

Estimation of Rumen Undegradable Protein with *In Situ* Nylon Bag and *In Vitro* Enzymatic Technique in Tropical Concentrate Feedstuffs

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ABSTRACT

Seventeen concentrate feedstuffs were used to study the relationships between the *in vitro* enzymatic technique and the *in situ* nylon bag technique for rumen undegradable protein determination. Feedstuffs were divided into 6 groups, 1) energy feed, 2) all protein feed, 3) feed higher than 15% crude protein (CP), 4) feed higher than 20% CP 5) feed lower than 20% CP and 6) all test feed. It was found that all test feed had the lowest relationship ($R^2 = 0.16$, $P < 0.1$, $n = 17$). On the other hand, the group of higher than 15% CP feed had the highest relationship ($R^2 = 0.79$, $P < 0.0002$, $n = 9$). The coefficient of determination (R^2) of the energy feed, all protein feed, higher than 20% CP feed and lower than 20% CP feed were 0.66, 0.73, 0.74 and 0.58, respectively. This result suggested that the enzymatic technique can be used to predict the *in situ* nylon bag technique when the group of feed was separated by protein levels. Therefore, the enzymatic technique is an alternative technique for rumen undegradable protein determination in tropical concentrate feedstuffs, particularly those with high protein feed ($>15\%CP$).

Key words: Rumen undegradable protein - Nylon bag technique -
Enzymatic technique - Concentrate feedstuffs

INTRODUCTION

Degradation and digestion characteristics are useful information to evaluate feedstuffs in ruminant nutrition. New feeding systems for ruminants evaluate feed protein from two different aspects; the first one is rumen microbial degradability of the protein and the second one is intestinal digestibility (1). Rumen protein degradability is estimated usually by the *in situ* nylon bag technique (2), by protein solubility (3), or by the enzymatic technique (4,5,6). Although, the *in situ* nylon bag technique is a standard method widely used for this purpose, it requires labor, cost and time. In addition it needs fistulated animals which may be limited in routine research (5). In recent years, a proteolytic enzymatic method as an alternative for routine estimation of effective degradability study has become widely accepted (4,5,6,7).

Currently, the varieties of proteases being used are ficin (8), bromelain (9), and rumen proteolytic bacteria (10) such as *Bacillus subtilis* (11) and *Streptomyces griseus* (7).

With respect to tropical feedstuffs, limited information is available on degradation characteristics and rumen undegradable protein value. The aim of this study was to investigate the relationship between the *in situ* nylon bag technique and *in vitro* enzymatic technique in evaluating the undegradable protein in tropical concentrate feedstuffs.

MATERIALS AND METHODS

Feedstuff Samples and Chemical Analysis

Seventeen concentrate feedstuffs were collected from various feed mills and organizations in the North East of Thailand. All concentrate feed samples (**Table 1**) were ground through a 1 mm screen. They were analyzed for dry matter (DM), crude protein (CP), ash (12), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (13).

Grouping of Feedstuffs

Feedstuffs were categorized into six groups according to the level of crude protein as: 1. energy feed, (corn meal, cassava chip, broken rice, rice pollard and rice bran), 2. all protein feed (palm meal mech-extd, palm meal solv-extd, kapok seed meal, soybean meal, leucaena leaf meal, coconut meal mech-extd, coconut meal solv-extd, peanut meal, mung bean meal, dried brewer's grain, whole cotton seed, and fish meal), 3. feed higher than 15% CP (palm meal solv-extd, kapok seed meal, soybean meal, coconut meal solv-extd, peanut meal, mung bean meal, dried brewer's grain, whole cotton seed, and fish meal) 4. feed higher than 20% CP (kapok seed meal, soybean meal, coconut meal solv-extd, peanut meal, whole cotton seed, and fish meal), 5. feed lower than 20% CP (palm meal mech-extd, palm meal solv-extd, leucaena leaf meal, coconut meal mech-extd, mung bean meal, dried brewer's grain), and 6. all test feed.

In Situ Nylon Bag Measurement

Two Brahman-Thai native crossbred beef steers with an average body weight of 250 ± 15 kg were fitted with a permanent rumen cannula and offered rice straw *ad libitum*. They were fed with concentrate (49.80% cassava chips, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0% mineral mix and 8.30% sugarcane molasses) at 0.5% body weight. A ruminal degradation measurement using the nylon bag technique was carried out after a two-week adaptation period.

