feed blocks group indicated the availability of more potentially N source with fermentable molasses for the proliferation of rumen microbes (Udén, 2006). Our results are in line with those obtained by Molina-Alcaide et al. (2010) who found that NDF digestibility of goats receiving feed blocks was higher than those without feed block supplement and this means that higher fiber digestibility could indicate higher energy availability. Increased CP digestibility in 180 g/kg of U-cas supplemented groups indicate sufficient supply of slowly release N and energy for the optimum growth of rumen microbes (Robinson, 2010; Calabrò et al., 2012; Cherdthong and Wanapat, 2013). In addition, molasses content in the feed blocks could influence on the readily available energy available for the microbes. Moreover, it could possibly be that NH_3 from U-cas was slowly released as compared to urea and could potentially be used more efficiently by rumen microorganisms (Galo et al., 2003). These results were in agreement with Cherdthong et al. (2011a), who reported that supplementation of U-cas product as a slow release NPN source in concentrate diet could improve digestibility and microbial mass in an *in vitro* experiment. Furthermore, the digestibility of aNDF and cellulolytic bacterial population were enhanced when dairy cows or beef cattle were fed with U-cas (Cherdthong et al. 2011b,c). In addition, a polymer-coated slow reduced urea was demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo et al., 2003).

Rumen fermentation

Ruminal pH and temperature generally were above 6.5 and 39.4 ^{O}C respectively during the 4 h post feeding and did not drop below 6.0 and 39 ^{O}C (Table 13). Similar results have been reported by Foiklang et al. (2011), when buffaloes were supplemented with the feed blocks. Cherdthong et al. (2011b-c) indicated that rumen fluid pH and temperature values were in range at 6.5 to 7.0 and 39.3 to 39.7 °C, respectively, and these ranges were considered as an optimal for microbial digestion of fiber and protein. Excessive N supply, a release of ruminal NH_3 that often exceeds its rate of incorporation into microbial protein, resulted in loss of a great part N as NH_3 absorbed from the rumen. Inclusion of U-cas in the feed blocks affected on ruminal NH_3–N concentrations and was slowly reduced, especially in 180 g/kg U-cas supplemented groups while NH_3–N concentrations tended to be higher in the urea when compared to U-cas groups. This could be explained by the effect of high slow release urea product in the feed blocks. Similarly, Cherdthong et al. (2011a-c) reported the finding in *in vitro* and *in vivo* that urea treatments rapidly increased the concentration of NH_3–N, but gradually increased in the U-cas treatments. Degree of U-cas protection, in term of NH_3 reduction when compared with urea at 2 h of fermentation, was reduced to 168 mg/dl in *in vitro* and 14.4 mg/dl in *in vivo* experiment (Cherdthong et al., 2011c). Similarly, Huntington et al. (2006) revealed that urea–calcium chloride product supplementation resulted in a lower concentrations of ruminal NH_3-N in the treatment that represented consumption of a CP equivalent to 220 to 460 g/d of CP in ruminants. In addition, Taylor-Edwards et al. (2009) reported that a slow release urea product reduced the rapidity of NH_3 production in the rumen without affecting other ruminal fermentation metabolites and it could be inferred that slow release urea diets could prolong microbial utilization of additional N sources during ruminal fermentation.

The concentration of total VFA was not changed by the feed blocks and the mean values ranged from 116.8-119.6 mmol/l and these were similar to those previously found by Foiklang et al. (2011) that total VFA concentrations in the rumen of buffalo fed with the feed blocks ranged from 102.2 to 116.0 mmol/l. Supplementation of U-cas in feed blocks enhanced the proportion of propionic acid while deceased acetic acid concentration. Similarly, Cherdthong et al. (2011a) reported that propionic acid concentrations were increased by U–cas supplementation in concentrate diet of dairy cows. This change might have helped to improve energy use for ruminant because propionic acid shows a positive relation with energy utilization efficiency. Thus, it means that higher propionic acid indicated a better energy yield while shifting in acetic: propionic acid and acetic plus butyric acid: propionic acid ratio explained better efficiency of energy use in 180 g/kg of U-cas. These findings agreed with Cherdthong et al. (2011a,b) who found higher propionic acid and thus a lower acetic: propionic acid ratio in the ruminal fluid of cows fed a high grain diet.

Purine derivatives and microbial crude protein

Excretion of urinary purine derivatives (PD), microbial crude protein (MCP) and efficiency of microbial N synthesis (EMNS) are presented in Table 14. Inclusion of U-cas in FB were altered concentration of allantoin and microbial protein in animals. Microbial crude protein yield (MCP) and EMNS were linearly increased when U-cas was included in FB at 180 g/kg DM ($p<0.05$). This increase in MCP in beef cattle fed the U-cas supplemented diet may have resulted from a slower rate of N release than urea and the better capture of these nutrients by rumen microbes (Infascelli et al. 2005; Südekum et al. 2006). Similarly, synchronization for rapid fermentation with highly degradable carbohydrate and N sources stimulated greater MCP when compared to diets with non synchronized N and energy release (Chanjula et al. 2003; Galina et al. 2003). Cherdthong et al. (2011b) reported that supplementation of U-cas in concentrate diet which containing a high level of cassava chip increased an efficiency of microbial protein synthesis from 12.9 to 18.2 g N/kg OM digested in the rumen of cattle. Therefore, in order to improve MCP, it seems that the manipulation of carbohydrate and N fermentation in the rumen should first be aimed at obtaining the most even ruminal carbohydrate supply pattern possible within a particular dietary regimen. The second goal is to supply the total daily amount of ruminally available N sufficient for use of the total amount of carbohydrate expected to be released in the rumen per day.

Ruminal microorganisms and predominant cellulolytic bacteria

The rumen of ruminant is a complex ecosystem in which diets consumed by the ruminant animal are digested by an active and diverse microorganism (Russell and Rychlik 2001; Simon and Igbasan 2002). Ruminal bacteria, protozoa and fungi degrade fibrous material, allowing ruminants to utilize plant fiber for nutrition (Koike and

Kobayashi 2001). The end products of these fermentations are volatile fatty acids (VFA) and MCP which are in turn used by the host. In the current study, it was found that viable population of protozoa was unaltered by dietary treatments (p>0.05) while bacteria and fungal zoospores population were changed by U-cas supplementation in FB (Table 15). Population of rumen bacterial increased (p<0.05) quadratically with FB inclusion of U-cas at 180 g/kg DM (7.2 x 10^{11} cell/ml), and protozoal population was linearly greatest (p<0.05) with the highest concentration of U-cas in FB (2.4 x 10^{4} cell/ml). An effect of hour after feeding (p<0.05) was observed, and there was no interaction of diet × hour. For 180 g/kg DM of U-cas in FB, rumen bacteria and protozoal population increased at 4 h after feeding. This observation can be explained by U-cas product is more slowly released in the rumen thus it may have provided to the continuous NH_3-N for microbial protein synthesis and improve microbial activities in the rumen (Russell and Rychlik 2001).

Bacteria in the rumen are considered more play important role than protozoa and fungal zoospores in determining the feed digestion and the production of microbial protein and VFA (Simon and Igbasan 2002; Stewart et al. 1998). Bacterial numbers in the rumen are very high (10^{10} to 10^{12} cell/ml of rumen fluid) and the complexity of ruminal bacteria was great (Russell and Rychlik 2001). Recent advances in molecular tools increasingly enable identification and characterization of the microbes in these bioreactors (Simon et al. 2005). Real time PCR technique has the ability to enumerate targeted cellulolytic bacteria with high sensitivity and has been used to analyze rumen digesta (Wanapat and Cherdthong 2009; Longo et al. 2013). This technique is both reliable and simple to perform (Koike and Kobayashi 2001). In this experiment, inclusion of U-cas in FB was linearly greatest (p<0.05) concentration means of total bacteria, whereas quadratic effects (p<0.05) were observed on *F. succinogenes* population with increasing U-cas concentration. Supplementation of 180 g/kg DM U-cas in FB were highly increased total bacteria and *F. succinogenes* at 8.2 x 10^{11} and 6.3 x 10^{9} copies/ml of rumen content, respectively. Interaction of the diet × hour after feeding on bacterial population were not differ (p>0.05) while effects of hour after feeding (p<0.05) were significantly observed with U-cas inclusion,. Possibly, U-cas in FB released NH_3-N more slowly than urea treatment, and can potentially be used more efficiently by rumen micro-organisms, especially incorporated with molasses as energy source in FB (Cherdthong et al. 2011ab). In addition, more available sulfur from $CaSO_4$ which consisted in U-cas could be an essential element for rumen bacterial growth and its metabolism is closely related to N metabolism. Thus, the continuous availability of N with sulfur for fermentation in the rumen is important and could enhance predominant cellulolytic bacterial population. Similar to those reports of Cherdthong et al. (2011ab) found supplementation of U-cas in concentrate mixture were greatest population of cellulolytic bacteria especially *F. succinogenes* in *in vitro* and *in vivo* study when compared with urea treatment. Furthermore, Koike and Kobayashi (2001) and Wanapat and Cherdthong (2009) confirmed that *F. succinogenes* was most dominant among the three species in ruminants, followed by *R. flavefaciens* and *R. albus*, respectively. However, this study *R. flavefaciens* and *R. albus* were not significantly different among treatments and concentrations were ranged from 8.3 to 8.7 x 10^8 and 1.3 to 1.4 x 10^8 copies/ml of rumen content, respectively.

N utilization

Moreover, N intake, fecal N excretion, urinary N excretion and N absorption were not altered by U-cas in FB (Table 16). N retention and proportion of N retention to N intake were significantly improved while total N excretion was reduced with the increasing level of U-cas in FB (P<0.05). Supplementation of U-cas at 180 g/kg DM in FB could reduce total N excretion to 4.1 g/d, and increased N retention and proportion of N retention to N intake up to 6.9 g/d and 14.9%, respectively. This could be explained that U-cas could control the rate of N degradation in the rumen and leaded to slow down the rates of total N excretion (Cherdthong et al. 2011b; Cherdthong et al. 2013). NH_3 is very volatile and disperses easily into the surrounding air, possibly acting as a pollutant of ground and surface water (Hünerberg et al. 2013). Thus, shifting total N excretion from the urine to the feces is recognized as a means of increasing the environmental stability of manure N (Hünerberg et al. 2013). As compared to the 0 g/kg U-cas in FB, the decrease in total N excretion was relatively to N retention observed in all three FB contained U-cas and this would likely reduce N losses in the form of NH_3, as direct and indirect N_2O emissions and leachate.

In consistency to Cherdthong et al. (2011b), it was reported that more positive N retention was obtained with the U-cas *versus* urea demonstrates the positive practical influence of U-cas with cassava chip based diets in a RS based feeding system. Galina et al. (2003) indicated that supplementation of slow release urea (SRU) with sugar cane tops could increase N retention from 36.11 g/d. In contrast, Taylor-Edwards et al. (2009) found that supplementation of SRU in steers did not affect on N retention and this could be due to the coating of SRU may hinder full release of urea and/or pass through the digestive tract.

Blood biochemistry

Feeding urea in FB resulted in greater PUN and total BP concentrations than those U-cas fed group (Table 17), and this clearly indicated that available N in excess of requirements was obtained. Higher PUN and total BP in urea fed group could be due to the result of NH_3 flux exceeding liver capacity for removal and this may also be the result of greater diffusion of NH_3 from the rumen wall directly into blood, thus bypassing the liver, especially at high ruminal NH_3 concentrations (Taylor-Edwards et al. 2009). Similarly, Kohn et al. (2005) reported that PUN and total BP are linearly related to total N excretion rate and this could be assumed that PUN and total BP concentration can be used to predict relative differences in total N excretion rate for animals of a similar stage of production within a study. The present data supports the general relationship between PUN concentration and total N excretion. Addition of U-cas at 180 g/kg DM in FB reduced total N excretion, concentration of PUN and total BP to 4.1 g/d, 3.7 mg/dl and 3.1 g/dl, respectively. Similar to previous study, supplementation of U-cas in concentrate diets could reduce PUN to 4.3 mg/dl in cows (Cherdthong et al. 2011b).

PGlu concentration at 4 h post feeding tended to increase when urea was added (85 mg/dl). The greater PGlu concentrations positively associated with NH_3 concentrations (Taylor-Edwards et al. 2009). This increase in PGlu concentrations that occurs within 4 h in response to PUN has been attributed at least partially to a reduction in glucose utilization rate or increased net hepatic glucose production or both, possibly because of an increased rate of hepatic glycogenolysis (Huntington et al. 2006). These results are in agreement with another experiment in which urea-calcium, a slow-release form of urea, prevented the marked increase in plasma glucose observed with dosing of urea treatment (Huntington et al. 2006). Moreover, Taylor-Edwards et al. (2009) found that supplementation of SRU could also decrease PGlu in steers and may diminish or abolish the aberrations in glucose homeostasis observed under conditions in which PUN concentrations are elevated. In our experiment confirmed that, concentration of PGlu was reduced 3.2 mg/dl at 4 h post feeding when 180 g/kg DM U-cas in FB was supplemented.

Plasma concentrations of Alb, PCre, GOT, γ-GTP, GPT and NEFA are indicators of liver function and elevated by liver disorders (Oguri et al. 2013). The obtained concentrations were not affected by treatment and were in the normal range as reported by Gupta et al. (2005) and Oguri et al. (2013), and this indicated that liver function was in normalwithout affected by the dietary treatments of U-cas. Therefore, supplementation of U-Cas at 180 g/kg DM in FB for cattle did not adversely affect on blood biochemistry parameters while concentration of PUN, PGlu and total BP were improved. However, effect of U-cas inclusion in the FB on BP and Alb was still unclear.

Hematological parameters

Table 18 presents the concentrations of PCV, RBC, Hb, MCV, WBC, lymphocyte, monocyte and platelet count. Hematological indices have been used to monitor and evaluate health and nutritional status of animals because they are correlated to nutritional status (Gupta et al. 2007). The assessment is normally done to determine the presence or prevalence of nutrient deficiencies and evaluate the efficacy of dietary supplementation or to compare available supplement (Gupta et al. 2005). Our current study, found that hematological parameters were not altered by U-cas supplementation (P>0.05). All U-Cas in FB did not change hematological variables in this study and remained within the normal range as compared to other reports (Gupta et al. 2005; Oguri et al. 2013). Thus, urea can be replaced by U-cas at 180 g/kg in FB without negatively affecting blood hematology.

Conclusions

In summary, inclusion of U-cas at 180 g/kg in the feed blocks resulted in improvement of feed intake, apparent nutrients digestibility and rumen fermentation rumen microorganism, microbial crude protein synthesis, predominant cellulolytic bacteria, improved N utilization, total BP and PGlu while it did not adversely affect on hematological parameters in Thai native beef cattle fed on rice straw. Based on this research it concluded that FB contained U-cas can be an effective strategic supplement for ruminant animals. However, the result should be repeated under farm conditions to show the effects of animal growth.

Table 10 Ingredient and chemical composition of concentrates and rice straw used in the experiment (g/kg dry matter (DM))

Ingredients, g/kg DM	Concentrate	Rice straw
Cassava chips	600	
Soybean meal (SBM), 440 g/kg CP solvent	190	
Rice bran	50	
Coconut meal, solvent	60	
Palm kernel meal, solvent	50	
Pure sulfur	10	
Mineral premix[a]	10	
Molasses, liquid	20	
Salt	10	
Chemical composition		
Dry matter, g/kg	962	980
Organic matter, g/kg DM	902	891
Ash, g/kg DM	98	109
aNeutral detergent fiber, g/kg DM	134	742
Acid detergent fiber, g/kg DM	79	534
Crude protein, g/kg DM	130	28
Metabolizable energy (ME)[b], MJ/kg DM	12	6

[a]Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

[b]Metabolizable energy (ME) was calculated according to the equation of Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×crude protein) + (3.9× ether extract) + (1.8×organic matter) ×*in vitro* organic matter digestibility)

Table 11 Ingredient and chemical composition of urea calcium sulphate mixture (U-cas) and feed block

	Supplementation of U-cas in FB, g/kg DM				U-cas
	0	120	150	180	
Ingredients, g/kg DM					
Rice bran	300	300	300	300	
Molasses, liquid	425	390	380	380	
Urea	105	35	20	-	
U-cas[a]	-	120	150	180	
Cement	110	105	100	90	
Pure sulfur	15	10	10	10	
Mineral premix[b]	15	10	10	10	
Salt	10	10	10	10	
Tallow	20	20	20	20	
Chemical composition					
Dry matter, g/kg	780	781	779	780	630
Organic matter, g/kg DM	700	701	703	704	820
Ether extract, g/kg DM	24	23	23	24	-
Ash, g/kg DM	300	299	297	296	180
aNeutral detergent fiber, g/kg DM	271	269	268	270	-
Acid detergent fiber, g/kg DM	211	213	212	211	-
Crude protein, g/kg DM	349	350	349	350	1690*
Metabolizable energy (ME)[c], MJ/kg DM	15.6	15.4	15.3	15.3	-

[a]U-cas was consisted an aqueous solution of $CaSO_4$ (1.35 g/mL) and 60 g urea.

[b]Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

[c]Metabolizable energy (ME) was calculated according to the equation of Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×crude protein) + (3.9× ether extract) + (1.8×organic matter)) ×*in vitro* organic matter digestibility)

where CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM.

*Urea N in U-cas is over-estimating CP.

Table 12 Influence of different levels of urea calcium sulphate mixture (U-cas) in feed block on feed intake, nutrient intake and apparent digestibility in Thai native beef cattle

	Supplementation of U-cas in FB, g/kg DM				SEM	P value
	0	120	150	180		
DM intake						
Rice straw						
kg/day	2.1[a]	2.1[a]	2.2[a]	2.5[b]	0.01	0.03
g/kg $BW^{0.75}$	75.4[a]	78.3[a]	82.1[ab]	94.8[b]	3.33	0.02
Concentrate						
kg/day	0.6	0.6	0.6	0.6	0.02	0.55
g/kg $BW^{0.75}$	21.3	23.2	23.2	24.2	4.32	0.65
Feed blocks						
kg/day	0.30	0.30	0.30	0.30	0.01	0.39
g/kg $BW^{0.75}$	10.8	11.2	11.2	11.4	2.98	0.58
Total intake						
kg/day	3.0	3.0	3.1	3.4	0.32	0.17
g/kg $BW^{0.75}$	107.4[a]	112.7[a]	116.5[ab]	130.3[b]	40.47	0.02
Nutrient intake, kg/d						
Dry matter	3.0	3.0	3.1	3.4	0.43	0.41
Organic matter	2.6	2.6	2.7	3.0	0.98	0.39
Crude protein	0.24	0.24	0.24	0.25	0.65	0.18
aNeutral detergent fiber	1.6	1.6	1.7	1.9	1.11	0.48
Acid detergent fiber	1.2	1.2	1.2	1.4	0.87	0.59
Estimated energy intake						
DOMI[c] , kg/d	1.8	1.8	1.9	2.2	0.27	0.67
DOMR[d], kg/d	1.2	1.2	1.3	1.4	0.13	0.38
ME, MJ/d	28.5[a]	28.5[a]	31.0[ab]	34.7[b]	1.51	0.02
ME, MJ/kg DM	9.6	9.6	10.0	10.0	0.56	0.45
Apparent digestibility, kg/ kg DM						
Dry matter	0.65[a]	0.65[a]	0.66[ab]	0.69[b]	0.012	0.03
Organic matter	0.69[a]	0.69[a]	0.72[ab]	0.73[b]	0.014	0.04
Crude protein	0.63[a]	0.64[a]	0.66[ab]	0.67[b]	0.012	0.02
aNeutral detergent fiber	0.54[a]	0.55[a]	0.61[b]	0.63[b]	0.011	0.03
Acid detergent fiber	0.43	0.42	0.44	0.44	0.096	0.08

[a,b]Means in the same row with different superscripts differ ($P<0.05$); [c]DOMI=Digestible organic matter intake; [d]DOMR=Digestible organic matter fermented in the rumen.

Table 13 Ruminal pH, rumen temperature and concentrations of rumen ammonia-N (NH_3-N) and volatile fatty acids (VFA), and of cattle fed different levels of urea calcium sulphate mixture (U-cas) in feed block

	Supplementation of U-cas in FB, g/kg DM				SEM	P value
	0	120	150	180		
Ruminal pH						
0 h post feeding	6.9	6.7	6.6	6.7	0.23	0.22
4 h post feeding	6.6	6.5	6.5	6.5	0.19	0.18
Changes (4 h–0 h)	-0.30	-0.20	-0.10	-0.20	0.09	0.08
Mean	6.8	6.6	6.6	6.6	0.51	0.37
Ruminal temperature, °C						
0 h post feeding	39.1	39.2	39.3	39.1	1.21	0.91
4 h post feeding	39.4	39.5	39.5	39.6	1.90	0.82
Changes (4 h–0 h)	0.30	0.30	0.20	0.50	0.23	0.29
Mean	39.3	39.4	39.4	39.4	1.54	0.46
NH_3-N, mg/dl						
0 h post feeding	16.2	16.8	15.9	16.1	0.98	0.19
4 h post feeding	25.9[a]	23.3[ab]	20.2[b]	19.7[b]	1.57	0.04
Changes (4 h–0 h)	9.7[a]	6.5[ab]	4.3[b]	3.6[b]	0.54	0.02
Mean	21.1[a]	20.1[ab]	18.1[b]	17.9[b]	1.00	0.03
Total VFA, mmol/l						
0 h post feeding	111.2	109.3	115.2	116.3	12.22	0.21
4 h post feeding	123.2	124.2	124.0	121.2	15.54	0.32
Mean	117.2	116.8	119.6	118.8	13.21	0.14
VFA, mol/ 100 mol						
Acetic acid						
0 h post feeding	70.1	71.2	70.1	69.3	7.87	0.89
4 h post feeding	72.7	73.4	72.0	71.1	9.76	0.55
Mean	71.4	72.3	71.1	70.2	8.23	0.24
Propionic acid						
0 h post feeding	18.6	17.9	19.9	19.8	0.74	0.22
4 h post feeding	18.5[a]	19.6[a]	21.4[ab]	23.1[b]	1.13	0.04

Table 13 Ruminal pH, rumen temperature and concentrations of rumen ammonia-N (NH_3-N) and volatile fatty acids (VFA), and of cattle fed different levels of urea calcium sulphate mixture (U-cas) in feed block (Cont.)

