Estimation of Ruminal Degradation and Intestinal Digestion of Tropical Protein Resources Using the Nylon Bag Technique and the Three-step *In vitro* Procedure in Dairy Cattle on Rice Straw Diets

C. Promkot[1], M. Wanapat* and P. Rowlinson[2]

[1] Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus, Thailand

ABSTRACT : The experiment was carried out using fistulated multiparous Holstein Friesian crossbred (75% Holstein Friesian and 25% Red Sindhi) dairy cows in their dry period fed on untreated rice straw to evaluate the nutritive value of local protein feed resources using the *in sacco* method and *in vitro* pepsin-pancreatin digestion. Experimental feeds were cottonseed meal (CSM), soybean meal (SBM), dried brewery's grains (DBG), palm kernel meal (PSM), cassava hay (CH), leucaena leaf meal (LLM). Each feedstuff was weighed into duplicate nylon bags and incubated in each of the two rumen fistulated cows for 0, 2, 4, 8, 16, 24, and 48 h. Rumen feed residues from bags of 16 h incubation were used for estimation of lower gut digestibility by the technique of *in vitro* pepsin-pancreatin digestion. Ruminal ammonia-nitrogen (NH_3-N) concentrations did not differ between treatments or time with a mean of 5.5 mg%. Effective degradability of DM of CSM, SBM, DBG, PSM, CH and LLM were 41.9, 56.1, 30.8, 47.0, 41.1 and 47.5%, respectively. Effective degradabilities of the CP in feedstuffs were 49.6, 59.2, 40.9, 33.5, 47.3 and 65.0% for the respective feedstuffs. The CP *in vitro* pepsin-pancreatin digestibility as ranked from the highest to the lowest were SBM, CSM, LLM, CH, DBG, PSM, respectively. The intestinal and total tract digestion of feedstuffs in the current study were relatively lower than that obtained from previous literature. The results of this study indicate that SBM and LLM were highly degradable in the rumen, while CH, CSM and DBG were less degradable and, hence resulted in higher rumen undegradable protein. Soybean meal and LLM could be used to improve rumen ecology whilst CH, CSM and DBG could be used as rumen by-pass protein for ruminant feeding in the tropics. (**Key Words :** Rumen Degradability, Rumen By-pass Protein, *In sacco*, Three-step Technique, Protein Sources, Digestibility, Rice Straw, Cassava Hay)

INTRODUCTION

The rate and extent of protein degradation in the rumen is very crucial, as it determines the availability of nitrogen to microorganisms and amino acids in the small intestine to the host animals. The protein consumed by the animal should be partly degradable in the rumen, as peptides, amino acids and NH_3-N derived from proteolysis and be used in microbial protein synthesis and to impove rumen ecology. It is, therefore, very important to determine the degradability and digestion of different feed ingredients which are grown and used in different locations. Incubation of feeds in nylon bags in the rumen of fistulated ruminants have been used to determine the extent of rumen degradation of the feed protein (Ørskov and McDonald, 1979; Rao and Prasad, 1989). The feed N which escapes rumen degradation and digestibility can be further measured by a three-step *in vitro* procedure (Calsamiglia and Stern, 1995). Ørskov et al. (1980) expressed the view that the nylon bag technique is not only a powerful tool for indexing the relative degradabilities of feedstuffs, but may also be used to study rumen processes, as it is possible to vary the factors within the bag, or within the rumen. *In sacco* digestibility has been used to determine the effect of physical and chemical treatments of forages on extent of rumen degradation of the feeds (Dong et al., 2005; Kamalak, 2005; Mahr-un-Nisa et al., 2005; Sacakli and Tuncer, 2006).