Approximately 5.0 g (as fed basis) of each test feed was accurately weighed into a synthetic bag with a mean pore size of 45 μ m (14). The bag plus the sample were placed into the rumen of the beef steers, 30 min after the morning meal and retrieved after periods of 2, 4, 6, 12, 24 and 48 h (four bags of each feed for each period). After removal from the rumen, the bags were rinsed with pipe line fresh water and washed by hand under tap water until the rinse water became clear. After washing,

the bags were placed into a hot, dry, forced air oven at 65°C for 48 h and weighed. To determine the content of water soluble material, four bags representing 0 h degradation were washed using the same washing procedure as the incubated bags. The dried residue from the same incubation period of each steer was pooled and analyzed for DM and CP. Crude protein disappearance values were calculated as the difference between the weight of the nutrient before and after incubation of each sample. Crude protein degradability of each feed were calculated from the equation $P = a + b(1 - e^{-ct})$ (2).

The effective degradability (ED) was calculated as $P = a + [(bc)/(c + k)]$, where a = rapidly soluble fraction, b = potentially degradable fraction, c = rate of degradation of b fraction, k = fractional passage rate (5%/h) (2). The rumen undegradable protein was calculated as %RUDP = 100 - %ED.

***In Vitro* Undegradable Intake Protein Measurement**

Crude protein contents of the feed were determined by the Kjeldahl method. Weigh out approximately 0.2 g feed protein of air dry sample into a 125 ml Erlenmeyer flask (example: soybean meal (54%CP) = 0.37 g of sample use). The sample was mixed with 40 ml borate-phosphate buffer (pH 6.7) and being incubated in a water bath at 39°C for 1 h. Then 10 ml of fresh protease solution was added and slightly swirled (protease solutions were prepared from protease type XIV from *Streptomyces griseus*, Sigma Chemical Co, Ltd.). The samples were removed after 18 h, filtered through Whatman 541, and washed with 250 ml distilled water. Nitrogen in the residue was estimate by the Kjeldahl method. Crude protein degradability was calculated as a percent of total CP: %CP degradability = [(initial CP - post incubate CP)/initial CP]×100. The undegrade intake protein (UIP) was calculated as %UIP = 100 - %CP degradability (4).

Statistical Analysis

Fractions a, b and c of *in situ* nylon bag were analyzed using Proc Nonlin of SAS. The regression procedure of SAS (15) was used to regress *in vitro* protease on *in sacco* nylon bag of undegradable protein for each group of feedstuffs.

RESULTS AND DISCUSSION

Chemical Composition of Feedstuffs

Chemical compositions of feedstuffs are shown in **Table 1**. Generally, there were wide variations in the chemical composition of the investigated feedstuffs. Ash contents ranged from 0.66% for broken rice to 27.84% for fishmeal. Crude protein contents ranged from 1.89% for cassava chip to 61.89% for fishmeal. Neutral detergent fiber contents ranged from 6.93% for cassava chip to 82.70% for palm meal solv-extd. Acid detergent fiber contents ranged from 0.65% for broken rice to 75.10% for palm meal mech-extd. Acid detergent lignin contents ranged from 0.1% for soybean meal to 33.47% for palm meal mech-extd. There are many factors that affect chemical composition of concentrate feedstuffs such as stage of growth maturity, species or

variety (16), drying method, growth environment (17), oil extraction process (18) and soil types (19). These factors may partially explain differences in chemical composition between our study and others.

Degradation Characteristics and Rumen Undegradable Protein of Concentrate Feedstuffs

The rumen undegradable protein (RUDP) determined by the *in situ* nylon bag technique and undegradable intake protein (UIP) determined by the *in vitro* enzymatic technique are shown in **Table 2**. There were high variations in RUDP and UIP for all test feed. A wide range of RUDP values was observed, ranging from 25.83% for whole cotton seed to 68.50% for palm meal solv-extd and dried brewer's grain. The UIP of all test feed ranged from 23.56% for kapok seed meal to 78.03% for broken rice. There are numerous factors affecting to protein degradation in rumen. They include the nature of feed protein, individual cow, feeding methods (20) and rate of passage (9). Therefore in this study, the evaluation was done at suitable rate of passage (5%/h). In the *in vitro* enzymatic technique, there were also numerous factors influencing the value, e.g. the time of incubation (6), group of feedstuffs (9), variety of enzyme (20), pH of buffer and enzyme concentration (21). In this study the standard method described by Roe et al (4) was used.