Mean	18.6[a]	18.8[a]	20.7[ab]	21.5[b]	0.81	0.03
Butyric acid						
0 h post feeding	11.3	10.9	10.0	10.9	1.21	0.10
4 h post feeding	8.8	7.0	6.6	5.8	1.08	0.49
Mean	10.1	9.0	8.3	8.4	1.19	0.77
Acetic: propionic acid ratio	3.8[a]	3.9[a]	3.4[b]	3.3[b]	0.05	0.02
Acetic plus butyric: propionic acid ratio	4.4[a]	4.3[a]	3.8[b]	3.7[b]	0.07	0.03

[a,b] Means in the same row with different superscripts differ ($P<0.05$)

Table 14 Excretion of urinary derivatives (PD), microbial crude protein and efficiency of microbial N synthesis as affect urea-calcium mixture (U-cas) in feed block (FB)

Items	Supplementation of U-cas in FB [g/kg DM]				SEM	Contrast		
	0	120	150	180		L	Q	C
PD [mmol/d]								
Allantoin excretion	133.1	133.5	145.3	155.6	3.32	0.01	0.09	0.07
Allantoin absorption	129.2	130.2	138.3	149.2	2.14	0.02	0.12	0.08
Microbial crude protein* [g/d]	336.5	340.1	390.8	421.1	10.32	0.01	0.08	0.06
EMNS† [g N/kg OMDR]	13.4	13.5	16.5	22.4	1.22	0.04	0.09	0.09

*Microbial crude protein (MCP) [g/d] =3.99 x 0.856 x mmoles of purine derivatives excreated (Cherdthong et al. 2011).

†Efficiency of microbial N synthesis (EMNS, g /kg of OM digested in the rumen (OMDR) = [(MCP [g/d] x 1000)/DOMR (g)], assuming that rumen digestion was 650 g/kg OM of digestion in total tract.

Table 15 Effect of urea-calcium mixture (U-cas) in feed block (FB) on total bacteria, population of *F. succinogenes*, *R. flavefaciens* and *R. albus* by using real-time PCR technique

Items	Supplementation of U-cas in FB [g/kg DM]				SEM	Contrast*				
	0	120	150	180		L	Q	C	H	DxH
	--copies/ml of rumen content--									
Total bacteria [x 10^{11}]	5.7	5.9	6.9	8.2	0.61	0.04	0.08	0.12	0.02	0.94
F. succinogenes [x 10^{9}]	3.8	3.9	5.0	6.3	0.55	0.12	0.03	0.18	0.01	0.44
R. flavefaciens [x 10^{8}]	8.3	8.7	8.6	8.6	1.43	0.39	0.48	0.43	0.22	0.87
R. albus [x 10^{8}]	1.3	1.3	1.3	1.4	0.33	0.11	0.20	0.15	0.33	0.54
Ruminal microbes [cell/ml]										
Bacteria [x $10^{11]}$	5.4	5.7	6.6	7.2	0.43	0.06	0.04	0.08	0.02	0.76
Protozoa [x 10^{6}]	4.5	4.7	4.2	4.4	0.68	0.43	0.55	0.19	0.33	0.44
Fungi [x 10^{4}]	1.4	1.4	1.6	2.4	0.19	0.03	0.12	0.08	0.03	0.54

*H = effect of hour after feeding; D × H = interaction of diet × hour after feeding.

Table 16 Effects of different levels of urea calcium sulphate mixture (U-cas) in feed block (FB) on feed intake and N utilization

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
N intake, g/d	38.5	39.2	39.6	41.3	2.23
Total N excretion, g/d	28.7[a]	27.8[a]	26.4[ab]	24.6[b]	1.02
Fecal excretion, g/d					
Output, kg/d	1.0	1.0	1.0	1.1	0.51
Total N, g/d	9.5	9.5	9.5	9.1	0.89
% N excretion	33.0	34.3	35.8	36.9	3.35
Urinary excretion					
Output, l/d	5.3	5.1	4.9	5.5	0.67
Total N, g/d	19.2	18.3	16.9	15.5	1.94
% N excretion	67.0	65.7	64.2	63.1	3.20
N absorption, g/d	29.1	29.6	30.2	32.2	2.12
N retention, g/d	9.8[a]	11.4[a]	13.2[ab]	16.7[b]	1.08
% of N retention to N intake	25.5[a]	29.0[a]	33.3[a]	40.4[b]	2.56

[a,b]Means in the same row with different superscripts differ (*P*<0.05).

Table 17 Effects of different levels of urea calcium sulphate mixture (U-cas) in feed block (FB) on blood biochemistry

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
H post feeding					
Plasma urea N, mg/dl					
0 h	11.2	10.7	11.6	11.1	1.14
4 h	20.6[a]	16.3[a]	14.3[ab]	13.2[b]	2.01
Mean	15.9[a]	13.5[ab]	13.0[ab]	12.2[b]	1.20
Plasma albumin, g/l					
0 h	30.9	31.5	32.1	30.2	3.54
4 h	40.4	37.1	39.4	39.1	4.33
Mean	35.7	34.3	35.8	34.7	3.96
Plasma creatinine, mg/dl					
0 h	0.2	0.4	0.1	0.2	0.09
4 h	2.9	2.1	2.6	2.7	1.32
Mean	1.6	1.3	1.4	1.5	1.02
Total blood proteins, g/dl					
0 h	6.4	6.8	6.1	4.2	0.98
4 h	9.9[a]	9.5[a]	6.7[ab]	6.0[b]	1.01
Mean	8.2[a]	8.2[a]	6.4[ab]	5.1[b]	0.91
Plasma glucose, mg/dl					
0 h	65.7	67.2	68.8	67.9	1.22
4 h	85.0[a]	82.8[ab]	82.3[ab]	80.8[b]	1.51
Mean	75.4	75.0	75.6	74.4	1.43
Glutamic oxaloacetic transaminase, IU/l					
0 h	62.8	63.5	65.4	64.5	13.30
4 h	91.8	89.7	86.9	85.8	18.77
Mean	77.3	76.6	76.2	75.2	15.56
Glutamate pyruvate transaminase, IU/l					
0 h	5.2	5.3	5.5	5.3	1.23
4 h	13.8	14.5	13.8	12.9	1.67
Mean	9.5	9.9	9.7	9.1	1.54
γ-glutamyl transpeptidase, IU/l					

Table 17 Effects of different levels of urea calcium sulphate mixture (U-cas) in feed block (FB) on blood biochemistry (Cont.)

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
0 h	12.1	12.6	13.0	12.7	1.98
4 h	18.7	17.5	18.0	17.6	2.12
Mean	15.4	15.1	15.5	15.2	2.01
Non-esterified fatty acid, mEq/l					
0 h	0.1	0.2	0.2	0.1	0.08
4 h	0.7	0.6	0.8	0.7	0.28
Mean	0.4	0.4	0.5	0.4	0.13

[a,b]Means in the same row with different superscripts differ (P<0.05).

Table 18 Effects of different levels of urea calcium sulphate mixture (U-cas) in feed block (FB) on hematological parameters

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
H post feeding					
Packed cell volume, %					
0 h	33.1	32.1	33.2	33.8	2.01
4 h	34.7	35.4	36.7	36.9	2.87
Mean	33.9	33.8	35.0	35.4	2.55
Red blood cell count, 10^{12}/l					
0 h	6.7	6.7	6.4	6.6	1.20
4 h	8.9	10.3	11.3	11.7	1.60
Mean	7.8	8.5	8.9	9.2	1.41
Hemoglobin, g/dl					
0 h	6.8	6.7	7.8	7.9	1.68
4 h	9.2	12.3	12.4	14.6	3.05
Mean	8.0	9.5	10.1	11.3	1.91
Mean corpuscular volume, 10^{-15}/l					
0 h	49.4	47.9	51.9	51.2	2.98
4 h	39.0	34.4	32.5	31.5	2.23
Mean	44.2	41.1	42.2	41.4	2.57
White blood cell count , 10^{9}/l					
0 h	8.1	7.9	7.8	8.2	1.52
4 h	14.5	15.4	16.5	15.7	1.98
Mean	11.3	11.7	12.2	12.0	1.77
Lymphocyte, 10^{9}/l					
0 h	5.4	5.1	6.5	6.1	1.22
4 h	8.6	8.7	8.7	9.0	1.54
Mean	7.0	6.9	7.6	7.6	1.34
Monocyte, 10^{9}/l					
0 h	0.2	0.1	0.3	0.2	0.07
4 h	0.6	0.6	0.7	0.8	0.09
Mean	0.4	0.4	0.5	0.5	0.08
Platelet count, 10^{9}/l					
0 h	387.1	380.1	378.9	386.9	4.44
4 h	430.2	443.6	445.8	447.8	7.54
Mean	408.7	411.9	412.4	417.4	5.05

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Appendices

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ จำนวน 4 เรื่อง ดังนี้

1) **Cherdthong, A.,** M. Wanapat, D. Rakwongrit, W. Khota, S. Khantharin, G. Tangmutthapattharakun, S. Kang, S. Foiklang, and K. Phesatcha. 2014. Supplementation effect with slow-release urea in feed blocks for Thai beef cattle-nitrogen utilization, blood biochemistry and hematology. **Tropical Animal Health and Production**. 46: 293-298. (Impact factor = 1.090).
2) **Cherdthong, A.,** and M. Wanapat. 2014. *In vitro* gas production in rumen fluid of buffalo as affected by urea-calcium mixture in high-quality feed block. **Animal Science Journal**. 85: 420-426. (Impact factor = 1.037).
3) **Cherdthong, A.,** and M. Wanapat. 2013. Rumen microbes and microbial protein synthesis in Thai native beef cattle fed with various feed block. **Archives of Animal Nutrition.** 67: 448-460. (Impact factor = 1.095).
4) **Cherdthong, A.,** M. Wanapat, W. Wongwungchun, S. Yeekeng, T. Niltho, D. Rakwongrit, W. Khota, S. Khantharin, G. Tangmutthapattharakun, K. Phesatcha and S. Kang. 2014. Effect of feeding feed blocks containing different levels of urea calcium sulphate mixture on feed intake, nutrients of digestibility and rumen fermentation in Thai native beef cattle fed on rice straw. **Animal Feed Science and Technology**. ***Revised#3***. (Impact factor = 1.608).

2. การนำผลงานวิจัยไปใช้ประโยชน์ ดังนี้

1) นำผลงานการวิจัยมาใช้ในการเรียนการสอนทั้งระดับปริญญาตรี และระดับบัณฑิตศึกษา เช่นรายวิชา Beef and Buffalo production, Tropical feed resources and feeding technology, Biochemistry in Nutritional Science เป็นต้น
2) สร้างนักวิจัยโดยผ่านกระบวนการฝึกจากผู้ช่วยวิจัย โดยเฉพาะอย่างยิ่งการคัดเลือกนักศึกษาระดับปริญญาตรีที่สนใจทำ Project ในเรื่องนี้ จากนั้นได้มีการฝึกฝนด้านการเก็บข้อมูล การวิเคราะห์ผลในห้องปฏิบัติการ อันทำให้นักศึกษามีใจรักในการเป็นนักวิจัย และสุดท้ายคือการเปิดโอกาสในนักศึกษาได้เข้ามาศึกษาต่อในระดับบัณฑิตศึกษาต่อไป
3) การนำผลงานการตีพิมพ์ไปใช้ประโยชน์ในการประกอบการขอกำหนดตำแหน่งทางวิชาการ

3. อื่น ๆ เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ ดังรายการต่อไปนี้

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REGULAR ARTICLE

Supplementation effect with slow-release urea in feed blocks for Thai beef cattle—nitrogen utilization, blood biochemistry, and hematology

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Abstract Four Thai male native beef cattle, initial body weight (BW) of 100±3.0 kg were randomly assigned in a 4×4 Latin square design to receive four dietary treatments with inclusion of urea calcium sulphate mixture (U-cas) in feed block (FB) at 0, 120, 150, and 180 g/kg dry matter (DM). Total intakes were increased with the increasing level of U-cas supplementation in FB and the result obtained the highest when supplementation of U-cas in FB at 180, followed by 150, 120, and 0 g/kg DM, respectively. Moreover, supplementation of U-cas in FB at 180 g/kg DM could reduce total N excretion (4.1 g/day), as compared to others treatments, while N retention and proportion of N retention to N intake were increased up to 6.9 g/day and 14.9 %, respectively. On the other hand, the blood biochemistry and hematological parameters were not different among treatments except concentration of plasma urea N, plasma glucose, and total blood protein were improved especially with U-cas supplementation at 180 g/kg DM in FB. In conclusion, supplementation of U-cas at 180 g/kg in FB improved feed intake, N utilization, and blood biochemistry in Thai native beef cattle fed on rice straw.

Keywords Block lick · Blood metabolite · Nitrogen utilization · Ruminant · Urea calcium mixture

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Introduction

Beef cattle are economically important domestic animals and have a long tradition in Thai agriculture. However, beef production in Thailand is often suboptimum, characterized by slow growth performance and low reproductive efficiency. This could be due to insufficient quantities of energy and protein during the dry season especially when cattle fed on rice straw (RS). Supplementation of feed blocks (FBs), a solidified mixture of unconventional feeds such as rice bran, molasses, binder, salt, mineral, and 10–15 g/kg dry matter (DM) of urea may help to overcome the situation and could improve the production performance of cattle (Foiklang et al. 2011). However, high amount of urea in the FB can be rapidly hydrolyzed upon entry into the rumen, hence resulting in peak rumen ammonia (NH_3) concentrations. NH_3 that is not utilized for microbial synthesis is absorbed across the gastrointestinal tract, with increasing ruminal NH_3 concentrations resulting in increased rate of absorption (Huntington et al. 2006). Increased blood NH_3 concentrations alter hepatic metabolism by increasing ureagenesis and may also affect glucose metabolism in the liver and peripheral tissues (Huntington et al. 2006; Taylor-Edwards et al. 2009). Blood examination is performed for screening procedure to monitor and evaluate health and nutritional status of animals (Aengwanich et al. 2009; Gupta et al. 2005). Hematological values such as total red blood cell count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and hemoglobin concentration, and white blood cell, i.e., lymphocyte and monocyte are indicated adaptability to adverse diet intake. It therefore becomes imperative to evaluate blood parameters of an organism particularly when unconventional feeds are fed to animals in order to determine the performance of the experimental animals as well as suitability of livestock (Garba and Abubakar 2010).

Slow urea release properties have been successful by using urea binding to calcium sulphate (U-cas) in order to control its release rate and improved N utilization as well as to enhanced blood metabolites in ruminants (Cherdthong et al. 2011b). Cherdthong et al. (2011a, b) reported that supplementation of U-cas in concentrate diet resulted in more efficiency than urea on N intake and N utilization. In earlier in vitro experiment, Cherdthong et al. (2013) reported that supplementation of U-cas at 180 g/kg DM in the FB resulted in improvement of in vitro NH_3-N utilization and digestibility of nutrients. Therefore, the objective of this study was to investigate the effect of U-cas supplementation in FB on N utilization, blood biochemistry, and hematology parameters in Thai native beef cattle when fed RS as a roughage source.

Materials and methods

Animals, experimental design, feed, and feeding

Four male Thai native beef cattle with initial body weight (BW) of 100±3.0 kg were randomly assigned in a 4×4 Latin square design to receive four treatments (inclusion of U-cas in FB at 0, 120, 150, and 180 g/kg DM, respectively). The U-cas was prepared according to Cherdthong et al. (2011a). The ingredients and chemical composition of FB and U-cas are reported in Table 1. All ingredients were mixed well together and then pressed into blocks using the procedure described by Foiklang et al. (2011). Concentrate (130 g/kg crude protein, CP; 12 MJ/kg DM metabolizable energy) was fed at 5 g/kg DM of BW daily. Concentrate consisted of cassava chips, soybean meal, rice bran, coconut meal, palm meal, sulfur, premix, molasses, and salt at 600, 190, 50, 60, 50, 10, 10, 10, and 10 g/kg DM, respectively. RS (28 g/kg CP; 6 MJ/kg DM metabolizable energy) was fed ad libitum allowing for 100 g/kg refusals. All animals were kept in individual pens, and clean fresh water and FB were available at all times. Individual FB intake was recorded daily by weighing the offered and refused quantities. The experiment lasted for four periods, and each lasted 21 days. During the first 14 days, all animals were fed their respective diets in the pens, while during the last 7 days, they were moved to metabolism crates for fecal and urine collection as the straws were fed by reducing 10 g/kg of voluntary straw intake of animals. This is to assure complete feed intake of animals when on the crates. The animals were allowed first 2 days for adjusting to the metabolism crates and then the samples of fecal, urine, and intake data were collected during the last 5 days. Moreover, all animals practically had an introductory phase for adjustment to the metabolism crate, which included 14 days before starting the experiment for the animal to become accustomed to being tethered.

Sample collection and sampling procedures

FB, concentrate, RS, refusals, and fecal samples were collected during the last 7 days of each period at the morning and afternoon feedings. The samples were analyzed using methods of AOAC (1995) for DM, CP, and ash. Acid detergent fiber and neutral detergent fiber were estimated according to Van Soest et al. (1991). Urine samples were analyzed for urinary N using the Kjeldahl procedure described by the AOAC (1995).

At the 21st day of each period, jugular vein blood samples (10 ml) were collected at 0 h (before feeding) and 4 h after feeding for determination of hematological parameters and blood chemistry. All samples were taken using a 21-ga needle, and the tubes containing 12 mg of EDTA as anticoagulant and plasma was separated by centrifugation at 500×g for 10 min at 4 °C and stored at −20 °C until used.

Concentrations of albumin (Alb), plasma urea N (PUN), plasma glucose (PGlu), and nonesterified fatty acid (NEFA) were determined using a diagnostic kit (Albumin-HRII, L type Wako UN, Glucose-HRII Wako, and NEFA-HR; Tokyo, Japan). Plasma creatinine (PCre) was measured by the Roche Hitachi 912 Plus automatic analyzer (Indianapolis, IN). Total blood protein (BP) concentrations were determined by a refract meter (SPR-Ne; Atago Co., Tokyo, Japan). Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and γ-glutamyl transpeptidase (γ-GTP) were analyzed according to the standard methods established by Oguri et al. (2013).

The packed cell volume (PCV) was determined by microhematocrit method (Igene and Iboh 2004). The hemoglobin (Hb) concentration was measured spectrophotometrically by the cyanmethemoglobin method using the SP6-500UV spectrophotometer (PYE, UNICAM, UK). The red blood cell (RBC) and white blood cell (WBC) counts were measured with the aid of Neubaur counter (hemocytometer) as reported by Oni et al. (2010). Differential leukocyte counts were analyzed by the ADVIA 120 hematology system (Tarrytown, NY). Platelet count was measured by the Roche Hitachi 912 Plus automatic analyzer (Indianapolis, IN). Mean corpuscular volume (MCV) was calculated from PCV, Hb, and RBC values (Schalm et al. 1986).

Statistical analysis

The data were analyzed as a 4×4 Latin square design using the GLM procedure of SAS (SAS Inc., Cary, NC, USA) using the model:

$$Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$$

where: Y_{ijk}, observation from animal j, receiving diet i, in period k; μ, the overall mean; M_i, effect of the different level

Table 1 Ingredient and chemical composition of urea calcium sulphate mixture and feed block

	Supplementation of U-cas in FB, g/kg DM				U-cas
	0	120	150	180	
Ingredients, g/kg DM					
Rice bran	300	300	300	300	
Molasses	425	390	380	380	
Urea	105	35	20	–	
U-cas	–	120	150	180	
Cement	110	105	100	90	
Sulfur	15	10	10	10	
Premix	15	10	10	10	
Salt	10	10	10	10	
Tallow	20	20	20	20	
Chemical composition					
Dry matter, g/kg	780	781	779	780	630
Organic matter	700	701	703	704	820
Ash	300	299	297	296	180
Neutral detergent fiber	271	269	268	270	–
Acid detergent fiber	211	213	212	211	–
Crude protein	349	350	349	350	1,690
Metabolizable energy, MJ/kg DM	15.6	15.4	15.3	15.3	–

U-cas urea calcium sulphate mixture, *FB* feed block

of U-cas (i=1, 2, 3, 4); A_j, the effect of animal (j=1, 2, 3, 4); P_k, the effect of period (k=1, 2, 3, 4); and ε_{ijk} the residual effect. Results are presented as mean values with the standard error of the means. Differences between treatment means were determined by Duncan's new multiple range test, and differences among means with $P<0.05$ were accepted as representing statistically significant differences.

Results and discussion

Feed intake, N metabolism, and utilization

Table 2 presents the total feed intake, N intake, total N excretion, and N utilization influenced by different levels of U-cas supplementation in FB. Total intakes were significantly different ($P<0.05$) among treatments, and the highest was found when cattle were supplemented with U-cas was at 180 g/kg DM, followed by 150, 120, and 0 g/kg DM, respectively. Increases in intake by supplementation of U-cas in FB licks probably was due to availability of essential nutrients and progressive changes in rumen fermentation and possibly resulted in an improvement of feed digestion; therefore, it could enhance the ruminant performance (Cherdthong et al. 2011b). Similarly, Liu et al. (1996) found that liveweight gains were higher in animals accessed to the block than in those without block. Moreover, N intake, fecal N excretion, urinary N excretion, and N absorption were not altered by U-cas in FB. N retention and proportion of N retention to N intake were significantly improved, while total N excretion was reduced with the increasing level of U-cas in FB ($P<0.05$). Supplementation of U-cas at 180 g/kg DM in FB could reduce total N excretion to 4.1 g/day and increased N retention and proportion of N retention to N intake up to 6.9 g/day and 14.9 %, respectively. This could be explained that U-cas could control the rate of N degradation in the rumen and leaded to slow down the rates of total N excretion (Cherdthong et al. 2011b; 2013). NH_3 is very volatile and disperses easily into the surrounding air, possibly acting as a pollutant of ground and surface water (Hünerberg et al. 2013). Thus, shifting total N excretion from the urine to the feces is recognized as a means of increasing the environmental stability of manure N (Hünerberg et al. 2013). As compared to the 0 g/kg U-cas in FB, the decrease in total N excretion was relative to N retention observed in all three FB contained U-cas, and this would likely reduce N losses in the form of NH_3, as direct and indirect N_2O emissions and leachate.