Many reports have been published on the CP degradability of feedstuffs for ruminants (Stern et al., 1983; Santos et al., 1984; Armentano et al., 1986; Voigt and Piatkowski, 1987; Arieli et al., 1989; Maiga et al., 1996; Gralak et al., 1997; Wanapat et al., 2000; Islam et al., 2002)

Table 1. Ingredients and estimated chemical composition of the concentrate diet consumed by ruminally fistulated crossbred dairy cows used for the in situ trial (% of DM basis)

Ingredient	% DM basis
Cassava chip	59.3
Palm kernel meal	10.9
Whole cottonseed	9.3
Soy bean meal	4.7
Course rice bran	7.7
Molasses	3.3
Urea	2.1
Ground oystershell	0.6
Salt	1.1
Dicalcium[1]	0.4
Sulphur	0.1
Dailymin[2]	0.6
Estimated values (total diet)	
DM (%)	89.3
CP (% of DM)	13.8
TDN (% of DM)	75.5
DE (Mcal/kg)	3.32

[1] Dicalcium (each kg contains): Calcium 300 g; Phosphorus 140 g.
[2] Mineral mix (Dailymin[5])(each kg contains): Iron 2.14 g; Iodin 0.15 g; sulphur 11.82 g; Copper 0.23 g; Magnesium 0.96 g; Sodium 2.68 g; Manganese 7.21 g; Cobalt 0.03 g; Phosphorus 19.60 g; Selenium 0.003 g; Zing 0.16; Calcium 204.03 g.
CP = Crude protein, TDN = Total digestible nutrient.
DE = 4.4× kg TDN in 1 kg of feed.

and intestinal digestibility (de Boer et al., 1987; Cros et al., 1991; Maiga et al., 1996). However, there is limited information available on characteristics of DM and crude protein degradation in the rumen and post-ruminal digestibility of protein sources locally used for livestock in the tropics. The major limitation to ruminant production in many tropical regions of Africa. Asia and Latin America is poor nutrition. The productivity of animals is restricted by the low nitrogen and high fiber content of the native grasses and crop residues. which form the basis of the diets in these regions (Leng and Preston 1983; Wanapat. 1994). Diets based on these feeds could result in lower ruminal ammonia nitrogen and might affect feed degradation. Nevertheless, various treatment methods of straw had relatively compared regarding the nutritive values and metabolizable energy (Wanapat et al., 1985; Wanapat el al., 1986; Wanapat et al., 1996). Mehrez et al. (1977) studied the rate of rumen fermentation in relation to ammonia concentration by varying the amount of urea added to a whole barley diet (from 0 to 10 g/kg diet), which was then incubated *in sacco* in the rumen of animals given these diets. They found that the disappearance of dry matter from the bag was positively correlated with increasing level of ruminal ammonia nitrogen from lower than 10 to 24 mg% where dry matter degradability reached a plateau. Erdman et al. (1986) similarly found that estimated effective dry matter degradation based on *in situ* rates of digestion were increased from 67.9 to 74.4% for corn and 77.5 to 80.3%

for soybean meal with increasing rumen ammonia nitrogen from 4.3 to 25.0 mg/dl. respectively.

Hence the hypothesis behind this study is that ruminants fed on low quality roughages (rice straw) would be potentially low in ruminal degradability and thus be improved by protein supplementation. Therefore, the objective of this study was to contribute to the knowledge of degradation characteristics and lower-gut digestibility of different protein sources in ruminants fed low quality roughages, since most of ruminant production in the tropic was based on low quality roughages.

MATERIALS AND METHODS

Experimental animals and design

Two. ruminally fistulated multiparous Holstein Fresian crossbred (75% Holstein Fresian and 25% Red Sindhi) dairy cows during their dry period (530 and 550 kg of BW) were used in this study. Six protein sources used in the experiment were as follows:

- Cassava hay (CH)
  (prepared according to Wanapat et al., 2000)
- Cottonseed meal (CSM)
- Dried brewers grains (DBG)
- Leucaena leaf meal (LLM)
- Palm kernel meal (PSM)
- Soybean meal (SBM)

All feed sources were ground to pass a 1-mm screen for use in the experiment. A Completely randomized design (CRD) was used. The cows were individually penned; rice straw, clean fresh water and mineral blocks were offered *ad libitum*. Concentrate feed (Table 1) was fed at maintenance level (0.5% body weight) in two equal portions. at 0830 and at 1630 h. Chemical composition of concentrate feed was analyzed for DM. CP using the procedure of AOAC (1990). Total digestible nutrient (TDN) of concentrate feed was calculated based on TDN content in each ingredient according to Kearl (1982). TDN was converted to DE by the relationship 1 kg of TDN = 4.4 Mcal of DE (NRC. 1984).