Relationship of Ruminal Undegradable Protein between *In Vitro* and *In Situ* Estimation

Regression of %UIP (determined by the *in vitro* enzymatic technique) on % RUDP (determined by the *in situ* nylon bag technique) are presented in **Table 3**. The results indicate that the highest relationship was in the group of feed higher than 15% CP ($R^2 = 0.79$, $P = 0.0002$, $n = 9$). The lowest relationship was found in the group of all test feed ($R^2 = 0.16$, $P = 0.1$, $n = 17$). The coefficient of determination (R^2) of energy feed, all protein feed, feed higher than 20% CP and feed lower than 20% CP were 0.66, 0.73, 0.74 and 0.58, respectively. Measurements of the rumen undegradable protein with proteolytic enzymes depend on the accurate estimate of regression equations between the enzymatic and *in situ* methods (20). These equations for suitable enzymes have high correlation coefficients (r) when small sets of feed are analyzed (11,22). When a larger set of feed is tested the correlation coefficients usually decrease (9,23). Tomankova and Kopečný (9) suggested that it was necessary to group feed into sets of similar nitrogen fraction properties. This is the reason for separating groups of feed in this experiment. Because, when feedstuffs were grouped by protein levels, the relationship was elevated.

Extensive research efforts have been directed to estimate undegradable protein in ruminant feed by the *in vitro* method based on the use of *S. griseus* protease (4). Cone et al (6) reported that the highest correlation ($R^2 = 0.77$) was observed between percentage of rumen undegradable protein determined with the nylon bag technique and *in vitro* undegradable protein after 1 h of incubation. While, longer incubation times weakened the relationship. Roe et al (20) compared three *in vitro* methods (*S. griseus*, ficin, and neutral protease) with the *in situ* nylon bag technique, and found that neutral protease shows the highest relationship with the *in situ* technique. Mathis et al (5) suggested the closed relationship between the 4 h and 48 h incubation time of *S. griseus* protease assays and average effective degradability value

of the *in situ* technique. The coefficients of determinations (R^2) were 0.88 and 0.87, respectively. It was in agreement with Licitra et al (21), who found that single time point of *S. griseus* assays between 4 h and 48 h are likely to be acceptable for routine analyses using the enzyme technique.

CONCLUSIONS

Rumen undegradable protein evaluated by the nylon bag and enzymatic technique in concentrate feedstuffs were found to be different. The relationship between the *in vitro* enzyme technique and the *in situ* nylon bag technique was lowest in the all test feed. However, when the group of feedstuffs was separated by protein levels, the relationship of the two measurement methods was elevated. Therefore, the enzymatic technique is an alternative technique for rumen undegradable protein determination in tropical concentrate feedstuffs particularly those of high crude protein concentration (>15% CP). This method could be more useful for routine feed evaluation without the need for a rumen fistulated animal.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. W. Angvanich for providing surgical fistulated animal, Mr. P. Rodthong for taking care of steers. We are grateful to the Department of Agricultural Technology, Faculty of Technology, Maha Sarakham University and Department of Animal Science, Faculty of Agriculture, Khon Kean University for supporting their facility. Financial support was provided by The Khon Kean University's Graduate Research Fund, Academic Year 2003.

Table 1. Chemical composition of various feedstuffs (%DM basis).