In consistency to Cherdthong et al. (2011b), it was reported that more positive N retention was obtained with the U-cas versus urea demonstrates the positive practical influence of U-cas with cassava chip based diets in an RS-based feeding system. Galina et al. (2003) indicated that supplementation of slow-release urea (SRU) with sugar cane tops could increase N retention from 36.11 g/day. In contrast, Taylor-Edwards et al. (2009) found that supplementation of SRU in steers did not affect on N retention, and this could be due to the

Table 2 Effects of different levels of urea calcium sulphate mixture in feed block on feed intake and N utilization

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
Total feed intake, g/kg $BW^{0.75}$	107.4a	112.7a	116.5ab	130.3b	7.47
N intake, g/day	38.5	39.2	39.6	41.3	2.23
Total N excretion, g/day	28.7a	27.8a	26.4ab	24.6b	1.02
Fecal excretion, g/day					
Output, kg/day	1.0	1.0	1.0	1.1	0.51
Total N, g/day	9.5	9.5	9.5	9.1	0.89
Percentage of N excretion	33.0	34.3	35.8	36.9	3.35
Urinary excretion					
Output, l/day	5.3	5.1	4.9	5.5	0.67
Total N, g/day	19.2	18.3	16.9	15.5	1.94
Percentage of N excretion	67.0	65.7	64.2	63.1	3.20
N absorption, g/day	29.1	29.6	30.2	32.2	2.12
N retention, g/day	9.8a	11.4a	13.2ab	16.7b	1.08
Percentage of N retention to N intake	25.5a	29.0a	33.3a	40.4b	2.56

Means in the same row with different letters differ ($P<0.05$)

U-cas urea calcium sulphate mixture, *FB* feed block

coating of SRU may hinder full release of urea and/or pass through the digestive tract.

Blood biochemistry

Feeding urea in FB resulted in greater PUN and total BP concentrations than those U-cas fed group (Table 3), and this clearly indicated that available N in excess of requirements was obtained. Higher PUN and total BP in urea fed group could be due to the result of NH_3 flux exceeding liver capacity for removal, and this may also be the result of greater diffusion of NH_3 from the rumen wall directly into blood, thus bypassing the liver, especially at high ruminal NH_3 concentrations (Taylor-Edwards et al. 2009). Similarly, Kohn et al. (2005) reported that PUN and total BP are linearly related to total N excretion rate, and this could be assumed that PUN and total BP concentration can be used to predict relative differences in total N excretion rate for animals of a similar stage of production within a study. The present data supports the general relationship between PUN concentration and total N excretion. Addition of U-cas at 180 g/kg DM in FB reduced total N excretion, concentration of PUN, and total BP to 4.1 g/day, 3.7 mg/dl, and 3.1 g/dl, respectively. Similar to previous study, supplementation of U-cas in concentrate diets could reduce PUN to 4.3 mg/dl in cows (Cherdthong et al. 2011b).

PGlu concentration at 4 h postfeeding tended to increase when urea was added (85 mg/dl). The greater PGlu concentrations positively associated with NH_3 concentrations (Taylor-Edwards et al. 2009). This increase in PGlu concentrations that occurs within 4 h in response to PUN has been attributed at least partially to a reduction in glucose utilization rate or increased net hepatic glucose production or both, possibly because of an increased rate of hepatic glycogenolysis (Huntington et al. 2006). These results are in agreement with another experiment in which urea–calcium, a slow-release form of urea, prevented the marked increase in plasma glucose observed with dosing of urea treatment (Huntington et al. 2006). Moreover, Taylor-Edwards et al. (2009) found that supplementation of SRU could also decrease PGlu in steers and may diminish or abolish the aberrations in glucose homeostasis observed under conditions in which PUN concentrations are elevated. Our experiment confirmed that concentration of PGlu was reduced 3.2 mg/dl at 4 h postfeeding when 180 g/kg DM U-cas in FB was supplemented.

Plasma concentrations of Alb, PCre, GOT, γ-GTP, GPT, and NEFA are indicators of liver function and elevated by liver disorders (Oguri et al. 2013). The obtained concentrations were not affected by treatment and were in the normal range as reported by Gupta et al. (2005) and Oguri et al. (2013), and this indicated that liver function was normal without being affected by the dietary treatments of U-cas. Therefore, supplementation of U-Cas at 180 g/kg DM in FB for cattle did not adversely affect on blood biochemistry parameters, while concentration of PUN, PGlu, and total BP were improved. However, effect of U-cas inclusion in the FB on BP and Alb was still unclear.

Hematological parameters

Table 4 presents the concentrations of PCV, RBC, Hb, MCV, WBC, lymphocyte, monocyte, and platelet count. Hematological indices have been used to monitor and evaluate health and nutritional status of animals because they are correlated to nutritional status (Gupta et al. 2005). The assessment is normally done to determine the presence or prevalence of nutrient deficiencies and evaluate the efficacy of dietary

Table 3 Effects of different levels of urea calcium sulphate mixture in feed block on blood biochemistry

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
H postfeeding					
Plasma urea N, mg/dl					
0 h	11.2	10.7	11.6	11.1	1.14
4 h	20.6a	16.3a	14.3ab	13.2b	2.01
Mean	15.9a	13.5ab	13.0ab	12.2b	1.20
Plasma albumin, g/l					
0 h	30.9	31.5	32.1	30.2	3.54
4 h	40.4	37.1	39.4	39.1	4.33
Mean	35.7	34.3	35.8	34.7	3.96
Plasma creatinine, mg/dl					
0 h	0.2	0.4	0.1	0.2	0.09
4 h	2.9	2.1	2.6	2.7	1.32
Mean	1.6	1.3	1.4	1.5	1.02
Total blood proteins, g/dl					
0 h	6.4	6.8	6.1	4.2	0.98
4 h	9.9a	9.5a	6.7ab	6.0b	1.01
Mean	8.2a	8.2a	6.4ab	5.1b	0.91
Plasma glucose, mg/dl					
0 h	65.7	67.2	68.8	67.9	1.22
4 h	85.0a	82.8ab	82.3ab	80.8b	1.51
Mean	75.4	75.0	75.6	74.4	1.43
Glutamic oxaloacetic transaminase, IU/l					
0 h	62.8	63.5	65.4	64.5	13.30
4 h	91.8	89.7	86.9	85.8	18.77
Mean	77.3	76.6	76.2	75.2	15.56
Glutamate pyruvate transaminase, IU/l					
0 h	5.2	5.3	5.5	5.3	1.23
4 h	13.8	14.5	13.8	12.9	1.67
Mean	9.5	9.9	9.7	9.1	1.54
γ-glutamyl transpeptidase, IU/l					
0 h	12.1	12.6	13.0	12.7	1.98
4 h	18.7	17.5	18.0	17.6	2.12
Mean	15.4	15.1	15.5	15.2	2.01
Nonesterified fatty acid, mEq/l					
0 h	0.1	0.2	0.2	0.1	0.08
4 h	0.7	0.6	0.8	0.7	0.28
Mean	0.4	0.4	0.5	0.4	0.13

Means in the same row with different letters differ ($P<0.05$)

U-cas urea calcium sulphate mixture, *FB* feed block

supplementation or to compare available supplement (Gupta et al. 2005). Our current study, found that hematological parameters were not altered by U-cas supplementation ($P>0.05$). All U-cas in FB did not change hematological variables in this study and remained within the normal range as compared to other reports (Gupta et al. 2005; Oguri et al. 2013).

Table 4 Effects of different levels of urea calcium sulphate mixture in feed block on hematological parameters

	Supplementation of U-cas in FB, g/kg DM				SEM
	0	120	150	180	
H postfeeding					
Packed cell volume, %					
0 h	33.1	32.1	33.2	33.8	2.01
4 h	34.7	35.4	36.7	36.9	2.87
Mean	33.9	33.8	35.0	35.4	2.55
Red blood cell count, 10^{12}/l					
0 h	6.7	6.7	6.4	6.6	1.20
4 h	8.9	10.3	11.3	11.7	1.60
Mean	7.8	8.5	8.9	9.2	1.41
Hemoglobin, g/dl					
0 h	6.8	6.7	7.8	7.9	1.68
4 h	9.2	12.3	12.4	14.6	3.05
Mean	8.0	9.5	10.1	11.3	1.91
Mean corpuscular volume, 10^{-15}/l					
0 h	49.4	47.9	51.9	51.2	2.98
4 h	39.0	34.4	32.5	31.5	2.23
Mean	44.2	41.1	42.2	41.4	2.57
White blood cell count , 10^{9}/l					
0 h	8.1	7.9	7.8	8.2	1.52
4 h	14.5	15.4	16.5	15.7	1.98
Mean	11.3	11.7	12.2	12.0	1.77
Lymphocyte, 10^{9}/l					
0 h	5.4	5.1	6.5	6.1	1.22
4 h	8.6	8.7	8.7	9.0	1.54
Mean	7.0	6.9	7.6	7.6	1.34
Monocyte, 10^{9}/l					
0 h	0.2	0.1	0.3	0.2	0.07
4 h	0.6	0.6	0.7	0.8	0.09
Mean	0.4	0.4	0.5	0.5	0.08
Platelet count, 10^{9}/l					
0 h	387.1	380.1	378.9	386.9	4.44
4 h	430.2	443.6	445.8	447.8	7.54
Mean	408.7	411.9	412.4	417.4	5.05

U-cas urea calcium sulphate mixture, *FB* feed block

Thus, urea can be replaced by U-cas at 180 g/kg in FB without negatively affecting blood hematology.

Conclusions

Supplementation of U-cas at 180 g/kg DM in FB improved N utilization, total BP, and PGlu, while it did not adversely affect on hematological parameters in Thai native beef cattle fed on RS.

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Conflict of interest None.

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ORIGINAL ARTICLE

In vitro gas production in rumen fluid of buffalo as affected by urea-calcium mixture in high-quality feed block

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ABSTRACT

This study aimed to determine the effect of urea-calcium sulphate mixture (U-cas) levels in high-quality feed block (HQFB) on ruminal digestibility, fermentation and gas kinetics in rumen fluid of swamp buffalo by using *in vitro* techniques. The treatments were seven levels of U-cas incorporated in HQFB at 0, 3, 6, 9, 12, 15 and 18% and the experimental design was a completely randomized design. Gas production rate constants for the insoluble fraction, potential extent of gas and cumulative gas were linearly increased with increasing levels of U-cas in HQFB. The *in vitro* dry matter digestibility, *in vitro* organic matter digestibility, true digestibility and microbial mass were altered by treatments and were greatest at 18% U-cas supplementation. Concentrations of propionate were linearly increased with increasing levels of U-cas and was highest with U-cas supplementation at 18%. The NH_3–N concentration was highest when urea was added in the HQFB while NH_3–N concentration tended to be reduced with increasing level of U-cas. The findings suggest supplementation of 18% U-cas in HQFB improves kinetics of gas production, rumen fermentation, digestibility and microbial mass as well as controlling the rate of N degradation in the rumen of swamp buffalo.

Key words: *feed block,* in vitro *gas production technique, ruminant, slow-release urea.*

INTRODUCTION

High-quality feed block (HQFB) has been used as strategic supplements for swamp buffaloes in the tropics, especially when fed with rice straw and other low-quality roughages-based diets (Wanapat *et al*. 1999; Foiklang *et al*. 2011). The HQFB may have provided additional and essential nutrients needed for swamp buffaloes. These enhancements were similar to those reported by Foiklang *et al*. (2011) who found that supplementation of HQFB as lick-blocks for swamp buffalo could improve feed intake, nutrient digestibility, rumen fermentation efficiency by increasing total volatile fatty acids (VFA), cellulolytic bacteria and remarkably decreased protozoal populations. Moreover, supplementation of lactating dairy cows' diets with HQFB increased milk yield and improved milk composition (Plaizier *et al*. 1999) and most importantly improved reproductive efficiency in terms of estrus length, conception rate and calving interval (Vu *et al*. 1999).

HQFB has been developed to contain local feed ingredients, particularly those from different energy sources (e.g. molasses, rice bran), essential minerals (S, Na, P) and non-protein nitrogen (NPN) source or urea. Use of urea is attractive in swamp buffalo diets because of its low cost, with high rumen digestibility (Wanapat 2009; Xin *et al*. 2010; Li *et al*. 2012). Urea is converted via ruminal NH_3 into microbial protein, thereby supplying additional microbial protein to the host (Nocek & Russell 1988; Ørskov 1994; Calsamiglia *et al*. 2010). However, the amount of urea that can be used in diets is limited due to its rapid hydrolysis to NH_3 in the rumen by microbial enzymes, resulting in its accumulation in the rumen and absorption through the rumen wall (Highstreet *et al*. 2010; Obitsu *et al*. 2011; Li *et al*. 2012).

Urea-calcium sulphate mixtures (U-cas), ruminal slow urea release properties, have been achieved by

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Table 1 Ingredients and chemical compositions of high-quality feed block (HQFB) used in the *in vitro* experiment

Items	% of urea-calcium sulphate mixture (U-cas) in HQFB						
	0	3	6	9	12	15	18
Ingredients, % dry matter							
Rice bran	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Molasses	42.5	41.0	40.0	39.5	39.0	38.0	38.0
Urea	10.5	9.0	7.0	5.5	3.5	2.0	0.0
U-cas	0.0	3.0	6.0	9.0	12.0	15.0	18.0
Cement†	12.0	12.0	12.0	12.0	11.5	11.0	10.0
Sulfur	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Mineral premix‡	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Tallow	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition							
Dry matter, %	74.2	74.0	73.9	73.6	73.3	73.1	73.0
				% of dry matter			
Crude protein	34.9	35.2	35.5	35.4	34.8	35.3	35.5
Crude ash	23.5	23.7	24.9	25.1	23.4	24.7	24.3
Neutral detergent fiber	14.2	15.6	15.4	14.5	15.8	14.9	14.6
Acid detergent fiber	8.2	8.6	8.5	9.0	9.2	8.3	9.4

†Cement was the fine powdery form, provides calcium and was used as the binding agent. ‡Minerals premix (each kg contains): vitamin A, 10 000 000 IU; vitamin E, 70 000 IU; vitamin D, 1 600 000 IU; Fe, 50 g; Zn, 40 g; Mn, 40 g; Co, 0.1 g; Cu, 10 g; Se, 0.1 g; I, 0.5 g.

using urea binding to substrates such as calcium sulphate to control its release rate (Cherdthong *et al*. 2011a). Cherdthong *et al*. (2011b) reported that supplementation of U-cas in the concentrate diets reduced ruminal NH_3 concentrations and improved feed intake, nutrient digestibility, cellulolytic bacterial populations, as well as milk yield in cattle.

Therefore, the aim of this study was to determine the effect of levels of U-cas in HQFB on ruminal digestibility of nutrients, fermentation end-products and kinetics of *in vitro* gas production by using rumen fluid from buffalo.

MATERIALS AND METHODS

Animals involved in this study were cared for according to the guidelines of the Khon Kaen University Animal Care and Use Committee. All standard procedures concerning animal care and management were taken throughout the entire period of the experiment.

Diets and experimental design

Seven HQFB were formulated and the experimental design was a completely randomized design (CRD). The dietary treatments were seven levels of U-cas mixtures (0, 3, 6, 9, 12, 15 and 18%) incorporated in HQFB. Rice straw and concentrate were used as substrates. U-cas products were prepared according to Cherdthong *et al*. (2011a) by, in brief, providing an aqueous solution (23 g $CaSO_4$ + 17 mL H_2O) of $CaSO_4$ at 50°C for 10 min and dissolving solid urea (60 g urea) in the aqueous $CaSO_4$, then heating and agitating the mixture at 50°C for 10 min prior to reducing the temperature of the solution to about 25°C. The proportions of ingredients in HQFB are reported in Table 1. All ingredients were mixed well together and then pressed into blocks of about 10 kg in a hydraulic compressive machine (Mineral Salt Block Hydraulic Press, Zhengzhou Rephale Machinery Company, He'nan, China) at 3 min per block and left to sun-dry for 2 to 3 days to reduce moisture. The sample of HQFB, rice straw and concentrate were dried at 60°C, then ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Höganäs, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were chemically analyzed (AOAC 1998) for dry matter (DM), crude ash and crude protein (CP). Acid detergent fiber (ADF) was determined according to an (AOAC 1998) method and is expressed inclusive of residual ash. Neutral detergent fiber (NDF) in samples was estimated according to Van Soest *et al*. (1991) with addition of α-amylase but without sodium sulphite. The proportions of ingredients in HQFB and nutrient contents of HQFB, concentrate and rice straw and chemical compositions of HQFB used in the *in vitro* gas production study are shown in Tables 1 and 2, respectively.

Preparation of rumen inoculum

Two male, rumen-fistulated swamp buffaloes with an initial body weight of 350 ± 50 kg were used as rumen fluid donors. Rumen fluid was collected from swamp buffaloes receiving concentrate (14% CP and 74% TDN) at 0.5% DM basis of body weight (BW) in two equal portions, at 07.00 and 16.00 hours and rice straw *ad libitum*. The animals were kept in individual pens and clean fresh water and mineral blocks (Sirichok Company, Shupan Buri, Thailand) were offered as free choice. The mineral blocks contained mainly calcium, trace elements (Cu, Mn, Zn and Se) and small amounts of phosphorus and sodium. On day 20, 1000 mL rumen liquor were withdrawn from each animal before the morning meal using a 60-mL hand syringe. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory. The artificial saliva was prepared according to Menke and Steingass (1988), but the medium did not include a nitrogen source in the buffer. The artificial saliva and rumen fluid was mixed in a 2:1 ratio to prepare a mixed rumen inoculum. One hour before filling with 40 mL of the mixed rumen inoculums, the serum bottles with the respective substrates were pre-warmed in a water bath at 39°C.

Table 2 Ingredient and chemical composition of concentrate and rice straw used in the experiment

Items	Concentrate	Rice straw
Ingredients, % dry matter		
Cassava chips	45.9	
Brewer's gain	13.7	
Rice bran	6.7	
Coconut meal	11.8	
Palm kernel meal	13.9	
Sulfur	0.5	
Mineral premix†	1.0	
Molasses	3.0	
Urea	3.0	
Salt	0.5	
Chemical composition		
Dry matter, %	93.1	97.0
	% of dry matter	
Crude protein	18.2	2.3
Crude ash	8.7	13.3
Neutral detergent fiber	18.0	75.1
Acid detergent fiber	9.0	54.4

†Minerals premix (each kg contains): vitamin A, 10 000 000 IU; vitamin E, 70 000 IU; vitamin D, 1 600 000 IU; Fe, 50 g; Zn, 40 g; Mn, 40 g; Co, 0.1 g; Cu, 10 g; Se, 0.1 g; I, 0.5 g.

In vitro fermentation of substrates

The 70:30 rice straw and concentrate ratio were used as substrates at 0.47 g with 0.03 g of respective HQFB and samples of 0.5 g were weighed into 50 mL serum bottles. For each treatment, five replications were prepared (five serum bottles per each U-cas treatment) and there were 35 sample bottles plus five blanks in total. The 40 bottles were incubated at 13 various incubation times. The amount of urea inclusion in HQFB and concentrate (substrates) were 14.0, 13.1, 11.9, 11.0, 9.8, 8.9 and 7.7 g/kg substrates for 0, 3, 6, 9 12, 15 and 18% of U-cas treatments, respectively. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39°C (96 h) for *in vitro* gas test. The bottles were gently shaken every 3 h. For each sampling time, five bottles containing only the rumen inoculums were included within each run and the mean gas production values of these bottles were used as blanks. The blank values were subtracted from each measured value to give the net gas production. The 84 bottles (3 bottles/treatment × 7 treatments x 4 sampling times (0, 2, 4 and 6 h incubation)) were separately prepared for NH_3-N and volatile fatty acids (VFAs) analysis. Digestibility analysis was prepared with another set for 42 bottles (3 bottles/ treatment × 7 treatments × 2 sampling times (12 and 24 h incubation)).

Sample and analysis

During the incubation, gas production data was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer (American Sensor Technologies, Inc., Mt Olive, NJ, USA) and a calibrated syringe (nSpire Health, Inc., Longmont, CO, USA). To describe the dynamics of gas production over time the following Gompertz function (Schofield *et al.* 1994) was chosen:

$$GP = A\exp\left\{-\exp\left[\frac{1+be\,(LAG-t)}{A}\right]\right\}$$

where *GP* is cumulative gas production (mL), *A* is the theoretical maximum of gas production, *b* is the maximum rate of gas production (mL/h) that occurs at the point of inflection of the curve, *LAG* is the lag time (h), which is defined as the time-axis intercept of a tangent line at the point of inflection, *t* is the incubation time (h) and *e* is the Euler constant. The parameters *A, b* and *LAG* were estimated by nonlinear regression analysis with weighted least squares means using the PROC NLIN (SAS 1998).

Inoculum ruminal fluid was sampled at 0, 2, 4 and 6 h post-inoculations and then filtered through four layers of cheesecloth for NH_3-N and VFA analyses. Samples were centrifuged at 16 000 × *g* for 15 min, and the supernatant was stored at −20°C before NH_3-N analysis using the micro-Kjeldahl methods of AOAC (1998). The VFAs were analyzed using high pressure liquid chromatography (600E system with 484 UV detector attached with Nova-Pak C18 column, 3.9 mm × 300 mm, Waters; mobile phase: 10 mmol/L H_2PO_4, pH 2.5) according to Samuel *et al.* (1997). *In vitro* digestibility was determined after termination of incubation at 12 and 24 h, when the contents were filtered through pre-weighed Gooch crucibles and residual DM was estimated. The percent loss in weight was determined and presented as *in vitro* DM digestibility (IVDMD). IVDMD (%) was calculated as follows: IVDMD = (((RS100– C) − (RB100 − C))/ WS) × 100, where RS100 is weight of the crucible and the residue after drying at 100°C, RB100 is weight of the crucible and the chemical reagent residue after drying at 100°C (blank), C is weight of the dried crucible and WS is weight of the sample (before incubation) on DM. The dried feed sample and residue left above was ashed at 550°C for 6 h for determination of *in vitro* organic matter digestibility (IVOMD) (Tilley & Terry 1963). At 48 h post-inoculation one bottle of each sample was determined *in vitro* true DM digestibility according to Van Soest *et al.* (1991). *In vitro* true DM digestibility (%) was calculated by the following equations: 100 – ((100-NDFD)*(NDF/100)), where NDF is neutral detergent fiber (% of DM) and NDFD is neutral detergent fiber digestibility (% of NDF).

The *in vitro* true DM digestibility was used to calculate microbial mass according to the method of Blümmel *et al.* (1997) and calculated as: microbial mass (mg) = mg substrate truly degraded − (mL gas volume × 2.2).

Statistical analysis

All data from the experiment were statistically analyzed as a CRD using the GLM procedure of SAS (1998). Data were analyzed using the model:

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

where Y_{ij} is dependent variable; μ is the overall mean, M_i is effect of the level of U-cas (i = 1–7), and ε_{ij} is the residual effect. Results are presented as mean values with the standard error of the means. Differences between mean control and U-cas supplementation group were determined by contrast. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. Orthogonal polynomial contrast was used to examine their responses.

RESULTS AND DISCUSSION

Chemical composition of the diets

Tables 1 and 2 showed the chemical compositions of HQFB, concentrate and rice straw. The concentrate

Table 3 The effect of levels of urea-calcium sulphate mixture (U-cas) in high-quality feed block (HQFB) on cumulative gas production (96 h), and parameters of gas production estimated with the Gompertz function

% of U-cas in HQFB	Parameters of Gompertz function†			Cumulative gas (mL) produced at 96 h
	A (mL)	*b* (mL/h)	*LAG* (h)	
0	63.2	1.8	2.9	65.7
3	66.1	2.1	2.6	68.4
6	66.3	2.0	3.0	68.4
9	67.6	2.1	2.8	69.7
12	70.3	2.2	3.2	73.4
15	70.1	2.2	3.0	73.0
18	72.3	2.4	3.1	75.7
SEM	0.4	0.1	0.9	1.1
Contrast				
Control vs. U-cas	*	*	ns	*
Orthogonal polynomials				
Linear	*	*	ns	ns
Quadratic	ns	*	ns	*
Cubic	ns	ns	ns	ns

*$P < 0.05$. †*A*, the theoretical maximum of gas production of 0.5 g dry matter basis; *b*, the maximum rate of gas production; *LAG*, the lag time; ns, non- significant; SEM, standard error of the mean.

diet and rice straw contained crude protein (CP) at 18.2 and 2.3% DM, respectively. While CP contents for HQFB products ranged from 34.8 to 35.5% and were similar to those reported by Wanapat *et al.* (1999) and Foiklang *et al.* (2011).

Cumulative gas and parameters of gas production

The cumulative gas production (96 h) and parameters of gas production estimated with the Gompertz function are presented in Table 3.The fermentation kinetics of feedstuffs can be determined from fermentative gas and the gas released from buffering of short chain fatty acids. Kinetics of gas production is dependent on the relative proportion of soluble, insoluble but degradable and undegradable particles of the feed. In this experiment, maximum gas volume (*A*) were linearly increased with U-cas in HQFB ($P < 0.05$) and was highest at 72.3 mL when supplemented with 18% of U-cas in the HQFB, while inclusion of only urea in HQFB was reduced in *A*. Similarly, the maximum rate of gas production (*b*) was highest ($P < 0.05$) for 18% U-cas than other levels. The lag time (*LAG*) was not altered among the levels of U-cas ($P > 0.05$). Under this study, improved performance of kinetics gas could be attributed by the slow release of N source from U-cas, thus providing continuous NH_3-N for microbial protein synthesis and improving microbial activities in the rumen (Wanapat 2009). These results were similar to our previous work reported by Cherdthong *et al.* (2011a), which supplemented U-cas with cassava chip as an energy source in concentrate diets, resulting in an increased gas production rate constant for the insoluble fraction and the potential extent of gas production value of the inoculums, as well as cumulative gas production.

In vitro digestibility and microbial biomass

As shown in Table 4, the IVDMD, IVOMD and true digestibility were altered by treatments ($P < 0.01$) and were greatest at 18% of U-cas supplementation. Moreover, supplementation of 18% U-cas in HQFB resulted in the highest concentration of microbial biomass. This could possibly be because U-cas was more slowly hydrolyzed to NH_3 concentration than urea treatment, which was used more efficiently by rumen microorganisms, leading to increase in *in vitro* digestibility (Galo *et al.* 2003; Cherdthong *et al.* 2011b). Furthermore, Cherdthong *et al.* (2011b) explained that the composition of the U–cas product contained sulfur to form $CaSO_4$ in which sulfur has long been recognized as essential amino acids (methionine and cysteine) for ruminant microorganism growth. Thus, the continuous availability of N with sulfur for ruminal fermentation is important and could improve rumen microbial populations as well as enhance *in vitro* digestibility. These results were in agreement with Cherdthong *et al.* (2011a), who reported that supplementation of urea-calcium mixture product as a slow release NPN source in concentrate diet could improve digestibility and microbial mass in *in vitro* rumen fluid of cattle. Moreover, the digestibility of fiber and cellulolytic bacterial population (*Fibrobacter succinogenes*) were enhanced when dairy cows or beef cattle were supplemented with U-cas (Cherdthong *et al.* 2011b).

In vitro VFAs and NH_3-N

The effect of levels of U-cas in HQFB on *in vitro* VFAs and NH_3-N production at 0, 2, 4 and 6 h of incubation is shown in Table 5. The mean values of total VFA, acetate and butyrate concentration were not different among treatments, while propionate concentration

Table 4 The effect of levels of urea-calcium sulphate mixture (U-cas) in high-quality feed block (HQFB) on *in vitro* digestibility dry matter (IVDMD) and organic matter (IVOMD), *in vitro* true digestibility dry matter (IVTDMD) and microbial mass

% of U-cas in HQFB	*In vitro* digestibility, %				IVTDMD, %	Microbial mass, mg/0.5 g dry matter substrate
	IVDMD		IVOMD			
	12 h	24 h	12 h	24 h		
0	50.2	60.4	52.3	62.3	57.4	18.7
3	50.4	61.3	52.3	63.4	58.9	18.9
6	51.6	62.5	53.4	64.5	59.1	19.0
9	53.4	65.4	54.8	66.6	62.1	19.0
12	54.7	66.6	55.8	67.9	62.0	22.2
15	57.6	67.5	58.0	69.2	65.4	22.8
18	57.4	67.7	58.9	69.9	65.7	25.6
SEM	5.0	1.9	4.3	1.6	1.5	0.4
Contrast						
Control *vs* U-cas	ns	*	ns	*	**	*
Orthogonal polynomials						
Linear	ns	*	ns	*	**	*
Quadratic	ns	ns	ns	Ns	ns	*
Cubic	ns	ns	ns	Ns	ns	ns

$^*P < 0.05$; $^{**}P < 0.01$. Microbial mass (mg) mg substrate truly digested – (mL gas volume × 2.2) (Blümmel *et al.* 1997); ns, non- significant; SEM, standard error of the mean.

and acetate to propionate concentration ratio were significantly different ($P < 0.05$). Inclusion of U-cas in HQFB at 18% DM increased propionate concentration in the rumen fluid of swamp buffaloes. This could be higher values of IVDMD, IVOMD, *in vitro* true digestibilities in U-cas than in the urea-fed group (Table 4). In addition, our previous study in dairy cows revealed that increasing propionate concentration could probably be due to higher populations of *F. succinogenes* in U-cas when compared with urea treatment (Cherdthong *et al.* 2011b). *F. succinogenes* is a major rumen cellulolytic species and produces succinate, formate and CO_2 and most propionate in the rumen is produced by the decarboxylation of succinate to propionate and CO_2 (Wolin 1974).

NH_3-N concentration was rapidly increased in urea treatment while the concentrations of NH_3-N were quite stable throughout the sampling periods when supplementation of 18% U-cas was made in HQFB ($P < 0.05$). This could be due to U-cas controlling the rate of N degradation in the rumen and leading to a slow rate of NH_3-N release when compared with 0% of U-cas in HQFB. Similar to previous reports by Chanjula *et al.* (2003) and Cherdthong *et al.* (2011a) who found that supplementation of urea as a rapidly fermentable N source in the concentrate diet could increase the NH_3-N concentration in the rumen both in *in vitro* and *in vivo* studies. Cherdthong *et al.* (2011a) explained that slow NH_3-N formation in the rumen of U-cas is likely due to hydrogen bonding in U-cas between the sulphate from $CaSO_4$ and amino group in the urea compound. Sulphate anions are linked between layers of sulphate and chelated by urea groups. The urea molecules take part in hydrogen bonding as both donors and acceptors, as described by Gale *et al.* (2010). Water molecules are also included, and form an additional hydrogen bond with sulphate. One water molecules further form hydrogen bonds to the urea CO group (Custelcean *et al.* 2007). In agreement with these observations, Cherdthong *et al.* (2011a, b) reported that supplementation of U-cas as a slow-release urea in concentrate diet reduces the rapidity of a NH_3 release in the rumen without affecting other ruminal fermentation parameters.

Conclusion

Based on the results of this experiment, it was confirmed that higher levels of U-cas in HQFB do not adversely affect *in vitro* fermentation. Supplementation of U-cas at 18% DM of HQFB improved *in vitro* kinetics of gas production, rumen fermentation, microbial mass and digestibility. Moreover, U-cas could control the rate of N degradation in the rumen and led to a slow rate of NH_3-N release.

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Table 5 The effect of levels of urea-calcium sulphate mixture (U-cas)in high-quality feed block (HQFB) on *in vitro* volatile fatty acids (VFAs) and NH_3-N at different times of incubation

% of U-cas in HQFB	*In vitro* VFA						NH_3-N, mg/dL
	Incubation time, h	Total mmol/L	C2,%	C3,%	C4,%	C2:C3 ratio	
0	0	42.3	65.5	21.1	13.4	3.1	18.2
	2	45.7	68.4	19.4	12.2	3.5	24.4
	4	50.3	69.2	19.3	11.5	3.6	29.5
	6	52.3	70.9	18.0	11.1	3.9	27.7
	Mean	47.7	68.5	19.5	12.1	3.5	25.0
3	0	42.9	64.3	23.4	12.3	2.7	16.7
	2	47.7	65.6	22.3	12.1	2.9	21.2
	4	52.4	65.7	21.5	12.8	3.1	25.6
	6	55.2	66.2	20.8	13.0	3.2	24.1
	Mean	48.3	65.5	22.0	12.6	3.0	21.9
6	0	43.1	64.2	23.7	12.1	2.7	16.3
	2	47.9	66.7	20.8	12.5	3.2	22.8
	4	53.6	67.5	21.1	11.4	3.2	24.5
	6	55.6	66.7	22.4	10.9	3.0	23.4
	Mean	50.0	66.3	22.0	11.7	3.0	21.8
9	0	42.8	63.2	25.6	11.2	2.5	15.6
	2	48.7	66.8	21.1	12.1	3.2	20.5
	4	53.9	66.7	22.9	10.4	2.9	24.5
	6	55.7	68.6	20.5	10.9	3.3	22.0
	Mean	50.3	66.3	22.5	11.2	3.0	20.7
12	0	42.3	65.5	23.6	10.9	2.8	14.2
	2	48.9	65.7	23.1	11.2	2.8	18.9
	4	54.4	66.6	23.2	10.2	2.9	22.3
	6	56.8	68.5	21.7	9.8	3.2	21.1
	Mean	50.6	66.6	22.9	10.5	2.9	19.1
15	0	42.8	64.2	24.9	10.9	2.4	14.5
	2	48.8	64.4	24.2	11.4	2.7	18.1
	4	55.4	65.4	24.5	10.1	2.7	19.8
	6	56.9	65.6	24.2	10.2	2.7	17.2
	Mean	51.0	64.7	24.3	10.7	2.6	17.4
18	0	43.0	63.7	26.6	9.7	2.4	13.3
	2	48.9	64.9	24.0	11.1	2.7	16.2
	4	56.8	65.5	24.7	9.8	2.7	18.1
	6	58.6	66.1	23.6	10.3	2.8	16.5
	Mean	51.8	65.1	24.7	10.2	2.6	16.0
SEM		5.5	2.7	0.9	3.3	0.3	1.5
Contrast							
Control vs. U-cas		ns	ns	**	ns	*	*
			Orthogonal polynomials				
Linear		ns	ns	**	ns	*	*
Quadratic		ns	ns	ns	ns	ns	ns
Cubic		ns	ns	ns	ns	*	ns

$^*P < 0.05$, $^{**}P < 0.01$. C2, acetate; C3, propionate; C4, butyrate; ns, non- significant; SEM, standard error of the mean.

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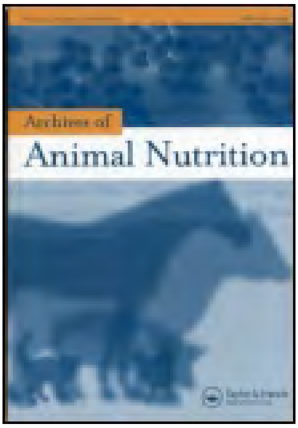

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Rumen microbes and microbial protein synthesis in Thai native beef cattle fed with feed blocks supplemented with a urea-calcium sulphate mixture

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Rumen microbes and microbial protein synthesis in Thai native beef cattle fed with feed blocks supplemented with a urea–calcium sulphate mixture

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The influence of slow-release urea (urea–calcium sulphate mixture; U–CaS) in feed blocks on rumen micro-organisms, predominant cellulolytic bacteria, microbial protein synthesis and ecology was studied in Thai native beef cattle. Four animals with an initial body weight of 100 ± 3.0 kg were randomly assigned to a 4 × 4 Latin square design with four dietary treatments (U–CaS in iso-nitrogen feed blocks at 0, 120, 150 and 180 g/kg dry matter (DM), respectively; U–CaS replaced urea). After 21 days of experimental feeding, rumen fluid was collected at 0 and 4 h after feeding. The mean intake of feed blocks and other feedstuffs offered (rice straw and concentrates) amounted to 0.3, 2.3 and 0.6 kg DM/day, respectively. Inclusion of U–CaS did not altered pH and temperature in the rumen. However, ruminal NH_3–N concentration decreased quadratically ($p < 0.05$) in response to U–CaS inclusion, with the lowest value at 180 g U–CaS per kg feed block. With inclusion of U–CaS, the populations of rumen bacteria increased quadratically ($p < 0.05$) and counts of fungal zoospores were linearly enhanced ($p < 0.05$), being highest at 180 g U–CaS per kg feed block. Supplementation of U–CaS increased the concentration of total bacteria linearly ($p < 0.05$) and of *Fibrobacter succinogenes* quadratically ($p < 0.05$), whereas *Ruminococcus flavefaciens* and *Ruminococcus albus* were not affected by dietary treatments. Microbial crude protein yield and efficiency of microbial nitrogen (N) synthesis were linearly increased with different levels of U–CaS addition. Furthermore, current data clearly indicate that inclusion of U–CaS in feed blocks can affect micro-organism diversity and major cellulolytic bacteria.

Keywords: cattle; feed blocks; microbial proteins; non-protein nitrogen; rumen bacteria; urea

1. Introduction

The complex symbiotic micro-organisms of the rumen are responsible for the breakdown of fibre which commonly occurs (Wanapat and Cherdthong 2009; Longo et al. 2013). These micro-organisms are highly responsive to changes in feed, age and the health of the host animal, which varies according to geographical location, season and feeding regimen (Hungate 1966). Anaerobic rumen fibrolytic bacteria, protozoa and fungi degrade fibrous material, allowing ruminants to utilise plant fibre for nutrition. Thai native beef cattle are one of the most economically important domestic animals and have a long tradition in Thai agriculture. However, beef cattle production is often suboptimal, characterised by slow growth and low reproductive efficiency. This can be due to insufficient energy and protein intake during dry season especially when cattle fed on rice straw, leading to a low

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microbial protein synthesis in the rumen. Moreover, the feed quality has been identified as one of the most important factors limiting animal productivity in tropical environments (McSweeney and Denman 2007).

A slow release of urea has been achieved by using urea linked to calcium sulphate (urea–calcium sulphate mixture; U–CaS) to control its release rate. Regarding microbial protein synthesis and diversity of rumen micro-organisms, Cherdthong et al. (2011a, 2011b) reported that a supplementation of U–CaS in concentrates was more efficient than urea supplementation. However, no information has been reported on inclusion of U–CaS in feed blocks for cattle. Therefore, the objective of this experiment was to study the effects of various feed blocks on feed intake, rumen ecology, diversity of rumen micro-organisms and predominant cellulolytic bacteria in Thai native beef cattle.

2. Materials and methods

2.1. Animals and experimental design

The experiment was carried out and proved according to the guidelines of the Khon Kaen University Animal Care and Use Committee. Four Thai native beef cattle, males about 1-year-old with an initial body weight (BW) of 100 ± 3.0 kg were used. The experiment was randomly assigned to a 4 × 4 Latin square design. The animals were placed in individual pens (concrete floor) and had free access to water and feed blocks. The dietary treatments differed in the inclusion level of U–CaS in feed blocks, which amounted to 0, 120, 150 and 180 g/kg DM, respectively. Concentrates were fed daily at 5 g/kg BW and were offered in two equal meals per day at 07:00 and 16:00 h. The experiment consisted of four periods of 21 days each. During the last 7 days of each period, the animals were moved to metabolism crates for total urine collection. The cattle was fed their respective diets *ad libitum*, which was achieved by offering the diets in excess of expected consumption and refusals of 100 g/day were considered as adequate for maximum feed intake in this study. Chemical composition of the concentrates, rice straw, feed blocks and U–CaS are presented in Tables 1 and 2.

2.2. Preparation of the U–CaS and the feed blocks

The U–CaS was prepared according to Cherdthong et al. (2011a). In brief, this mixture was produced by an aqueous solution of $CaSO_4$ (23 g $CaSO_4$ + 17 ml H_2O), which was kept for 10 min at 50°C and dissolving 60 g solid urea in the aqueous $CaSO_4$ and then heating and agitating the mixture for 10 min at 50°C prior to reducing the temperature of the solution to about 25°C. The other ingredients of the feed blocks are reported in Table 2. All components were mixed well and were then pressed into blocks of about 10 kg in a hydraulic compressive machine for 3 min per block, were sun-dried for 2–3 days and stored until use.

2.3. Sample collection and sampling procedures

Feed blocks, concentrates and rice straw were collected during the last 7 days of each period at the morning and afternoon feeding. The samples were dried at 60°C and ground (1 mm screen using a Cyclotech Mill, Tecator, Sweden) and analysed using standard methods of AOAC (1995) for DM, crude protein (CP) and ash. Acid detergent fibre (ADF) was determined according to an AOAC method (1995) and was expressed

Table 1. Ingredient and chemical composition of concentrates and rice straw used in the experiment [g/kg dry matter (DM)].

	Concentrate	Rice straw
Ingredients [g/kg DM]		
Cassava chips	600	
Soybean meal (440 g crude protein/kg)	190	
Rice bran	50	
Coconut meal	60	
Palm kernel meal	50	
Sulphur	10	
Premix*	10	
Molasses, liquid	20	
Salt	10	
Chemical composition		
Dry matter [g/kg]	962	980
Organic matter [g/kg DM]	902	891
Ash [g/kg DM]	98	109
Neutral detergent fibre [g/kg DM]	134	742
Acid detergent fibre [g/kg DM]	79	534
Crude protein [g/kg DM]	130	28
Metabolisable energy† [MJ/kg DM]	12	6

Note: *Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g Fe; 40 g Zn; 40 g Mn; 0.1 g Co; 10 g Cu; 0.1 g Se; 0.5 g I; †Calculated according to the equation described by Robinson et al. (2004).

Table 2. Ingredients and chemical composition of feed blocks and the U–CaS.

	U–CaS in feed blocks [g/kg DM]				
	0	120	150	180	U–CaS
Ingredients [g/kg DM]					
Rice bran	300	300	300	300	
Molasses, liquid	425	390	380	380	
Urea	105	35	20	–	
U–CaS	–	120	150	180	
Cement	110	105	100	90	
Sulphur	15	10	10	10	
Premix*	15	10	10	10	
Salt	10	10	10	10	
Tallow	20	20	20	20	
Chemical composition					
Dry matter [g/kg]	780	781	779	780	630
Organic matter [g/kg DM]	700	701	703	704	820
Ash [g/kg DM]	300	299	297	296	180
Neutral detergent fibre [g/kg DM]	271	269	268	270	–
Acid detergent fibre [g/kg DM]	211	213	212	211	–
Crude protein [g/kg DM]	349	350	349	350	1690
Metabolisable energy† [MJ/kg DM]	15.6	15.4	15.3	15.3	–

Note: *Contains per kilogram premix: 10,000,000 IU vitamin A; 70,000 IU vitamin E; 1,600,000 IU vitamin D; 50 g Fe; 40 g Zn; 40 g Mn; 0.1 g Co; 10 g Cu; 0.1 g Se; 0.5 g I; †Calculated according to the equation described by Robinson et al. (2004).

inclusive of residual ash. Neutral detergent fibre (NDF) in samples was estimated according to Van Soest et al. (1991) with the addition of α-amylase but without sodium sulphite and the results are expressed with residual ash. Metabolisable energy (ME) was calculated according to the equation described by Robinson et al. (2004) as follows:

$$\mathrm{ME[MJ/kg\,DM]} = 0.82(2.4\,\mathrm{CP} + 3.9\,\mathrm{EE} + 1.8\,\mathrm{OM})\,\mathrm{ivOMD}$$

where CP is crude protein [g/kg DM], EE is ether extract [g/kg DM], OM is organic matter [g/kg DM] and ivOMD is the *in vitro* OM digestibility obtained from our previous *in vitro* study with mean values of 530 g/kg DM.

Urine samples were analysed for urinary nitrogen (N) using the Kjeldahl procedure described by the AOAC (1995) and allantoin in urine was determined by high-performance liquid chromatography (HPLC) as described by Chen and Gomes (1995). The amount of microbial purines absorbed and the efficiency of microbial N synthesis were calculated from the excretion of purine derivates based on the relationship derived by Chen and Gomes (1995).