Feedstuffs	DM (%)	CP	Ash	NDF	ADF	ADL
	% DM basis.....					
Corn meal	92.20±0.05	8.53±0.10	1.69±0.02	13.25±0.17	3.63±0.06	0.41±0.03
Cassava chip	93.40±0.39	1.89±0.07	2.01±0.08	6.93±0.68	6.35±0.22	1.87±0.13
Broken rice	92.06±0.38	7.80±0.15	0.66±0.02	9.28±0.11	0.65±0.08	0.12±0.01
Rice bran	91.70±0.06	14.26±0.32	6.31±0.07	20.29±0.24	8.12±0.10	2.61±0.008
Rice pollard	90.49±0.02	8.46±0.28	14.08±0.08	61.18±0.30	45.96±1.27	11.91±0.32
Palm meal (mech-extd)	92.58±0.39	11.31±0.17	3.58±0.07	82.47±0.41	57.23±0.20	20.32±0.61
Palm meal (solv-extd)	91.04±0.02	16.68±0.08	6.94±0.04	82.70±1.70	51.41±0.88	9.09±0.02
Kapok seed meal	91.01±0.11	28.09±0.06	8.91±0.07	42.50±0.07	29.49±0.55	16.34±0.01
Soybean meal	91.31±0.03	47.24±0.33	7.12±0.01	12.84±1.15	8.26±0.16	0.10±0.002
Leucaena leaf meal	90.88±0.03	10.28±0.09	9.86±0.01	58.01±1.04	50.79±0.51	17.00±0.62
Coconut meal (mech-extd)	92.18±0.05	10.93±0.32	3.32±0.13	67.30±0.67	42.69±1.05	8.06±1.19
Coconut meal (solv-extd)	86.01±0.01	24.69±0.90	8.59±0.10	80.80±2.17	43.45±0.83	7.94±1.28
Peanut meal	92.24±0.07	40.79±0.04	8.72±0.02	28.22±1.24	13.25±0.72	4.95±0.13
Mung bean meal	90.65±0.01	18.46±0.78	5.83±0.01	32.26±0.07	29.53±0.53	3.31±0.04
Dried brewer's grain	90.28±0.05	19.56±0.45	6.18±0.11	74.65±0.59	29.12±0.08	5.06±1.58
Whole cotton seed	92.64±0.23	21.75±0.02	3.86±0.04	52.28±0.82	37.80±0.27	11.67±0.39
Fish meal	90.01±0.10	61.89±0.52	27.84±0.01	-	-	-

Note: DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin

Table 2. Rate and constant of ruminal degradation characteristics of crude protein in concentrate feedstuffs (a, b, and c), rumen undegradable protein determined by the *in situ* nylon bag technique (RUDP) and undegradable intake protein determined by the *in vitro* enzymatic technique (UIP).

Feedstuffs	a	b	c	<i>In situ</i> RUDP, %	<i>In vitro</i> UIP, %
Corn meal	29.75±1.25	45.50±1.40	0.051±0.02	47.21±0.71	67.52±0.27
Cassava chip	60.06±1.90	19.81±0.64	0.065±0.004	28.77±3.75	46.60±0.48
Broken rice	6.77±2.15	82.56±0.98	0.058±0.018	48.75±3.45	78.03±0.28
Rice bran	36.73±0.61	42.81±0.51	0.156±0.038	30.86±0.44	58.48±0.41
Rice pollard	36.48±0.58	29.39±0.185	0.41±0.008	37.33±0.39	46.07±1.39
Palm meal (mech-extd)	18.04±1.11	27.32±0.52	0.028±0.11	58.25±0.16	54.23±1.06
Palm meal (solv-extd)	13.75±0.85	78.95±2.13	0.015±0.01	68.50±1.97	61.6±1.39
Kapok seed meal	10.22±1.44	61.80±1.60	0.264±0.03	37.82±0.64	23.56±0.29
Soybean meal	10.98±0.38	89.02±1.57	0.038±0.005	30.90±0.86	25.32±1.96
Leucaena leaf meal	27.00±0.59	42.42±3.20	0.061±0.005	49.73±0.68	40.47±1.30
Coconut meal (mech-extd)	15.16±0.17	72.16±4.77	0.017±0.006	66.59±2.17	43.45±0.35
Coconut meal (solv-extd)	16.01±0.37	65.38±2.24	0.017±0.009	67.39±1.09	54.23±2.27
Peanut meal	15.14±1.43	84.86±1.43	0.028±0.004	54.66±3.29	48.67±0.54
Mung bean meal	27.15±3.44	53.00±2.24	0.133±0.03	34.31±0.88	35.23±0.33
Dried brewer's grain	4.90±0.66	58.50±1.70	0.042±0.01	68.50±0.60	51.42±0.14
Whole cotton seed	37.68±1.39	46.60±3.62	0.181±0.03	25.83±0.68	31.82±0.30
Fish meal	27.85±1.44	36.40±1.70	0.058±0.01	52.68±0.66	36.62±1.11