At the end of each period, rumen fluid was collected at 0 and 4 h after feeding. Approximately 100 ml rumen fluid was taken from the middle part of the rumen by a stomach tube (12.7 mm i.d., 19 mm e.d.; Regular Plastic Stomach Tube, CDMV Inc., St-Hyacinthe, QC, USA) connected to a vacuum pump (Model DOA-P104-AA, GAST Manufacturing Inc., Benton Harbor, MI, USA). Immediately after withdrawal, rumen fluid was measured for pH and temperature using HI 8424 microcomputer (Hanna Instruments, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into three portions; first portion was used for NH_3–N analysis with 5 ml of 1 mol H_2SO_4 added to 45 ml of rumen fluid. The mixture was centrifuged at 16,000 *g* for 15 min and the supernatant was stored at −20°C before NH_3–N analysis using the Kjeltech Auto 1030 Analyzer (Tecator, Inc., Herndon, VA, USA). A second portion was fixed with a 10% formalin solution in sterilised 0.9% saline solution. The total direct count of bacteria, protozoa and fungal zoospores were made by the methods of Galyean (1989) based on the use of a haemocytometer (Boeco, Hamburg, Germany). The third portion was cultured for groups of bacteria using a roll-tube technique (Hungate 1969) for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria). Another portion was stored at −20°C for DNA extraction (Yu and Morrison 2004). Community DNA was extracted from 0.25 ml aliquots of each sample by the repeated bead beating plus column (RBB+C) method (Yu and Morrison 2004), which was shown to substantially increase DNA yields. In total, 32 samples belonging to four treatments, four periods and two times of rumen fluid sampling (0 and 4 h post-feeding). The quality and quantity of these DNA samples were also determined by agarose gel electrophoresis and spectrophotometry. The primers used for the real-time polymerase chain reaction (PCR) were as follows: primers for *Fibrobacter succinogenes*, Fs219f (5′GGT ATG GGA TGA GCT TGC-3′) and Fs654r (5′-GCC TGC CCC TGA ACT ATC-3′), were selected to allow amplification (446-bp product) of all 10 *F. succinogenes* strains deposited in GenBank. For *Ruminococcus albus* primers, Ra1281f (5′-CCC TAA AAG CAG TCT TAG TTC G-3′) and Ra1439r (5′CCT CCT TGC GGT TAG AAC A-3′) (175-bp product) were used. *Ruminococcus flavefaciens* primers, Rf154f (5′-TCT GGA AAC GGA TGG TA-3′) and Rf425r (5′-CCT TTA AGA CAG GAG TTT ACA A-3′), were also selected to allow species–species amplification (295 bp) of all seven *R. flavefaciens* strains deposited in GenBank. All these primer sets were previously published by Koike and Kobayashi (2001).

Regular PCR conditions for *F. succinogenes* were as follows: 30 s at 94°C for denaturing, 30 s at 60°C for annealing and 30 s at 72°C for extension (48 cycles), except for 9 min denaturation in the first cycle and 10 min extension in the last cycle. Amplification of 16S rRNA for the other two species was carried out similarly, with the exception that an annealing temperature of 55°C was used. Quantification of total bacteria population, primer and condition, was previously published by Kongmun et al. (2010). Four sample-derived standards were prepared from treatment pool, a set of community DNA. The regular PCR was used to generate sample-derived DNA standards for each real-time PCR assay. Then the PCR product was purified using a QIA quick PCR purification kit (QIAGEN, Inc., Valencia, CA, USA) and quantified using a spectro-photometer. For each sample-derived standard, the copy number concentration was calculated based on the length of the PCR product and the mass concentration. Tenfold serial dilution was made in Tri-EDTA prior to real-time PCR (Yu et al. 2005). In total, four real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the same as those of the regular PCR described above. Biotools QuantiMix EASY SYG KIT (B&M Labs, S. A., Spain) was used for real-time PCR amplification. All PCRs were performed in duplicate.

2.4. Statistical analysis

The rumen pH, temperature, excretion of urinary purine derivatives, microbial CP and efficiency of microbial N synthesis data were analysed using the MIXED procedure (SAS 1996) as a 4 × 4 Latin square design with four treatments, four animals and four periods, according to the following model:

$$Y_{ijk} = \mu + D_i + A_j + P_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observation from animal *j*, receiving diet *i*, in period *k*; μ is the overall mean; D_i is the effect of the different level of U–CaS ($i = 1, 2, 3, 4$); A_j is the effect of animal ($j = 1, 2, 3, 4$); P_k is the effect of period ($k = 1, 2, 3, 4$); and ε_{ijk} is the residual effect. The LSMeans option was used to generate individual diet means. Orthogonal polynomials for diet responses were determined by linear, quadratic and cubic effects.

Ruminal micro-organism measures were analysed as repeated measures over time by using the MIXED procedure (SAS 1996), according to the following model:

$$Y = \mu + D_i + A_j + P_{ij} + H_k + (\mathrm{DT})_{jk} + \varepsilon_{ijk},$$

where Y_{ijk} is the observation from animal *j*, receiving diet *i*, in period k; μ is the overall mean; M_i is the effect of the different level of U–CaS ($i = 1, 2, 3, 4$); A_j is the effect of animal ($j = 1, 2, 3, 4$); P_k is the effect of period ($k = 1, 2, 3, 4$); H_k is the effect of time after feeding ($k = 1$ and 4); $(\mathrm{DT})_{jk}$ is the interaction of U–CaS level × time after feeding; and ε_{ijk} the residual effect.

The best-fitted covariance structure for bacteria, fungal zoospore, total bacteria and *F. succinogenes* was the autoregressive. The unstructured covariance was used for ruminal protozoal concentration, whereas the ante dependence structure was adopted for *R. flavefaciens* and *R. albus*. The LSMeans option (SAS 1996) was used to generate individual diet means. Effects of diet, time and the interaction of diet × time were defined by the *F*-test of ANOVA. The SLICE command (SAS 1996) was used to separate the significant interactions of diet × time. Comparisons among diets within time after feeding were performed by Tukey's test. Orthogonal polynomials for diet responses were determined by linear, quadratic and cubic effects.

3. Results and discussion

3.1. Chemical composition of experimental feedstuffs

The chemical compositions of experimental feedstuffs are presented in Tables 1 and 2. The concentrates, which were offered at 5 g/kg BW per animal and day, contained CP at 130 g/kg DM. Rice straw containing a high amount of NDF and ADF was fed as roughage source. Moreover, the U–CaS consisted of 1690 g CP per kg DM and the CP contents of all feed blocks amounted to 349–350 g CP per kg DM (Table 2). The concentrate consisted a high level of cassava chip at 600 g/kg DM. Cassava (*Manihot esculenta*, Crantz) is grown widely in tropical areas and the price is generally relatively low (Wanapat 2009). Cassava chips contain high levels of non-structural carbohydrates and are highly degradable in the rumen compared with other energy sources, including corn meal (Chanjula et al. 2003). Recently, Cherdthong et al. (2011a, 2011b) demonstrated that concentrates based on a high proportion of cassava chips with a high level of slow-release urea (urea calcium products) could improve the efficiency of ruminal fermentation and the ruminal synthesis of microbial CP in dairy cows or steers. Moreover, in our study, the CP content of feed blocks was similar with those reported by Khampa et al. (2009) and Foiklang et al. (2011) (340–370 g/kg DM of CP). Mean intakes of rice straw, concentrate, feed blocks and total intake were 2.3, 0.6, 0.3 and 3.2 kg/day, respectively. Feeding Thai native cattle with a roughage diet based on rice straw led to reduced a total DM intake. Rice straw used in this experiment was a single-crop variety of *Oryza sativa* indica. Single-crop varieties have a higher stem proportion, lower *in vitro* DM digestibility and degradability but a higher protein content than double-crop varieties, although differences in leaf–stem proportion, chemical composition and *in vitro* or *in sacco* DM and fibre digestibility have also been reported between double-crop varieties (Wanapat 2009). However, rice straw has a low nutritive value with low level of protein, high fibre and lignin content and low DM digestibility, thus resulting in low feed intake (McSweeney and Denman 2007; Wanapat 2009). Although, the rice straw used in our study consisted a low level of CP (28 g/kg dry matter (DM)), it may improved by supplementation with feed blocks, leading to manipulate rumen ecology and rumen micro-organisms of cattle (Cherdthong et al. 2013).

3.2. Rumen ecology and microbial protein synthesis

Rumen pH, temperature, NH_3–N and rumen micro-organisms of cattle fed different levels of U–CaS in feed blocks are presented in Table 3. The pH and temperature in the rumen were ranged from 6.6–6.8°C and 39.3°C–39.4°C, respectively, and were not significantly different among treatments. The fact that an inclusion of U–CaS in feed blocks does not alter pH and temperature in the rumen of cattle and that the values were in the normal ranged were also reported by Wanapat and Cherdthong (2009). This demonstrates that animals were well adapted to the experimental diets. Therefore, supplementation of feed blocks in beef cattle fed on rice straw could maintain normal ruminal pH and temperature. In addition, Cherdthong et al. (2011b) indicated that ruminal pH and temperature values were stable at pH 6.5–7.0 and temperature of 39.3–39.7°C,

Table 3. Rumen condition and rumen micro-organisms of cattle fed different levels of U–CaS in feed blocks.

	U–CaS in feed blocks [g/kg DM]					Contrasts*				
	0	120	150	180	SEM	Linear	Quadratic	Cubic	T	D × T
Ruminal ecology										
pH	6.8	6.6	6.6	6.6	0.51	0.22	0.43	0.32	nd‡	nd
Temperature [°C]	39.3	39.4	39.4	39.4	1.54	0.89	0.91	0.55	nd	nd
NH_3-N [mg/dl]	21.1	20.1	18.1	17.9	1.00	0.07	0.06	0.02	nd	nd
Ruminal microbes [cells/ml]										
Bacteria ($\cdot 10^{11}$)	5.4	5.7	6.6	7.2	0.43	0.06	0.04	0.08	0.02	0.76
Protozoa ($\cdot 10^{6}$)	4.5	4.7	4.2	4.4	0.68	0.43	0.55	0.19	0.33	0.44
Fungal zoospore ($\cdot 10^{4}$)	1.4	1.4	1.6	2.4	0.19	0.03	0.12	0.08	0.03	0.54

Note: **T*, Effect of time after feeding; *D* × T, Interaction of diet × time after feeding; ‡nd, Not determined.

respectively, when animals were fed with an U–CaS in concentrate mixture, and these ranges were considered optimal for microbial digestion of fibre and protein. However, ruminal NH_3–N concentration showed a quadratic effect ($p < 0.05$) in response to U–CaS inclusion, with the lowest value being observed for 180 g U–CaS per kg feed block, while the inclusion of the highest amount of urea without U–CaS in feed blocks caused the significant highest concentration of NH_3–N ($p < 0.05$). This observation may be due to supplementation of U–CaS products in feed blocks that could control the rate of NH_3–N degradation from urea in the rumen and lead to slow rates of NH_3–N released when compared with urea treatment. These results were in agreement with Liu et al. (1996) and Wu et al. (2005), who revealed that the NH_3–N concentration was significantly improved in animals with access to lick block supplementation than in those with no block. Similarly, in a previous study of our group was shown that treatments with urea rapidly increased in the concentration of NH_3–N, which was in contrast to U–CaS treatments, where NH_3–N was slowly increased (Cherdthong et al. 2011a, 2011b). This effect was observed in *in vitro* and *in vivo* studies. It seems that U–CaS in feed blocks, which have lower rates of ruminal degradation, tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released N by rumen microbes (Śliwiński et al. 2002; Huntington et al. 2006; Südekum et al. 2006).

Excretion of urinary purine derivatives, microbial crude protein (MCP) yield and efficiency of microbial protein synthesis (EMNS) are presented in Table 4. Inclusion of U–CaS in feed blocks altered the absorption and excretion of allantoin and MCP of animals. MCP and EMNS were linearly increased when U–CaS was included in feed blocks at 180 g/kg DM ($p < 0.05$). This increase in MCP in beef cattle may a result from a slower rate of N release than for urea and the better capture of these nutrients by rumen microbes (Infascelli et al. 2005; Südekum et al. 2006). Similarly, synchronisation for rapid fermentation with highly degradable carbohydrates and N sources stimulated greater MCP when compared to diets with non synchronised N and energy release (Chanjula et al. 2003; Galina et al. 2003). Cherdthong et al. (2011b) reported that supplementation of U–CaS to concentrates containing a high level of cassava chips increased the efficiency of microbial protein synthesis from 12.9 to 18.2 g N/kg OM digested in the rumen of cattle. Therefore, in order to improve MCP, it seems that the manipulation of carbohydrate and N fermentation in the rumen should first be aimed at obtaining the most even ruminal carbohydrate supply pattern possible within a particular dietary regimen. The second goal is to supply the total daily amount of ruminally available N sufficient for use of the total amount of carbohydrate expected to be released in the rumen per day (Śliwiński et al. 2002).

3.3. *Ruminal micro-organisms*

The rumen of ruminants is a complex ecosystem in which diets consumed by the ruminant are digested by active and diverse micro-organisms. Ruminal bacteria, protozoa and fungi degrade fibrous material, allowing ruminants to utilise plant fibre for nutrition (Koike and Kobayashi 2001). The end products of these fermentations are volatile fatty acids and MCP which are in turn used by the host. In the current study, it was found that viable population of protozoa was unaltered by dietary treatments, while the count of bacteria and fungal zoospores were changed by U–CaS supplementation in feed blocks (Table 3). Population of rumen bacterial increased quadratically ($p < 0.05$) with increasing amounts of U–CaS in feed blocks reaching the highest value ($7.2 \cdot 10^{11}$ cells/ml) at 180 g U–CaS per kg DM. The count of fungal zoospores was also linearly increased be U–CaS

Table 4. Excretion of urinary purine derivatives (PD), microbial crude protein and efficiency of microbial N synthesis as affect U–CaS in feed block.

	U–CaS in feed blocks [g/kg DM]					Contrast		
	0	120	150	180	SEM	Linear	Quadratic	Cubic
PD [mmol/day]								
Allantoin excretion	133.1	133.5	145.3	155.6	3.32	0.01	0.09	0.07
Allantoin absorption	129.2	130.2	138.3	149.2	2.14	0.02	0.12	0.08
MCP* [g/day]	336.5	340.1	390.8	421.1	10.32	0.01	0.08	0.06
EMNS† [g N/kg OMDR]	13.4	13.5	16.5	22.4	1.22	0.04	0.09	0.09

Note: *MCP, Microbial crude protein, calculated as: MCP [g/day] = 3.99 · 0.856 PD excreted [mmol/day] (Cherdthong et al. 2011b); †EMNS, Efficiency of microbial N synthesis, calculated as: EMNS [g N/kg organic matter digested in the rumen {OMDR}] = (MCP [g/day] · 1000)/OMDR [g], assuming that rumen digestion was 650 g/kg OM of digestion in total tract.

supplementation ($p < 0.05$) and the highest value ($2.4 \cdot 10^4$ cells/ml) was reached with the highest U–CaS concentration in feed blocks. Furthermore, for bacteria and fungal zoospores an effect of feeding time ($p < 0.05$) was observed, but there was no interaction of diet × time. At 180 g U–CaS per kg DM in feed blocks, rumen bacteria and fungal zoospores increased at 4 h after feeding. This observation can be explained by the slower release of the U–CaS product in the rumen, thus it may have caused a continuous NH_3–N supply for microbial protein synthesis and improved microbial activities in the rumen (Russell and Rychlik 2001).

3.4. Predominant cellulolytic bacterial population in the rumen

Bacteria in the rumen are considered to play a more important role than protozoa and fungal zoospores in feed digestion and the production of microbial protein and volatile fatty acids (Stewart et al. 1998). Bacterial numbers in the rumen are very high (10^{10} to 10^{12} cells/ml of rumen fluid) and the complexity of ruminal bacteria is great (Russell and Rychlik 2001). Recent advances in molecular tools increasingly enable identification and characterisation of the microbes in these bioreactors (Simon et al. 2005). Real-time PCR technique has the ability to enumerate targeted cellulolytic bacteria with high sensitivity and has been used to analyse rumen digesta (Wanapat and Cherdthong 2009; Longo et al. 2013). This technique is both reliable and simple to perform (Koike and Kobayashi 2001). In this experiment, increasing levels of U–CaS in feed blocks caused a linear increase of total bacteria ($p < 0.05$), whereas a quadratic effects ($p < 0.05$) were observed for *F. succinogenes* population (Table 5). The highest values for total bacteria and *F. succinogenes* with $8.2 \cdot 10^{11}$ and $6.3 \cdot 10^9$ copies/ml of rumen content, respectively, were observed at 180 g U–CaS per kg DM. Regarding these effects on bacterial populations, no interactions of diet × time of feeding existed, while significant effects of time after feeding were observed in case of U–CaS inclusion. Possibly, the NH_3–N release from U–CaS was slower than from urea, and can potentially be used more efficiently by rumen micro-organisms, especially if feed blocks contain molasses as energy source (Cherdthong et al. 2011a, 2011b). In addition, U–CaS contains also $CaSO_4$, a good available source of sulphur, which is an essential element for rumen bacterial growth and its metabolism is closely related to N metabolism. Thus, the continuous availability of N and sulphur for ruminal fermentation is important and could enhance predominant cellulolytic bacterial population. These finding are similar to the results of Cherdthong et al. (2011a, 2011b), who found in their *in vitro* and *in vivo* studies that a supplementation of U–CaS in concentrate mixture caused greater populations of cellulolytic bacteria, especially *F. succinogenes,* when compared with urea supplementation. Furthermore, Koike and Kobayashi (2001) and Wanapat and Cherdthong (2009) confirmed that *F. succinogenes* was most dominant among the three investigated species in ruminants, followed by *R. flavefaciens* and *R. albus*. However, in this study *R. flavefaciens* and *R. albus* were not significantly different among treatments and concentrations ranged from 8.3 to $8.7 \cdot 10^8$ and 1.3 to $1.4 \cdot 10^8$ copies/ml of rumen content, respectively.

The study revealed that an inclusion of 180 g U–CaS per kg DM of feed blocks could improve rumen ecology, composition of rumen micro-organism, MCP synthesis and predominant cellulolytic bacteria in Thai native beef cattle fed on rice straw as roughage. Based on this research it can be concluded that feed blocks containing U–CaS can be an effective feed supplement for ruminants. However, the result should be repeated under farm conditions to show the effects of animal growth.

Table 5. Effect of U–CaS in feed block on total bacteria, population of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* by using real-time PCR technique in Thai native beef cattle [copies/ml of rumen content].

	U–CaS in feed blocks [g/kg DM]					Contrast*				
	0	120	150	180	SEM	Linear	Quadratic	Cubic	T	D × T
Total bacteria (· 10^{11})	5.7	5.9	6.9	8.2	0.61	0.04	0.08	0.12	0.02	0.94
Fibrobacter succinogenes (· 10^{9})	3.8	3.9	5.0	6.3	0.55	0.12	0.03	0.18	0.01	0.44
Ruminococcus flavefaciens (· 10^{8})	8.3	8.7	8.6	8.6	1.43	0.39	0.48	0.43	0.22	0.87
Ruminococcus albus (· 10^{8})	1.3	1.3	1.3	1.4	0.33	0.11	0.20	0.15	0.33	0.54

Note: **T*, Effect of time after feeding; *D* × *T*, Interaction of diet × time after feeding.

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Anusorn Cherdthong

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Article Type: Research Paper

Section/Category: Ruminants

Keywords: slow release urea; feed block; rumen fermentation; growth performance; cattle

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Abstract: This experiment evaluated the effect of urea calcium sulphate mixture (U-cas) level in feed blocks on feed intake, apparent digestibility of nutrients and rumen fermentation in Thai native beef cattle fed rice straw. Four Thai native beef cattle were randomly assigned to receive four dietary treatments with various levels of U-cas in feed blocks was 0, 120, 150 and 180 g/kg dry matter (DM) in a 4x4 Latin square design. The present results revealed that rice straw intake was increased with the increasing level of U-cas inclusion in the feed blocks. Total intakes of DM and energy (ME, MJ/d) were the highest with U-cas inclusion at 180 g/kg DM fed group, followed by 150, 120 and 0 g/kg DM, respectively. Apparent digestibility of nutrients other than ADF was enhanced with the increasing level of U-cas supplementation. Rumen pH and temperature were not changed by U-cas levels inclusion. The concentration of ruminal NH3-N at 4 h post feeding was decreased with the increasing level of U-cas supplementation ($P<0.05$). Inclusion of U-cas at 180 g/kg DM in the feed blocks could increased the propionic acid concentration in the rumen at 4 h post feeding which resulted in lower ratio of acetic: propionic acid and acetic plus butyric: propionic acid ($P<0.05$). Inclusion of U-cas at 180 g/kg DM in the feed blocks resulted in improved feeding value of the diets based on rice straw.

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Was intake of straw measured-not clear.
☑ We have explained in sub-topic 2.2 as: "Individual intakes of rice straw, concentrate and feed blocks were recorded daily by weighing the offered and refused feeds."

Line 110-111: Define equation.
☑ We have defined as: "Metabolizable energy (ME) was calculated according to the equation described by Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×CP) + (3.9× EE) + (1.8×organic matter) ×*in vitro* organic matter digestibility (ivOMD))
where: CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM.
"

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Effect of feeding feed blocks ~~with~~ containing different levels of urea calcium sulphate mixture on feed intake, nutrients of digestibility ~~of nutrients,~~ and rumen fermentation ~~and growth performance~~ in Thai native beef cattle fed on rice straw

A. Cherdthong*, M. Wanapat, W. Wongwungchun, S. Yeekeng, T. Niltho, D. Rakwongrit, W. Khota, S. Khantharin, G. Tangmutthapattharakun, K. Phesatcha, S. Kang

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Abstract

This experiment was to evaluated the effect of urea calcium sulphate mixture (U-cas) level containing in feed blocks (FB) on feed intake, apparent digestibility of nutrients and, rumen fermentation and growth performance in Thai native beef cattle fed on rice straw., base. Four Thai native beef cattle with initial body weight (BW) of 100±3.0 kg were randomly assigned according to receive a 4×4 Latin square design. The four dietary treatments were with variousthe inclusion levels of U-cas in FBfeed blocks wasat 0, 120, 150 and 180 g/kg dry matter (DM) in a 4x4 Latin square design., respectively. The present results revealed that rice straw intake were was increased with the increasing level of U-cas inclusion in the FBfeed blocks. Total intakes of DM and energy (ME, MJ/d) were significantly different (P<0.05) among treatments and the highest with was in s U-cas inclusion at 180 g/kg DM fed group, followed by 150, 120 and 0 g/kg DM, respectively. Moreover, aApparent digestibility of nutrients other than ADF was enhanced with the increasing level of U-cas supplementation in FB, except ADF digestibility. Rumen pH and temperature were not changed by U-cas levels inclusion. while tThe concentration of ruminal NH_3-N at 4 h post feeding was and the mean values were slowly decreased with the increasing level of U-cas level in FBsupplementation (P<0.05). On the other hand, iInclusion of U-cas at 180 g/kg DM in the FBfeed blocks could increased concentration of the propionic acid (C3)concentration in the rumen at 4 h post feeding which resulted in lower ratio of acetic acid: (C2) to C3 propionic acid and acetic C3 plus butyricplus butyric: propionic acid acid (C4) to C3 (P<0.05). The feed conversion ratio (FCR)was not different among treatments while final BW, BW change and mean average daily gain (ADG) were increased in cattle received FB containing U-cas at 180 g/kg. Based on this study, it could be concluded that iInclusion of U-cas at 180 g/kg DM in the FBfeed blocks resulted in improvement improved feeding value of the diets based on rice straw. of feed intake, apparent digestibility and rumen fermentation of Thai native beef cattle fed rice straw.

Keywords: cattle, feed block, rumen fermentation, slow release urea

Abbreviations: ~~ADG; average daily gain,~~ ADF, acid detergent fiber; BW, body weight; DM, dry matter; ~~FCR; feed conversion ratio, FB; feed blocks,~~ aNDF, neutral detergent fiber; NH_3-N; ammonia nitrogen, VFA, volatile fatty acid; U-cas; urea calcium sulphate mixture

1. Introduction

Rice straw is the main source of roughage, particularly during dry season, for Thai native beef cattle in Thailand (Wanapat, 2009). Feeding ~~only~~ rice straw alone does not provide enough nutrients to ~~the~~ ruminants due to its low content of nitrogen (N), ~~low intake~~ and poor digestibility associated with low intake (Liu et al., 2002). Therefore, to improve the productive and reproductive capacity of ~~smallholder owned~~ ruminant animals ~~,~~on small-holder farms there is a need to develop feeding strategies that will enhance the quality and sustained availability of feed resources such as rice straw produced on-farm (Calabrò et al., 2008; Wanapat, 2009). Feed blocks ~~(FB), a~~are solidified mixture of unconventional feeds such as rice bran, molasses, binder, salt, mineral and ~~10-15 g/kg DM of~~ urea which have been shown ~~has been reported to overcome the situation and could~~ to improve production of ruminants fed with rice straw (Wanapat and Khampa et al., 2006; Foiklang et al., 2011). However, inclusion of urea in the ~~FB~~feed blocks is still limited because of the rapid hydrolysis of urea to NH_3-N, which is rapidly absorbed~~causes accumulation and escape~~ from the rumen (Galo et al., 2003). The net result is that a potentially large part of the N from NPN sources such as urea being ~~-is~~ excreted in the urine and faeces, which is a loss of potential nutrient for production and can contribute to environmental pollution (Broderick et al., 2009).

Recently, slow release urea ~~property~~ has been achieved by binding urea to calcium sulphate (~~urea calcium sulphate mixture;~~ U-cas) in order to ~~control its release rate and~~ improve N utilization in the rumen by~~,~~ increasing microbial protein synthesis, hence resulted in~~-as well as~~

milk yield in ruminants via improved nutrition (Cherdthong et al., 2011a-c). ~~Furthermore, in an earlier *in vitro* experiment,~~ Cherdthong and Wanapat~~et al.~~ (2013) found that the inclusion of U-cas at 180 g/kg DM in ~~FB~~the feed blocks resulted in improvement of *in vitro* rumen fermentation, microbial mass and digestibility. However, replacement of urea by U-cas in the ~~FB~~feed blocks in *in vivo* work has not yet been investigated. Therefore, the present study was to investigate the effect of U-cas level inclusion in the ~~FB~~feed blocks on feed intake, digestibility of nutrients and rumen fermentation in Thai native beef cattle fed on rice straw.

2. Materials and methods

2.1 Dietary treatments preparation

Rice straw and concentrate were obtained from the Ruminant Metabolism Center, Tropical Feed Resources Research and Development Center (TROFREC), Khon Kaen University, Thailand. Rice straw was a single-crop variety of *Oryza sativa indica*. The U-cas was prepared according to Cherdthong et al. (2011a) by, ~~in brief, providing~~producing an aqueous solution ~~(23 g $CaSO_4$ + 17 ml H_2O)~~ of $CaSO_4$ (1.35 g/mL) ~~at 50 °C for 10 min~~ and dissolv~~ing~~ed with 60 g urea in the aqueous $CaSO_4$ and then ~~heating and~~ agitat~~ing~~ed the mixture at 50 °C for 10 min prior to reduc~~ing~~e the temperature of the solution to about 25°C. ~~The proportions of ingredients in FBs are reported in Table 1.~~ All ingredients in the feed block (Table 2) were mixed ~~well~~ together and then pressed into blocks of about 10 kg by ~~in a~~ hydraulic compressi~~ve~~on ~~machine at~~ for 3 min per block ~~and~~ then left to dry in the sun ~~under sun drying~~ for 2 to 3 days or under open room with roof. ~~as to reduce moisture and stored until use.~~

2.2 Animals, experimental design and feeding

Four, Thai native beef cattle with initial body weight (BW) of 100±3.0 kg were randomly assigned according to a 4×4 Latin square design to receive ~~four different dietary treatments. The dietary treatments were inclusion of~~ U-cas supplementation in ~~FB~~feed blocks at 0, 120, 150 and

180 g/kg DM~~, respectively~~. A ~~C~~concentrate mixture (Table 1) was fed to animals at 5 g/kg of BW daily and offered in two equal meals per day at 7:00 and 16:00 hours. Rice straw was fed ~~*ad libitum*~~ by allowing for refusals of 100 g/kg~~ refusals~~. All animals were kept in individual ~~pens, and clean~~pens. Clean fresh water and ~~FB~~feed blocks were available at all times. Individual ~~FB~~ intakes of rice straw, concentrate and feed blocks ~~was~~ were recorded daily by weighing the offered and refused feeds. The experiment was conducted for 4 periods, lasting 21 days per each. ~~During t~~The first 14 days were an adaptation period~~, all animals were fed their respective diets on *ad libitum*, whereas during~~ and ~~the~~ last 7 days animals~~ they~~ were moved to metabolism crates and fed the straw at ~~for fecal collection during as animals were restricted to~~ 900 g/kg of the previous voluntary feed intake of straw. ~~, while c~~Concentrate was still offered at 5 g/kg of BW daily and~~, while~~ ~~FB~~feed blocks were available at all times during which animals were in metabolism crates.

The animals were weighed on d 21, 42, 63 and 84.~~ for BW change, average dairy gain (ADG) and feed conversion ratio (FCR) calculation. Chemical compositions of the experimental diets are shown in Tables 1 and 2.~~

2.3 Sample collection and sampling procedures

Feed~~s~~ offered, refusals and fecal samples were collected during the last 7 days of each period at morning and afternoon feedings. The samples were firstly dried at 60°C and ground (1 mm screen using a Cyclotech Mill, Tecator, Sweden) and then analyzed using ~~standard methods of~~ AOAC (1995) method for DM (ID 967.03), N (ID 984.13), EE (ID 954.02), ash (ID 942.05), and ADF (ID 973.18). Neutral detergent fiber (aNDF) in samples was estimated according to Van Soest et al. (1991) with addition of α-amylase but without sodium sulphite and results are expressed ~~with~~ inclusive of residual ash. Metabolizable energy (ME) was calculated according to the equation described by Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×CP) + (3.9× EE) + (1.8×organic matter) ×*in vitro* organic matter digestibility (ivOMD))

Formatted: Normal, Justified, Indent: First line: 1.27 cm, Line spacing: Double, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers where: CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM. ~~.~~

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Digestible organic matter fermented in the rumen (DOMR) was calculated according to the equation described by ARC (1984) as:

DOMR (kg/d) = digestible organic matter intake (DOMI, kg/d) x 0.65

where: DOMI=[digestibility of organic matter (kg/kg DM) x organic matter intake (kg/d)]/100, 1 kg DOMI = 15.9 MJ ME/kg (Kearl, 1982).

~~Rumen fluid samples were collected at 0 and 4 h after feeding at the end of each period.~~ Approximately, 45 ~~ml~~mL of rumen fluid was taken from the rumen by a stomach tube connected ~~with~~ to a vacuum pump at 0 and 4 h after feeding on the last day of each period~~each time of collection~~. Ruminal pH and temperature were determined using a portable pH and temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore). Ruminal NH_3-N concentration was analyzed according to the Kjeltech Auto 1030 Analyzer (AOAC, 1995; ID 973.18). Volatile fatty acids (VFAs) were analyzed using high pressure liquid chromatography ~~according~~ using the method~~to~~ of Samuel et al. (1997).

2.4 Statistical analysis

~~The data from the experiment were subjected for statistical analysis following~~Statistical analysis accounted for the ~~a~~ 4×4 Latin square design using the GLM procedure of SAS (1996). Data were analyzed using the model:

$$Y_{ijk}=\mu+M_i+A_j+P_k+\varepsilon_{ijk}$$

where: Y_{ijk}, observation from animal j, receiving diet i, in period k; μ, the overall mean, M_i, effect of the different level of U-cas (i=1, 2, 3, 4), A_j, the effect of animal (j=1, 2, 3, 4), P_k, the effect of period (k=1, 2, 3, 4), and ε_{ijk} the residual effect. Results are presented as mean values with the standard error of the means. Differences between treatment means were determined by

Duncan's New Multiple Range Test (Steel and Torrie, 1980), and differences among means with P<0.05 were represented as statistically significant differences.

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3. Results

3.1 Chemical composition of feeds

The composition of U-cas and FB is presented in Table 2. U-cas contained CP at 1690 g/kg U-cas while FB contained 349-350 g/kg DM of CP.

3.21 Intake of rice straw, concentrate, and FBfeed blocks

DM intake of rice straw, concentrate, FB and intake of nutrients of Thai native beef cattle fed different levels of U-cas inclusion in FB are presented in Table 3. Rice straw intake of beef cattle offered feed block containing U-cas at 120, 150 and 180 g/kg of U-cas inclusion in FB was were 78.3, 82.1 and 94.8 g/kg $BW^{0.75}$, respectively, and werewas significantly (P<0.05) higher (P<0.05) than the intake of cattle received FBfeed blocks containing 0 g/kg of U-cas supplementation (75.4 g/kg $BW^{0.75}$). Total and eEnergy (ME, MJ/d) intake were was significantly different (P<0.05) among treatments and found the highest was foundwith in cattle consumed consuming FBfeed blocks containing U-cas at 180 g/kg DM, followed by 150, 120 and 0 g/kg DM, respectively. However, supplementation of U-cas containing in FBfeed blocks did not change intake of concentrate, FBfeed blocks and nutrients (DM, OM, CP, aNDF and ADF) intake in the present study..

3.3 2 Apparent digestibility of nutrients

The results of apparent nutrient digestibility of DM, OM, CP, aNDF and ADF in Thai native beef cattle as affected by U-cas levels supplemented in FB are presented in Table 3. Digestibility of nutrients was enhanced with the increasing level of U-cas supplementation in FB, except ADF digestibility. Cattle fed with FBfeed blocks contained containing 180 g/kg DM of U-cas had the a highreest digestibility of DM, OM, CP and aNDF among treatmentscompared to cattle received food block containing lower levels of U-cas (Table 3; P<0.05).

3.43 Rumen fermentation

Rumen pH, temperature, NH_3-N and VFA concentrations affected by dietary treatments are presented in Table 4. It was found that rumen pH and temperature were not changed by U-cas levels supplemented in FBfeed blocks. while tThe concentration of ruminal NH_3-N at 4 h post feeding and mean values werewas lower when cattle was offered feed block included with the increasing of U-cas at 120 and 150 g/kg DMlevel in FB (P<0.05). Inclusion of U-cas at 180 g/kg DM in FBfeed blocks increased the concentration of C3 propionic acid (4 h post feeding) and decreased the ratio of C2/C3 and C2+C4/C3. acetic: propionic acid and acetic plus butyric: propionic acid (P<0.05).

3.5 Performance responses of cattle

Significant performance responses of cattle to U-cas supplementation in FB were obtained in the present study, but the mean feed conversion ratio (FCR) was not different among treatments (5.8, 5.9, 4.9 and 5.6 kg/kg for 0, 120, 150 and 180 U-cas in FB, respectively). Mean average daily gain (ADG) were increased when cattle was fed FB containing U-cas at highest level (180 g/kg DM). Supplementation of U-cas at 180 g/kg in FB increased (P<0.05) mean ADG at 8 kg (84 days) and 23 g/d, respectively when compared with 0 U-cas supplement.

4. Discussion

4.1 Intake of rice straw, concentrate and FBfeed blocks

Supplementation of FBfeed blocks with containing different levels of U-cas inclusion influenced on the intake of rice straw, total feed and energy in Thai native beef cattle. FBFeed blocks intake by cattle in the present study was 0.3 kg/d and this was inicated, suggesting that U-cas can replace urea in FBfeed blocks for Thai native beef cattle without adverse intake effects.

The~~se~~ ~~FB~~feed blocks intake~~s results~~ in the present result were similar to Foiklang et al. (2011) who reported ~~found that~~ the range of fee block intake of swamp buffalo~~FB~~feed blocks ranged from 0.27 to 0.31 kg/d~~ in swamp buffaloes~~. Though the intake of ~~FB~~feed blocks was not changed as a result of supplementation of U-cas~~,~~; however, the ~~FB~~feed blocks supplementation enhanced the intake of basal roughage significantly ($P<0.05$). Increase~~d~~~~s~~ in DMI of rice straw by supplementation of U-cas in the ~~FB~~feed blocks licks ~~were~~ was due to the availability of limiting nutrients (sulfur from $CaSO_4$) and progressive change in rumen fermentation. Similar results to present study were also reported by Wanapat and Khampa (2006) and Foiklang et al. (2011) who found that supplementation of ~~FB~~feed blocks could increase feed intake of urea-lime treated rice straw and total intakes while on change on ~~FB~~feed blocks intakes were found ~~not significantly affected~~ among treatments. Moreover, steers consuming ~~cooked~~ formulated molasses block; containing base ingredients of beet molasses, cane molasses, or concentrated separator by-product (Greenwood et al., 2000) and varying levels of urea and/or feed grade biuret as CP sources (Löest et al., 2001)~~,~~ increased intake of low-quality prairie hay. In contrast, Wu et al. (2005) revealed that the DM intake of roughages slightly decreased with urea-minerals lick block supplementation.~~ but the differences were not significant.~~ Furthermore, it was reported that the use of cement as binding agent in feed block is necessary in order to solidify the blocks (Foiklang et al., 2011). In this study, supplementation of cement at 90-105 g/kg DM have not shown such adverse effects on intake of feed blocks and were similar to those previously reported by Hadjipanayiotou et al. (1993) who found that 100 g/kg of cement in the formula provided good blocks quality.

4.2 Apparent digestibility of nutrients

The improvement in nutrients digestibility in 180 g/kg of U-cas supplement in ~~FB~~feed blocks group indicated the availability of more potentially N source with fermentable molasses for the proliferation of rumen microbes (Udén, 2006). Our results are in line with those obtained by Molina-Alcaide et al. (2010) who found that NDF digestibility of goats receiving FBfeed blocks diets was higher than that those without feedof no block supplemented in goats and this means that higher fiber digestibility could indicate higher energy availability. Increased CP digestibility in 180 g/kg of U-cas supplemented groups indicating indicate sufficient supply of slowly release N and energy for the optimum growth of rumen microbes (Robinson, 2010; Calabrò et al., 2012; Cherdthong and Wanapatet al., 2013). In addition, molasses content in the FBfeed blocks could influence on the readily available energy available to for the microbes. Moreover, it could possibly be that ammonia NH_3 released from U-cas was slowly released to ammonia thanas compared to urea and could potentially be used more efficiently by rumen microorganisms (Galo et al., 2003). These results were in agreement with Cherdthong et al. (2011a), who reported that supplementation of U-cas product as a slow release NPN source in concentrate diet could improve digestibility and microbial mass in an *in vitro* experiment. Furthermore, the digestibility of aNDF and cellulolytic bacterial population were enhanced when dairy cows or beef cattle were fed with U-cas (Cherdthong et al. 2011b,c). In addition, a polymer-coated slow reduced urea was demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo et al., 2003).

4.3 Rumen fermentation

Ruminal pH and temperature generally were above 6.5 and 39.4 °C respectively during the 4 h post feeding and did not drop below 6.0 and 39 °C. Similar results have been reported by Foiklang et al. (2011), when buffaloes were supplemented with the FBfeed blocks. Also, Cherdthong et al. (2011b-c) indicated that rumen fluid pH and temperature values were in rangestable at pH 6.5 to 7.0 and temperature of 39.3 to 39.7 °C, respectively, and these ranges were considered as an optimal for microbial digestion of fiber and protein. Excessive N supply, a release of ruminal NH_3 that often exceeds its rate of incorporation into microbial protein, resulted in loss of a great part of this N as NH_3 absorbed from the rumen. There were effect of

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Inclusion of U-cas in the ~~FB~~feed blocks ~~supply~~ affected on ruminal NH_3–N concentrations and was slowly reduced, especially in 180 g/kg U-cas supplemented groups while NH_3–N concentrations tended to be higher in the urea when compared to U-cas groups. This could be explained by the effect of high slow release urea product in the ~~FB~~feed blocks. Similarly, Cherdthong et al. (2011a-c) reported the finding ~~that~~ in *in vitro* and *in vivo*~~,~~ that urea treatments rapidly increased the concentration of NH_3–N, but gradually increased in the U-cas treatments. Degree of U-cas protection, in term of NH_3 reduction when compared with urea at 2 h of fermentation, was reduced ~~at~~ to 168 mg/dl in *in vitro* and 14.4 mg/dl in *in vivo* experiment (Cherdthong et al., 2011c). Similarly, Huntington et al. (2006) revealed that urea–calcium chloride product supplementation resulted in a lower concentrations of ruminal NH_3-N in ~~a~~ the treatment that represented consumption of a CP equivalent to 220 to 460 g/d of CP in ruminants. In addition, Taylor-Edwards et al. (2009) reported that a slow release urea product reduced the rapidity of NH_3 production in the rumen without affecting other ruminal fermentation metabolites and it could be inferred that slow release urea diets could prolong microbial utilization of additional N sources during ruminal fermentation.

The concentration of total VFA was not changed by the ~~FB~~feed blocks and the mean values ranged from 116.8-119.6 mmol/l and these were ~~close~~ similar to those previously found by Foiklang et al. (2011) that total VFA concentrations in the rumen of buffalo fed with the ~~FB~~feed blocks ranged from 102.2 to 116.0 mmol/l. Supplementation of U-cas in ~~FB~~feed blocks enhanced the proportion of ~~C3~~ propionic acid while ~~depressed~~ deceased ~~C2~~acetic acid concentration. Similarly, Cherdthong et al. (2011a) reported that propionic acid~~C3~~ concentrations were ~~the greatest for the~~increased by U–cas supplementation in concentrate diet of dairy cows. This change might have helped ~~in~~ to improv~~e~~~~ing~~ energy use for ruminant because propionic acid~~C3~~ shows a positive relation with energy utilization efficiency. Thus, it means that higher propionic acid~~C3~~ ~~was~~ indicated a better energy yield while shifting in acetic:~~C2/~~

propionic acid~~C3~~ and acetic plus butyric acid:~~C2+C4/~~ propionic acid~~C3~~ ratio explained better efficiency of energy use in 180 g/kg of U-cas. These findings agreed with Cherdthong et al. (2011a,b) who found ~~more~~ higher propionic acid~~C3~~ and thus a lower acetic: ~~C2to~~ propionic acid~~C3~~ ratio in the ruminal fluid of cows fed a high grain diet.

~~*4.4 Performance responses of cattle*~~

~~FB contained 180 g/kg DM of U-cas could be used efficiency as a strategic supplement to improve ADG especially when cattle are fed on rice straw supplemented with a low level of concentrate. Moreover, it also enhanced roughage intake in maintaining normal fermentation and establishing a more rumen ecology balance (Cherdthong et al., 2011a-c). Briefly, U-cas in FB allow, through appropriate formulation and making procedures, a balanced, synchronised and fractionated supply of the major nutrients (mainly energy, slow release nitrogen and minerals) resulting in an improvement in the digestion of rice straws and, therefore, in an increase of ruminant performance (Gasmi-Boubaker et al., 2006). The positive performance response of cattle consuming FB containing U-cas was in agreement with Titgemeyer et al. (2004) who revealed that gain efficiencies were increased from −0.03 to 0.05 when supplementation with cooked molasses blocks in cattle fed on alfalfa. Similarly, Liu et al. (1996) found that BW gains were significantly higher in animals with access to block than in those without no block; 370 *vs.* 203 g/d for cattle and 95 *vs.* 73 g/d for goats, respectively. The use of blocks with and without polyethylene glycol (PEG) avoided BW loss of the goats under dry season, but increased at 12 g/d (−PEG) and 24 (+PEG) g/d, while the control group lost 19 g day−1 (Gasmi-Boubaker et al., 2006).~~

5. Conclusions

In summary, inclusion of U-cas at 180 g/kg in the ~~FB~~feed blocks resulted in improvement of feed intake, apparent nutrients digestibility and rumen fermentation. Therefore, ~~FB~~feed blocks containing U-cas could be used as a supplementary feed resource when rice straw was fed to Thai native beef cattle. However, ~~more~~ additional works on inclusion of U-cas in the ~~FB~~feed blocks should be investigated further ~~-~~under field conditions.

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*Table (Marked/Corrections highlighted)

Table 1
Ingredient and chemical composition of concentrates and rice straw used in the experiment (g/kg dry matter (DM))

Ingredients, g/kg DM	Concentrate	Rice straw
Cassava chips	600	
Soybean meal (SBM), 440 g/kg CP solvent	190	
Rice bran	50	
Coconut meal, solvent	60	
Palm kernel meal, solvent	50	
Pure sulfur	10	
Mineral premix[a]	10	
Molasses, liquid	20	
Salt	10	
Chemical composition		
Dry matter, g/kg	962	980
Organic matter, g/kg DM	902	891
Ash, g/kg DM	98	109
aNeutral detergent fiber, g/kg DM	134	742
Acid detergent fiber, g/kg DM	79	534
Crude protein, g/kg DM	130	28
Metabolizable energy (ME)[b], MJ/kg DM	12	6

[a]Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.
[b]Metabolizable energy (ME) was calculated according to the equation of Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×crude protein (CP)) + (3.9× ether extractEE) + (1.8×organic matter (OM))) ×*in vitro* organic matter digestibility (ivOMD))
where CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM.