Note: a = rapidly soluble fraction

b = potentially degradable fraction

c = rate of degradation of fraction b

RUDP = Effective rumen undegradable protein calculated at 5%/h out flow rate

UIP = Undegradable intake protein

Table 3. Relationship of the *in vitro* value and the *in situ* nylon bag value of rumen undegradable protein of different feed sources.

Group of feedstuffs	Regression equation	R ²	n	P
All test feed	%RUDP = 0.1665 (%UIP) + 27.751	0.16	17	0.10
Energy feed	%RUDP = 0.5413 (%UIP) + 6.4636	0.66	5	0.09
All protein feed	%RUDP = 1.1031 (%UIP) + 4.6938	0.73	12	0.0004
Feed higher than 15% CP	%RUDP = 1.1363 (%UIP) + 2.4314	0.79	9	0.0002
Feed higher than 20% CP	%RUDP = 1.1021 (%UIP) + 4.4284	0.74	6	0.02
Feed lower than 20%CP	%RUDP = 1.0629 (%UIP) + 6.9126	0.58	6	0.07

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**การประเมินค่าโปรตีนที่ไม่ถูกย่อยสลายในกระเพาะหมักโดยเทคนิคดุงไनोंและเทคนิค
 เอนไซม์ในวัตถุดิบอาหารชั้นเขตร้อน**

การประเมินค่าโปรตีนที่ไม่ถูกย่อยสลายในกระเพาะหมักด้วยเทคนิคดุงไनोंและหาความสัมพันธ์กับเทคนิคเอนไซม์ ได้ทำการศึกษาในวัตถุดิบอาหารชั้นจำนวน 17 ชนิด โดยจัดกลุ่มวัตถุดิบอาหารชั้นออกเป็น 6 กลุ่มคือ 1) วัตถุดิบพลังงาน 2) วัตถุดิบแหล่งโปรตีนทั้งหมด 3) วัตถุดิบที่มีโปรตีนหยาบสูงกว่า 15% 4) วัตถุดิบที่มีโปรตีนหยาบสูงกว่า 20% 5) วัตถุดิบที่มีโปรตีนหยาบต่ำกว่า 20% 6) วัตถุดิบอาหารทั้งหมด (ไม่จัดกลุ่ม) ผลการทดลองพบว่า กลุ่มอาหารทั้งหมดมีค่าสัมประสิทธิ์การตัดสินใจต่ำที่สุด ($R^2 = 0.16$) และกลุ่มที่มีโปรตีนหยาบสูงกว่า 15 % มีค่าสัมประสิทธิ์การตัดสินใจสูงที่สุด ($R^2 = 0.79$) นอกจากนั้นยังพบว่ากลุ่มวัตถุดิบที่มีโปรตีนหยาบต่ำกว่า 20%, กลุ่มอาหารพลังงาน, กลุ่มวัตถุดิบโปรตีนทั้งหมด, กลุ่มวัตถุดิบที่มีโปรตีนหยาบสูงกว่า 20% มีค่าสัมประสิทธิ์การตัดสินใจ (R^2) เท่ากับ 0.58, 0.66, 0.73 และ 0.74 ตามลำดับ ผลจากการทดลองครั้งนี้ชี้ให้เห็นว่าการใช้เทคนิคเอนไซม์เพื่อทำนายค่าของเทคนิคดุงไनोंสามารถใช้ได้ดีในกลุ่มวัตถุดิบที่มีค่าโปรตีนหยาบใกล้เคียงกัน ดังนั้นเทคนิคเอนไซม์จึงเป็นเทคนิคทางเลือกหนึ่งในการนำมาหาค่าโปรตีนที่ไม่ถูกย่อยสลายในกระเพาะหมักโดยเฉพาะอย่างยิ่งเมื่อวัตถุดิบที่มีโปรตีนสูงกว่า 15%

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