Table 2
Ingredient and chemical composition of urea calcium sulphate mixture (U-cas) and feed block (FB)

	Supplementation of U-cas in feed blocks FB, g/kg DM				U-cas
	0	120	150	180	
Ingredients, g/kg DM					
Rice bran	300	300	300	300	
Molasses, liquid	425	390	380	380	
Urea	105	35	20	-	
U-cas[a]	-	120	150	180	
Cement	110	105	100	90	
Pure sulfur	15	10	10	10	
Mineral premix[ab]	15	10	10	10	
Salt	10	10	10	10	
Tallow	20	20	20	20	
Chemical composition					
Dry matter, g/kg	780	781	779	780	630
Organic matter, g/kg DM	700	701	703	704	820
Ether extract, g/kg DM	24	23	23	24	-
Ash, g/kg DM	300	299	297	296	180
aNeutral detergent fiber, g/kg DM	271	269	268	270	-
Acid detergent fiber, g/kg DM	211	213	212	211	-
Crude protein, g/kg DM	349	350	349	350	1690*
Metabolizable energy (ME)[cb], MJ/kg DM	15.6	15.4	15.3	15.3	-

[a]U-cas was consisted an aqueous solution of $CaSO_4$ (1.35 g/mL) and 60 g urea.
[b]Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.
[b]Metabolizable [c]Metabolizable energy (ME) was calculated according to the equation of Robinson et al. (2004) as: ME (MJ/kg DM) = 0.82×(((2.4×crude protein (CP)) + (3.9× ether extractEE) + (1.8×organic matter (OM))) ×*in vitro* organic matter digestibility (ivOMD)) where CP, EE and OM are in g/kg DM and ivOMD values obtained from our previous *in vitro* study with mean values of 540 g/kg DM.
*Urea N in U-cas is over-estimating CP. U-cas contained CP at 1690 g/kg U-cas.

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Table 3
Influence of different levels of urea calcium sulphate mixture (U-cas) in feed block ~~(FB)~~ on feed intake, nutrient intake and apparent digestibility in Thai native beef cattle

	Supplementation of U-cas in feed blocks~~FB~~, g/kg DM				SEM	P value
	0	120	150	180		
DM intake						
Rice straw						
kg/day	2.1^{a}	2.1^{a}	2.2^{a}	2.5^{b}	0.~~05~~01	0.03
g/kg BW$^{0.75}$	75.4^{a}	78.3^{a}	82.1^{ab}	94.8^{b}	~~5.50~~3.33	0.02
Concentrate						
kg/day	0.6~~0~~	0.6~~0~~	0.6~~0~~	0.6~~0~~	0.02	0.55
g/kg BW$^{0.75}$	21.3	23.2	23.2	24.2	4.32	0.65
Feed blocks~~HQFB~~						
kg/day	0.30	0.30	0.30	0.30	0.01	0.39
g/kg BW$^{0.75}$	10.8	11.2	11.2	11.4	2.98	0.58
Total intake						
kg/day	3.0	3.0	3.1	3.4	0.32~~02~~	0.17
g/kg BW$^{0.75}$	107.4^{a}	112.7^{a}	116.5^{ab}	130.3^{b}	~~7~~40.47	0.02
Nutrient intake, kg/d						
Dry matter	3.0	3.0	3.1	3.4	0.43	0.41
Organic matter	2.6	2.6	2.7	3.0	0.98	0.39
Crude protein	0.24	0.24	0.24	0.25	0.65	0.18
aNeutral detergent fiber	1.6	1.6	1.7	1.9	1.11	0.48
Acid detergent fiber	1.2	1.2	1.2	1.4	0.87	0.59
Estimated energy intake						
DOMI^{c} , kg/d	1.8	1.8	1.9	2.2	0.27	0.67
DOMR^{d}, kg/d	1.2	1.2	1.3	1.4	0.13	0.38
ME, MJ/d	28.5^{a}	28.5^{a}	31.0^{ab}	34.7^{b}	1.51	0.02
ME, MJ/kg DM	9.6	9.6	10.0	10.0	0.56	0.45
Apparent digestibility, kg/ kg DM						
Dry matter	0.65^{a}	0.65^{a}	0.66^{ab}	0.69^{b}	0.012	0.03
Organic matter	0.69^{a}	0.69^{a}	0.72^{ab}	0.73^{b}	0.014	0.04
Crude protein	0.63^{a}	0.64^{a}	0.66^{ab}	0.67^{b}	0.012	0.02
aNeutral detergent fiber	0.54^{a}	0.55^{a}	0.61^{b}	0.63^{b}	0.011	0.03
Acid detergent fiber	0.43	0.42	0.44	0.44	0.096	0.08

[a,b]Means in the same row with different superscripts differ ($P<0.05$)
[c]DOMI=Digestible organic matter intake.
[d]DOMR=Digestible organic matter fermented in the rumen.

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Table 4
Ruminal pH, rumen temperature and concentrations of rumen ammonia-N (NH_3-N) and volatile fatty acids (VFA), and of cattle fed different levels of urea calcium sulphate mixture (U-cas) in feed block ~~(FB)~~

	Supplementation of U-cas in ~~FB~~ feed blocks, g/kg DM				SEM	P value
	0	120	150	180		
Ruminal pH						
0 h post feeding	6.9	6.7	6.6	6.7	0.23	0.22
4 h post feeding	6.6	6.5	6.5	6.5	0.19	0.18
Changes (4 h–0 h)	-0.30	-0.20	-0.10	-0.20	0.09	0.08
Mean	6.8	6.6	6.6	6.6	0.51	0.37
Ruminal temperature, °C						
0 h post feeding	39.1	39.2	39.3	39.1	1.21	0.91
4 h post feeding	39.4	39.5	39.5	39.6	1.90	0.82
Changes (4 h–0 h)	0.30	0.30	0.20	0.50	0.23	0.29
Mean	39.3	39.4	39.4	39.4	1.54	0.46
NH_3-N, mg/dl						
0 h post feeding	16.2	16.8	15.9	16.1	0.98	0.19
4 h post feeding	25.9[a]	23.3[ab]	20.2[b]	19.7[b]	1.57	0.04
Changes (4 h–0 h)	9.7[a]	6.5[ab]	4.3[b]	3.6[b]	0.54	0.02
Mean	21.1[a]	20.1[ab]	18.1[b]	17.9[b]	1.00	0.03
Total VFA, mmol/l						
0 h post feeding	111.2	109.3	115.2	116.3	12.22	0.21
4 h post feeding	123.2	124.2	124.0	121.2	15.54	0.32
Mean	117.2	116.8	119.6	118.8	13.21	0.14
VFA, mol/ 100 mol						
Acetic acid ~~(C2)~~						
0 h post feeding	70.1	71.2	70.1	69.3	7.87	0.89
4 h post feeding	72.7	73.4	72.0	71.1	9.76	0.55
Mean	71.4	72.3	71.1	70.2	8.23	0.24
Propionic acid ~~(C3)~~						
0 h post feeding	18.6	17.9	19.9	19.8	0.74	0.22
4 h post feeding	18.5[a]	19.6[a]	21.4[ab]	23.1[b]	1.13	0.04
Mean	18.6[a]	18.8[a]	20.7[ab]	21.5[b]	0.81	0.03
Butyric acid ~~(C4)~~						
0 h post feeding	11.3	10.9	10.0	10.9	1.21	0.10
4 h post feeding	8.8	7.0	6.6	5.8	1.08	0.49
Mean	10.1	9.0	8.3	8.4	1.19	0.77
Acetic: propionic acid~~C2/C3~~ ratio	3.8[a]	3.9[a]	3.4[b]	3.3[b]	0.05	0.02
Acetic plus butyric: propionic acid~~C2+C4/C3~~ ratio	4.4[a]	4.3[a]	3.8[b]	3.7[b]	0.07	0.03

[a,b] Means in the same row with different superscripts differ (P<0.05)

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การใช้ยูเรียปลดปล่อยช้าแทนยูเรียในอาหารอัดก้อนต่อการย่อยสลายได้ของโภชนะและคุณลักษณะกระบวนการหมักในรูเมนของโคพื้นเมืองไทย

Replacement of urea with slow-release urea in feed block on nutrient digestibility and rumen fermentation characteristics in Thai native cattle

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บทคัดย่อ: การวิจัยครั้งนี้มีวัตถุประสงค์ เพื่อศึกษาผลของการทดแทนยูเรียด้วยสารผสมยูเรีย-แคลเซียมซัลเฟต (urea-calcium sulphate mixture; U-cas) ในสูตรอาหารอัดก้อน ต่อการย่อยสลายได้ของโภชนะ และคุณลักษณะกระบวนการหมักในรูเมนโดยใช้โคเนื้อพื้นเมืองไทย เพศผู้ จำนวน 4 ตัว น้ำหนักเฉลี่ย 100±3.0 กิโลกรัมวางแผนการทดลองแบบ 4x4 Latin Square โดยมีปัจจัยทดลอง ได้แก่การทดแทนยูเรียด้วย U-cas สูตรอาหารอัดก้อนที่ระดับ 0, 12, 15 และ 18% ตามลำดับผลการทดลองพบว่าการกินได้รวมของอาหารหยาบมีค่าสูงในกลุ่มที่ทดแทนด้วย U-cas เมื่อเปรียบเทียบกับกลุ่มที่ได้รับการเสริมยูเรียเพียงอย่างเดียว อย่างไรก็ตาม ปริมาณการกินได้ของอาหารอัดก้อนไม่มีความแตกต่างกัน การย่อยได้ของวัตถุแห้ง อินทรียวัตถุ โปรตีนหยาบ และ เยื่อใย NDF มีค่าสูงขึ้นในสัตว์ที่ได้รับการเสริม U-cas ในอาหารอัดก้อนที่ระดับ 18% ในขณะที่การเสริม U-cas ไม่มีผลต่อการย่อยได้ของเยื่อใย ADF การทดแทนยูเรียด้วย U-cas ที่ระดับ 18% ทำให้ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจนลดลง (P<0.05) ขณะเดียวกันการเสริม U-cas ที่ 18% สามารถเพิ่มประสิทธิภาพในการสังเคราะห์จุลินทรีย์ 84.6 กรัมต่อวันเมื่อเปรียบเทียบกับกลุ่มที่ได้รับการเสริมยูเรียเพียงอย่างเดียว (P<0.05) นอกจากนี้การเสริม U-cas ทำให้ประชากรของแบคทีเรียและเชื้อรามีค่าเพิ่มขึ้นรวมทั้งสามารถปรับปรุงสัดส่วนความเข้มข้นของกรดโพรพิโอนิก ดังนั้นการทดแทนยูเรียด้วย U-cas ในสูตรอาหารอัดก้อนสามารถปรับปรุงความสามารถในการย่อยได้ของโภชนะ และกระบวนการหมักในรูเมนในโคพื้นเมืองไทยที่ได้รับฟางข้าว

คำสำคัญ: กระบวนการหมัก, การย่อยได้, รูเมน, สารผสมยูเรีย-แคลเซียมซัลเฟต, โคเนื้อ

ABSTRACT: The objective of this study was to evaluate the replacement effect of urea with urea-calcium sulphate mixture (U-cas)in feed block on nutrient digestibility and rumen fermentation characteristics in Thai native cattle. Four Thai native cattle with 100±3.0 kg BW were randomly assigned in a 4×4 Latin square design. The replacement of urea with U-cas in feed block at 0, 12, 15 and 18% DM, respectively. It was found that total intake of roughage was higher in U-cas when compared with urea treatment while feed block intake was not changed. Digestibilities of DM, OM, CP and NDF were increased in the replacement with U-cas whereas digestibility of ADF was similar among treatments. Replacement of urea by U-cas could reduce ammonia-N concentration (P<0.05) and supplementation of U-cas at 18% improved efficiency of microbial protein synthesis at 84.6 g/d when compared with urea fed group. Moreover, bacterial population and fungal zoospores were enhanced with U-cas inclusion. Proportion of propionic acid was improved when animal fed with U-cas. Therefore, replacement of urea with U-cas could improve nutrient digestibility and rumen fermentation in Thai cattle fed with rice straw

Keywords: fermentation, digestibility, urea-calcium sulphate mixture, cattle

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บทนำ

อาหารอัดก้อน (feed block) เป็นผลิตภัณฑ์อาหารสำหรับโคเนื้อ ผลิตขึ้นโดยใช้ กากน้ำตาล และยูเรียเป็นหลัก (Foiklang et al., 2011) ซึ่งทำให้มีปริมาณโภชนะด้านไนโตรเจนและแหล่งพลังงานที่สลายได้ง่าย อันเป็นประโยชน์เมื่อนำไปเสริมให้กับโคเนื้อได้เลียกิน โดยเฉพาะอย่างยิ่งการนำไปใช้สำหรับการสังเคราะห์จุลินทรีย์ในรูเมน อย่างไรก็ตามการที่อาหารอัดก้อน ประกอบด้วยแหล่งไนโตรเจนจากยูเรียในปริมาณที่สูง (15-18% DM) อาจจะส่งผลทำให้การย่อยสลายของยูเรียสู่แอมโมเนียมีอัตราที่รวดเร็ว และส่งผลให้แบคทีเรียในกระเพาะรูเมนไม่สามารถนำแอมโมเนียไปใช้ในการสังเคราะห์ของจุลินทรีย์อย่างมีประสิทธิภาพได้ ดั้งนั้น จึงเกิดการสะสมของแอมโมเนียขึ้นในร่างกายของสัตว์ นอกจากนี้แอมโมเนียที่ผลิตขึ้นและมีการขับออกนอกร่างกาย อาจส่งผลต่อการเพิ่มการผลิตแก๊สเรือนกระจก (greenhouse gas) และนำไปสู่การเกิดสะภาวะโลกร้อนได้ (Wanapat, 2009)

สำหรับทางเลือกในการใช้ประโยชน์จากยูเรียสำหรับอาหารสัตว์เคี้ยวเอื้องนั้นคือ การใช้ผลิตภัณฑ์ยูเรียที่มีการปลดปล่อยช้าๆ (slow-release urea) เช่น สารผสมยูเรีย-แคลเซี่ยมซัลเฟส (urea calcium sulphate mixtures; U-cas) ซึ่งจากการศึกษาในห้องปฏิบัติการของ Cherdthong et al. (2011a) รายงานว่าการเสริม U-casในอาหารข้นของสัตว์เคี้ยวเอื้องสามารถลดอัตราการปลดปล่อยแอมโมเนีย-ไนโตรเจนได้ นอกจากนี้ Cherdthong et al. (2011a,b) พบว่าการเสริม U-casสามารถเพิ่มประสิทธิภาพการสังเคราะห์จุลินทรีย์โปรตีน และประสิทธิภาพในการเจริญเติบโตและการให้ผลผลิตของโคเนื้อและโคนมได้ อย่างไรก็ตาม การใช้U-casแทนยูเรียในสูตรอาหารอัดก้อนยังไม่มีการศึกษามาก่อน ดังนั้นจุดประสงค์ในการวิจัยครั้งนี้ เพื่อศึกษาผลของการทดแทนยูเรียด้วย U-cas ในสูตรอาหารอัดก้อน ต่อการย่อยสลายได้ของโภชนะ และคุณลักษณะของกระบวนการหมักในรูเมนของโคพื้นเมืองไทย

วิธีการศึกษา

ใช้โคเนื้อพื้นเมืองไทย เพศผู้ จำนวน 4 ตัว น้ำหนักเฉลี่ย 100±3.0 กิโลกรัมอายุ 2 ปี วางแผนการทดลองแบบ 4x4 Latin Square โดยปัจจัยทดลองที่ศึกษาได้แก่การทดแทนยูเรียด้วย U-cas ในสูตรอาหารอัดก้อนที่ระดับ 0, 12, 15 และ 18% ตามลำดับ สำหรับ urea calcium sulphate mixtures (U-cas) จัดเตรียมตามวิธีการของ Cherdthong et al. (2011a) ส่วนอาหารอัดก้อน จัดเตรียมตามวิธีการของ Foiklang et al. (2011) และองค์ประกอบอาหารอัดก้อนแสดงดัง Table1 สัตว์ทุกตัวจะได้รับการเสริมอาหารอัดก้อนแบบเลียกินอย่างอิสระ มีการเสริมอาหารข้น (13% โปรตีน) ที่ระดับ 0.5% น้ำหนักตัว และให้ฟางกินแบบเต็มที่การทดลองออกเป็น 4 ระยะๆ ละ 21 วัน ในช่วง 14 วันแรกจะทำการเก็บข้อมูลการกินได้ และย้ายสัตว์ขึ้นกรงเมแทบอลิซึมในช่วง 7 วันสุดท้ายเพื่อเก็บมูลและปัสสาวะทั้งหมด (total collection technique) รวมทั้งเก็บตัวอย่างอาหาร โดยตัวอย่างที่ได้นำไปวิเคราะห์องค์ประกอบทางเคมี ได้แก่ วัตถุแห้ง อินทรียวัตถุโปรตีนหยาบ เยื่อใย NDF เยื่อใย ADF ตามวิธีการของ AOAC (1995) และ Van Soest et al. (1991)ในวันสุดท้ายของแต่ละช่วงการทดลองเก็บของเหลวในรูเมนโดยการสอดท่อทางปากร่วมกับปั๊มดูด เพื่อวัดความเป็นกรด-ด่าง และอุณหภูมิของของเหลวจากรูเมนแบ่งเก็บของเหลวในรูเมนเพื่อรอการวิเคราะห์แอมโมเนีย-ไนโตรเจน กรดไขมันที่ระเหยได้ง่าย ประชาการจุลินทรีย์ในรูเมนและการสังเคราะห์จุลินทรีย์โปรตีนวิเคราะห์ข้อมูลที่ได้ทั้งหมดแบบ ANOVAโดยใช้ GLM procedure ของ SAS (1996)

ผลการศึกษาและวิจารณ์

ผลการทดลองพบว่าการกินได้รวมของอาหารหยาบมีค่าสูงในกลุ่มที่ทดแทนด้วย U-cas เมื่อเปรียบเทียบกับกลุ่มที่ได้รับการเสริมยูเรียเพียงอย่างเดียว (Table 2)ทั้งนี้อาจเป็นเพราะ การเสริม U-casจะส่งผ

ลทำให้สัตว์ได้รับคุณค่าทางโภชนะที่เหมาะสม โดยเฉพาะอย่างยิ่งแหล่งของไนโตรเจนที่ปลดปล่อยอย่างช้าๆ จึงทำให้สัตว์สามารถกินอาหารหยาบได้เพิ่มมากขึ้น อย่างไรก็ตาม ปริมาณการกินได้ของอาหารอัดก้อนไม่มีความแตกต่างกัน และมีค่าใกล้เคียงกันกับการรายงานของ Foiklang et al. (2011) ที่ร่ายงานไว้ที่ระดับ 0.27-0.31 กิโลกรัมต่อวันเช่นเดียวกันกับการกินได้ของไนโตรเจนซึ่งไม่มีความแตกต่างกันทางสถิติ การย่อยได้ของวัตถุแห้ง อินทรียวัตถุ โปรตีนหยาบ และเยื่อใย NDF มีค่าเพิ่มขึ้น($P<0.05$) ในสัตว์ที่ได้รับการเสริม U-casในอาหารอัดก้อนที่ระดับ 18% ในขณะที่การเสริม U-casไม่มีผลต่อการย่อยได้ของเยื่อใย ADF ในการปรับปรุงความสามารถการย่อยได้ของโภชนะเมื่อสัตว์ได้รับการเสริม U–casที่ระดับ 18% แสดงให้เห็นว่าสัตว์ได้รับแหล่งของไนโตรเจนที่ใช้ประโยชน์ได้อย่างเพียงพอเพื่อนำไปใช้ในการสังเคราะห์จุลินทรีย์ร่วมกับแหล่งของพลังงานในสูตรอาหารอัดก้อน ดั้งนั้นจึงส่งผลโดยตรงต่อการย่อยสลายโภชนะเพิ่มขึ้น ที่สัตว์ได้รับเข้าไป(Cherdthong et al., 2013) นอกจากนี้ การเสริมกากน้ำตาลในสูตรอาหารอัดก้อนอาจเป็นอีกปัจจัยหนึ่งที่จะส่งผลต่อการนำไปใช้เป็นแหล่งพลังงานในกระบวนการสังเคราะห์จุลินทรีย์ได้ สอดคล้องกับการทดลองของ Cherdthong et al. (2011a) รายงานว่าการเสริม U-casในสูตรอาหารข้นสำหรับโคเนื้อสามารถปรับปรุงความสามารถในการย่อยได้ของโภชนะในโคที่ได้รับฟางข้าวเป็นแหล่งอาหารหยาบหลัก

ความเป็นกรดด่างในรูเมน และอุณหภูมิ มีค่าเฉลี่ยที่ 6.5 และ 39.4 °C ซึ่งถือว่าอยู่ในช่วงที่ปกติ และเหมาะสมต่อการทำงานของจุลินทรีย์ในกระเพาะรูเมน (Table 3)การทดแทนยูเรียด้วย U-casที่ระดับ 18% ในอาหารอัดก้อนพบว่าจะทำให้ค่าความเข้มข้นของแอมโมเนีย-ไนโตรเจนมีค่าลดลง ($P<0.05$)อาจเนื่องมากจากการเสริม U-casซึ่งเป็นผลิตภัณฑ์ที่มีคุณสมบัติในการปลดปล่อยไนโตเจนอย่างช้าๆ เมื่อเปรียบเทียบกับยูเรีย ดังนั้นเมื่อมีการเสริมยูเรียอย่างเดียวจึงทำให้ค่าแอมโมเนียไนโตรเจนสูงกว่า และอาจจะส่งผลในทางลบต่อการนำไปใช้ประโยชน์ในตัวสัตว์เองได้ ในขณะเดียวกันการที่มีไนโตรเจนปลดปล่อยอย่างช้าจะทำให้จุลินทรีย์สามารถนำไนโตรเจนไปใช้ในการสังเคราะห์เซลล์จุลินทรีย์ได้ทัน และลดการสูญเสียของไนโตรเจนได้อีกทางหนึ่ง สอดคล้องกับการทดลองของ Cherdthong et al. (2011a) พบว่าการเสริม U-casเพื่อเป็นแหล่งยูเรียปลดปล่อยอย่างช้าๆ ในอาหารข้น พบว่าความเข้มข้นของแอมโมเนีย-ในโตรเจนในรูเมนมีค่าต่ำกว่าเมื่อเปรียบเทียบในกลุ่มที่มีการเสริมยูเรีย และนำไปสู่การเพิ่มขึ้นของการสังเคราะห์จุลินทรีย์ในรูเมนได้โดยจากผลการทดลองครั้งนี้พบว่าการเสริม U-casที่ 18% ในอาหารอัดก้อนสามารถเพิ่มประสิทธิภาพในการสังเคราะห์จุลินทรีย์ถึง 84.6 กรัมต่อวันเมื่อเปรียบเทียบกับกลุ่มที่ได้รับการเสริมยูเรียเพียงอย่างเดียว ($P<0.05$) นอกจากนี้การเสริม U-cas ยังทำให้ประชากรของแบคทีเรียและเชื้อรามีค่าเพิ่มขึ้นด้วย (Table 3)

การทดแทนยูเรียด้วย U-casในสูตรอาหารอัดก้อนสามารถปรับปรุงค่าความเข้มข้นของกรดไขมันที่ระเหยได้ง่าย โดยพบว่าการเพิ่มระดับของ U-casจะทำให้สัดส่วนของกรดโพรพิโอนิกสูงขึ้น ซึ่งสอดคล้องกับงานวิจัยของ Cherdthong et al. (2011b) ที่รายงานว่าการเสริม U-casในอาหารข้นสำรับโคนมสามารถเพิ่มสัดส่วนของกรดโพรพิโอนิคในรูเมนสำหรับการเพิ่มขึ้นของกรดโพรพิโอนิกจากการทดลองนี้ อาจเนื่องจากการเพิ่มขึ้นของปริมาณการกินได้และการย่อยสลายได้ในโคเนื้อ อย่างไรก็ตาม จากการทดลองนี้พบว่าการเสริม U-cas จะไม่ส่งผลต่อกรดไขมันที่ระเหยได้ง่ายรวม กรดอะซิติก และกรดบิวทิริก

สรุป

การทดแทนยูเรียด้วย U-casที่ระดับ 18% ในสูตรอาหารอัดก้อนสามารถปรับปรุงความสามารถในการย่อยได้ของโภชนะ นิเวศวิทยา กระบวนการหมักและการสังเคราะห์จุลินทรีย์ในรูเมนได้

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ขอขอบคุณสำนักงานคณะกรรมการการอุดมศึกษา (สกอ.) และสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่สนับสนุนทุนวิจัยโดยผ่านโครงการทุนอาจารย์รุ่นใหม่ในสถาบันอุดมศึกษา (สัญญารับทุนเลขที่ MRG5580077) และ ศูนย์วิจัยและพัฒนาทรัพยากรอาหารสัตว์เขตร้อน ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่นขอนแก่นที่สนับสนุนอุปกรณ์และสิ่งอำนวยความสะดวกต่างๆ

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Influence of Urea-Calcium Mixture in High-Quality Feed Block on Ruminal Fermentation in Swamp Buffalo

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ABSTRACT

The effect of levels of urea calcium sulphate mixture (U-cas) in high-quality feed block (HQFB) on ruminal digestibility of nutrients, fermentation end-products and kinetics of gas production in rumen fluid of swamp buffalo by using in vitro gas production techniques were investigated. The dietary treatments were 7 levels of U-cas supplementation in HQFB at 0, 3, 6, 9, 12, 15 and 18%. The result revealed that gas production from soluble fractions (a) and gas production from the insoluble fraction (b) were not changed (P>0.05), while gas production rate constants for the insoluble fraction (c) and potential extent of gas production (a+b) were linearly increased when increasing level of U-cas in HQFB (P<0.05). The c value was highest at 0.09 ml/h when supplementation 18% of U-cas in the HQFB. The cumulative gas production (96 h) was significantly different among treatments and was linearly highest when HQFB contained of 18% U-cas (102.3 ml/0.5 g DM substrate). The in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), true digestibility and microbial mass were altered by treatments (P<0.01) and were greatest at 18% of U-cas supplementation. The NH_3–N concentration were highest when urea was supplemented in HQFB while concentrations tended to be reduced with increasing level of U-cas (P<0.05). The finding suggests that the supplementation of U-cas in HQFB resulted in improved in vitro kinetics gas production, rumen fermentation, microbial mass and digestibility as well as could control the rate of N degradation in the rumen and leading to a slow rate of NH_3-N released.

Keywords: Ammonia-nitrogen, buffalo, feed block, gas production technique, in vitro digestibility, slow-release urea

INTRODUCTION

High-quality feed block (HQFB) have been used as strategic supplements for ruminants and have been developed to contain local feed ingredients particularly those from different energy sources (e.g. molasses, rice bran), essential minerals (S, Na, P) and NPN source or urea (Foiklang et al., 2011). Use of urea is attractive in ruminant diets because of its low cost, with high rumen degradability (Wanapat, 2009). However, the amount of urea that can be used in diets is limited due to its rapid hydrolysis to NH_3 in the rumen by microbial enzymes, resulting in its accumulation in the rumen and absorption though the rumen wall (Cherdthong et al., 2011a). Urea calcium sulphate mixtures (U-cas), ruminal slow urea release properties, have been achieved by using urea binding to substrates such as calcium sulphate to control its release rate (Cherdthong et al., 2011a). Cherdthong et al. (2011b) reported that supplementation of U-cas in the concentrate diets were shown to reduce ruminal NH_3 concentrations, improve feed intake, nutrient digestibility, the cellulolytic bacterial population, as well as milk yield in ruminants.

Therefore, the aim of this study was designed to determine effect of levels of U-cas in HQFB on ruminal digestibility of nutrients, fermentation end-products and kinetics of gas production by using in vitro gas production techniques.

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MATERIALS AND METHODS

Seven high-quality feed blocks (HQFB) were formulated and the experimental design was a completely randomized design (CRD). The dietary treatments were 7 levels of urea calcium sulphate mixture (U-cas; 0, 3, 6, 9, 12, 15 and 18%) in HQFB. U-cas products were prepared according to Cherdthong et al. (2011a). The amounts of ingredients for producing HQFB were shown in Table 1. All ingredients were mixed well together and then were pressed into blocks (Foiklang et al., 2011). The sample of HQFB, roughage and concentrate were dried at 60ºC, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the in vitro gas test.

Two male, rumen-fistulated swamp buffaloes with an initial body weight of 350± 50 kg) were used as rumen fluid donors. Swamp buffalo rumen fluid was collected and was prepared for artificial saliva was done according to Menke and Steingass (1988). The 70:30 roughage and concentrate ratio were used as substrates at 0.47 g with 0.03 g of respective HQFB and samples of 0.5 mg were weighed into 50 ml serum bottles. For each treatment, five replications were prepared. Ruminal fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (ml/ml) at 39 ºC under continuous flushing with CO_2 and 40 ml of rumen inoculum mixture were added into each bottle under CO_2 flushing.

During the incubation, data of gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows: $y = a+b\ (1-e^{-ct})$. Inoculum ruminal fluid was sampled at 0, 2, 4, 6, 12 and 24 h post inoculations were analysed for ammonia- nitrogen (NH_3-N), in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), in vitro true digestibility and microbial mass by using standard method.

All data from the experiment were analyzed as a completely randomized design using the GLM procedure of SAS (1998).

RESULTS AND DISCUSSIONS

Table 1 presents the chemical compositions of HQFB and crude protein (CP) contents for HQFB products were ranged from 34.8 to 35.5% and were similar to those reported by Foiklang et al. (2011). Urea was replaced by U-cas up to 100% in HQFB (18% of U-cas) and, therefore, relatively more slowly release to ammonia than urea and could potentially be used more efficiently by rumen microorganisms (Cherdthong et al., 2011a,b). The gas kinetics and cumulative gas production of substrates studied are presented in Table 2. Gas production from soluble fractions (a) and gas production from the insoluble fraction (b) were not changed (P>0.05), while gas production rate constants for the insoluble fraction (c) and potential extent of gas production (a+b) were linearly increased when increasing level of U-cas in HQFB (P<0.05). The c value was highest at 0.09 ml/h when supplementation 18% of U-cas in the HQFB. In vitro cumulative gas production techniques were developed to predict fermentation of ruminant feedstuffs. Similarly, cumulative gas production (96 h) was significantly different among treatments and was linearly highest when HQFB contained of 18% U-cas (102.3 ml/0.5 g DM substrate). The in vitro gas production technique has a remarkable boost and data on fermentation kinetics of numerous feeds are available (Infascelli et al., 2005). Under this study, improved performance of kinetics gas could be due attributed to fermentable energy as molasses and NPN source from 18% U-cas in HQFB, may have provided on a continuous NH_3-N basis, additional and essential nutrients needed for rumen microbes. These results were similar to our previous work by Cherdthong et al. (2011a), which supplemented U-cas with cassava chip as an energy source in concentrate diets results in an increased c and a+ b value of the inoculums as well as cumulative gas production. This will indicate the availability of readily fermentable materials as a ready energy and protein sources which will stimulate the activity of the rumen microorganisms which in turn would accelerate the production of gas volumes.

The in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), true digestibility and microbial mass were altered by treatments ($P<0.01$) and were greatest at 18% of U-cas supplementation. As the result, high gas production in 18% of U-cas indicated high digestibility of substrates. Moreover, higher in vitro true digestibility reflects higher microbial biomass, the result was also found in 18% of U-cas supplementation in HQFB (Table 2). This could possibly be that U-cas was more slowly release to ammonia than urea and could potentially be used more efficiently by rumen microorganisms. These results were in agreement with Cherdthong et al. (2011a), who reported that supplementation of urea calcium mixture product as a slow release NPN source in concentrate diet could improve digestibility and microbial mass in in vitro experiment. Moreover, the digestibility of fiber and cellulolytic bacterial population were enhanced when dairy cows or beef cattle fed with U-cas (Cherdthong et al., 2011b).

The NH_3–N concentration were highest when urea was supplemented in HQFB while concentrations tended to be reduced with increasing level of U-cas ($P<0.05$). This could be due to U-cas that could control the rate of N degradation in the rumen and leading to a slow rate of NH_3-N released when compared with 100% of urea in HQFB. In agreement with these observations, Cherdthong et al. (2011a,b) reported that supplementation of U-cas as slow-release urea in concentrate diet reduces the rapidity of ammonia release in the rumen without affecting other ruminal fermentation parameters.

Based on the results of this experiment, supplementation of U-cas supplementation at 18% DM in high quality feed block resulted in improved in vitro kinetics gas production, rumen fermentation, microbial mass and digestibility. Moreover, U-cas could control the rate of N degradation in the rumen and leading to a slow rate of NH_3-N released. However, in in vivo study in order to improve production efficiency of ruminant animals still warrant further research.

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Table 1. Ingredients and chemical compositions of high-quality feed block (HQFB) were used an in vitro experiment.

Items	% of urea-calcium mixture (UCM) in HQFB						
	0	**3**	**6**	**9**	**12**	**15**	**18**
Ingredients, kg DM	--------------------------------%DM------------------------------------						
Rice bran	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Molasses	42.5	41.0	40.0	39.5	39.0	38.0	38.0
Urea	10.5	9.0	7.0	5.5	3.5	2.0	0.0
UCM	0.0	.0	6.0	9.0	12.0	15.0	1 .0
Cement	12.0	12.0	12.0	12.0	11.5	11.0	10.0
Sulfur	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Mineral premix	1.5	1.5	1.5	1.0	1.0	1.0	1.0
Tallow	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chemical composition							
Dry matter	74.2	74.0	73.9	73.6	73.3	73.1	73.0
	--------------------------------%DM--------------------------------------						
Organic matter	76.5	76.3	75.1	74.9	76.6	75.3	75.7
Crude protein	34.9	35.2	35.5	35.4	34.8	35.3	35.5
Ash	23.5	23.7	24.9	25.1	23.4	24.7	24.3
Neutral detergent fiber	14.2	15.6	15.4	14.5	15.8	14.9	14.6
Acid detergent fiber	8.2	8.6	8.5	9.0	9.2	8.3	9.4

Table 2. The effect of levels of urea-calcium mixture (UCM) in high-quality feed block (HQFB) on gas kinetics, ruminal fermentation and digestibility.

Items	% of UCM in HQFB							SEM	P-value
	0	**3**	**6**	**9**	**12**	**15**	**18**		
Gas kinetics									
A	-2.3	-2.1	-2.4	-2.6	-2.2	-2.4	-2.3	2.1	ns
B	103.1	108.7	110.2	109.9	109.7	110.8	112.4	7.2	ns
C	0.03	0.03	0.06	0.07	0.07	0.06	0.09	0.03	*
a+b	100.1	103.4	106.8	107.4	107.4	109.9	110.2	2.5	**
Gas volume, ml	93.4	93.2	95.5	95.6	94.3	97.8	102.3	2.4	*
In vitro degradability, %									
IVDMD	55.3	55.9	57.1	59.4	60.7	62.6	62.6	1.5	*
IVOMD	57.3	57.9	59.0	60.7	61.9	63.6	64.4	2.1	*
True digestibility, %	57.4	58.9	59.1	62.1	62	65.4	65.7	1.5	**
Microbial mass, mg	18.7	18.9	19.0	19.0	22.2	22.8	25.6	0.4	*
NH_3-N, mg%									
0 h incubation	18.2	16.7	16.3	15.6	14.2	14.5	13.3	3.5	ns
2 h incubation	24.4	21.2	22.8	20.5	18.9	18.1	16.2	2.1	*
4 h incubation	29.5	25.6	24.5	24.5	22.3	19.8	18.1	1.4	*
6 h incubation	27.7	24.1	23.4	22.0	21.1	17.2	16.5	2.3	*
Mean	25.0	21.9	21.8	20.7	19.1	17.4	16.0	1.2	*

*$p < 0.05$, **$p < 0.01$, ns = non- significant differences.

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Nutritional status of some trace minerals of water buffaloes in Egypt

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ABSTRACT

The domesticated water buffalo (Bubalus bubalis) is very important animal in Egypt as it serves as dual purpose animal providing meat and milk for human and as draft animal. Despite the economical importance of buffaloes in Egypt, they are subjected to different environmental, managemental and nutritional stressors which negatively affect their performance. A study was conducted to evaluate the nutritional status of some trace-minerals (copper, iron &zinc) of male buffalo calves in relation to their contents in the commonly available feedstuffs in Mid-Delta district of Egypt. Fifty blood samples were collected from apparently healthy male buffalo calves with an average age ranged between 5 to8 months during the winter season from several private farms at the designated district of the study. The present study indicated that the commonly cultivated feedstuffs in the Mid- Delta district of Egypt have critical levels of copper and zinc reflected in the marginal levels of such minerals in the serum of the examined male buffalo calves which exhibited silent symptoms except of low gain. Iron content in the available feedstuffs supplied surplus Fe, so that the animals did not exhibiting any symptoms of iron deficiency. In conclusion, the levels of the copper and zinc in the Egyptian feedstuffs cultivated in the district of the study should be investigated on soil basis so mineral supplements must to be added. Moreover, it seemed that the requirements of buffalo calves for these minerals are less than expected and buffaloes adapted well with such marginal deficiencies in feedstuffs.

Keywords: Buffalo, Egypt, Copper, Iron, Zinc, Feedstuffs

INTRODUCTION

The domesticated water buffaloes (Bubalus bubalis) account for 170million in the world (FAO, 2004), with 97% in Asia and 2% in Africa mainly Egypt. There are two general types; the Swamp buffalo and River buffaloes. In Egypt, River buffaloes play an important role in the rural economy as suppliers of milk and draft power. Despite the economical importance of buffaloes in Egypt, they are subjected to different environmental, managemental and nutritional stressors which negatively affect their performance. Essential trace minerals such as copper, iron, and zinc are of utmost importance in regulating animals metabolism and their deficiencies cause great economical losses in animal production. Copper is essential for osteogenesis, hematopoiesis and mylination of nerve cells. Cu deficiency is the most common micro-mineral deficiency for the grazing livestock worldwide. Cu deficiency exhibits different symptoms ranged from anemia, retard growth, diarrhea, loss of hair growth and pigment, long bone affection to silent infertility (McDowell, 1997). In many species, hidden (subclinical) copper deficiency is far less dramatic, but economically very important as it effects on live weight gain, especially cattle. Iron is a component of heme compounds, and it enters in the several enzyme systems regulating body metabolism. Commonly, Fe deficiency is greatly related to great morbidity and mortality associated with depressed immunity

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Welcome to WCAP 2013 in Beijing – China

On behalf of the Chinese Association of Animal Science and Veterinary Medicine (CAAV), I would like to invite you to the 11th World Conference on Animal Production (11th WCAP) which will be held in Beijing from October 15 to 20, 2013. This is the first time that China has hosted such a grand international conference in the area of animal production.

The theme of the Conference is "Animal, People and Environment in Harmony for Progress". This theme is focusing on the competition among animals, humans and their living environment. This is a crucial topic. In the last 30 years, China has made magnificent progress in animal production increasing the world's production. This program will cover all scientific achievements and efforts that we have conduced to both raising humans and protecting our living environment.

Since the 10th WCAP in Cape Town, South Africa when CAAV earned the right to host the 11th WCAP, the Organizing Committee has worked to prepare this grand scientific conference. During the preparation, we have received considerable enthusiastic help from China Association for Science and Technology, the Ministry of Agriculture, China Agricultural University and the WAAP president and vice presidents, the Korean Society of Animal Science and Technology, and the American Society of Animal Science. To express our grateful appreciation to them, we have invited all vice presidents in charge of five continents of WAAP to this Conference. We have provided financial aid to 15 young scientists, 20 graduate students and partly supported 2 students from Asian developing countries. We have invited 54 senior scientists to present talks at the Conference. We have attempted to create a grand unite for the worldwide scientists in Beijing. We thus are happy to see delegates from 54 countries with more than 1057 contributed papers at the Conference.

During the Conference, beside the thorough communication on world animal production, we will have a day for industry communication and exhibition to demonstrate the fabulous development of industry in the area of animal production. We would also like to offer several opportunities for technical visits for your experience in Beijing. All delegates and accompanying people will enjoy numerous local tours and pre-/post- conference tours.

Lastly, representing the organizing committee, I would like to express our heartily welcome to all of you to this grand unite. Our efforts will contribute to the brilliant future of worldwide animal production.

Defa Li *Defa Li*
President, 11th WCAP Organizing Committee
Vice President, Chinese Association of Animal Science and Veterinary Medicine (CAAV)
Professor, China Agricultural University (CAU)

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Abstract No.	Presenter	Title
WCAP2013-2-02-084	*Daiwen Chen*	Effects of excess daidzein supplementation in diets on growth performance, organic and intestinal health and tissue distribution of several primary Isoflavones in pigs
WCAP2013-2-02-085	*Huiling Mao*	Synergistic effect of cellulase and xylanase on *in vitro* rumen fermentation and microbial population with rice straw as substrate
WCAP2013-2-02-086	*Wenhan Yang*	High-level exprssion of *Aspergillus sulphureus* xylanase gene xynA in *pichia pastoris*
WCAP2013-2-02-087	*Woong B. Kwon*	Effect of various inclusion levels of β-mannanase on nutrient digestibility in diets consisted of corn, soybean meal, and palm kernel expeller fed to pigs
WCAP2013-2-03-002	*Máikal S. Borja*	Development of fungi used in enzymatic industry decrease the fiber in physic nuts cake
WCAP2013-2-03-003	*Anusorn Cherdthong*	Potential use of a slow release urea product in a high-quality feed block as strategic supplements for Thai-native beef cattle fed on rice straw
WCAP2013-2-03-004	*Seyed A. Mirghelenj*	Correlation between protein solubility in KOH of full fat soybean extruded at three temperatures and biological performance of broiler chickens
WCAP2013-2-03-005	*Safa Zhaleh*	Intestinal microflora and anti-oxidative status of broiler chickens fed extruded soybeans
WCAP2013-2-03-007	*Mao Li*	Effect of additives on silage fermentation and *in vitro* gas production of King Grass
WCAP2013-2-03-008	*Viengsakoun Napasirth*	The use of lactic acid bacterial inoculants on grass and forage silage fermentation characteristics in Lao PDR.
WCAP2013-2-03-011	*Jianmin Yuan*	Effects of storage time on the nutritional value of maize, and performance and meat quality of broilers
WCAP2013-2-03-012	*Xijiu Jin*	Effects of fertilization and preparation after harvesting rice on dietary cation anion differences and feed composition of rice straw
WCAP2013-2-03-013	*HongLiang Li*	Effect of physically effective neutral detergent fiber in total mixed ration on chewing activity, digestibility and ruminal pH and VFA concentration in Hanwoo (*Bos taurus coreanae*) cattle
WCAP2013-2-03-014	*Baiyila Wu*	Bacterial community associated with aerobic stability and instability of alfalfa silage
WCAP2013-2-03-015	*Naoki Nishino*	Ensiling total mixed rationa as a means of preservation and ensuring high aerobic stability of silage in the tropics

Potential Use of Slow Release Urea product in High-Quality Feed Block as Strategic Supplements for Thai-Native Beef Cattle Fed on Rice Straw

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Abstract
This research was conducted to investigate the effect of urea calcium sulphate mixture (U-cas) as slow release urea in high-quality feed block (HQFB) on intake of feeds, digestibilities of nutrients, ruminal fermentation and body weigh change in Thai-native beef cattle. Four animals with initial body weight (BW) of 101±10.2 kg were randomly assigned in a 4×4 Latin square design. The dietary treatments were U-cas supplementation in HQFB at 0, 12, 15 and 18% with rice straw fed to allow ad libitum intake and were supplemented with concentrate at 5 g/kg of BW daily. Individual block intake was recorded daily by weighing the offered and refused quantities. The findings revealed significant improvements in dry matter intake, apparent digestibilities of DM and NDF and was highest when supplementation of U–Cas in HQFB at 18% ($P<0.05$). Ruminal pH and temperature were not changed among treatments ($P>0.05$) and were resulted in normal ranges. Mean values of ruminal NH_3–N concentrations tended to be increased in the control diet. However, NH_3–N concentrations with 18% of U-cas in HQFB were slower released than those treatments ($P<0.05$) and were very stable throughout the sampling periods (12-15 mg%). Protozoal population and fungal zoospores were not significantly among treatments, while bacterial population was increased when supplementation of U-cas at the highest level in HQFB. BW change were altered with dietary treatments and was improved when HQFB contained 18% U-cas ($P<0.05$). Based on this research, it could be concluded that supplementation of U–Cas at 18% in HQFB manipulated feed intake, digestibilities of nutrients, ruminal NH_3–N concentrations, bacterial population and BW change in Thai-native beef cattle fed on rice straw.

Keywords: Body weigh change, feed intake, ruminal fermentation, ruminant, urea calcium sulphate mixture