The animals were on diets for 14 days before the nylon bag studies were started.

Ruminal disappearance study

Samples of protein feed sources were obtained from local market in Khon Kaen. Thailand. Samples were oven-dried at 60°C for 48 h, and milled to a 1-mm screen (Polymix® PX-MFC. Kinematica AG. Switzerland). All the samples were analyzed for DM. Ash and CP using the procedure of AOAC (1990), NDF and ADF according to Goering and Van Soest (1970). Residues after ruminal incubation were also analyzed for DM and CP (AOAC.

1990).

Dry matter and CP disappearances were estimated for each feed source using the nylon bag technique (Ørskov and McDonald, 1979). The bags were made from dacron cloth with a pore size of 38-μm. Five grams of each feed (DM basis) were placed in each bag, which was anchored with a 30-cm length of braided fishing line. All the samples were prepared in duplicate and incubated in the rumen of each animal for 0, 2, 4, 6, 8, 16, 24 and 48 h. Nylon bags were incubated at respective hours in the rumen and were removed from the rumen simultaneously as suggested by Vanzant et al. (1998) in order to reduce the error.

Ruminal pH values of each incubation time were measured immediately after bags were removed using a glass electrode pH meter. Rumen fluid was collected at each incubation time from both the beef cattle. Ruminal fluid samples were then filtered through four layers of cheesecloth and were centrifuged (3,000×g, 4°C for 15 min) immediately and supernatant of centrifuged rumen fluid was kept in a plastic bottle to which 5 ml of 1 M H_2SO_4 was added and stored at -20°C. These were subsequently used to determine NH_3-N using the procedure of AOAC (1990).

After the specific incubation periods, the bags were removed from the rumen and were immediately washed with cold tap water until clear and then dried in a forced air oven at 60°C for 72 h. Control, bags without incubation (0 h) were washed and dried in the same conditions. Dry matter and CP of residual feeds were estimated by the procedure of AOAC (1990). The percentages of disappearance of DM and CP at each incubation time were calculated from the proportion remaining after incubation in the rumen using the equation of Ørskov and McDonald (1979).

$$p = a+b(1-e^{-ct})$$

where p = disappearance rate at time t, a = intercept representing the portion of DM or CP solubilized at initiation of incubation (time 0), b = the fraction of DM or CP potentially degradable in the rumen, c = a rate constant of disappearance of fraction b, and t = time of incubation.

The nonlinear parameters a, b, and c are estimated by an iterative least squares procedure. The effective degradability of DM (EDDM) or of CP (EDCP) were calculated using the following equation.

$$EDDM \text{ or } EDCP = a+(bc/(c+k))$$

where k is the estimated rate of out flow from the rumen, and a, b, and c are the same parameters as described earlier.

Effective degradability of DM or CP was estimated for each ingredient assuming rumen solid out flow rate from

the previous experiment (Ørskov and McDonald, 1979). Undegradable protein digestion of each sample residue from the at 16 h incubation was used to estimate *in vitro* protein digestion in the lower-gut using a three-step *in vitro* procedure as described by Calsamiglia and Stern (1995).

In vitro pepsin-pancreatin digestion procedure

The rumen feed residues from bags following the 16 h incubation time, after N determination were weighed to contain 15 mg of residual N into a 50-ml centrifugation tube. 10 ml of a pH 1.9, 0.1 N HCl solution containing 1 g/L of pepsin (sigma P-7012, Sigma), were added, vortexed, and incubated for 1 h in a 38°C shaker water bath. After incubation, 0.5 ml of a 1 N NaOH solution and 13.5 ml of a pancreatin solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin (Sigma P-7545, Sigma)) were added. Samples were vortexed and incubated at 38°C for 24 h in a shaker water bath and vortexed every 8 h. After incubation, 3 ml of a 100% (wt/vol) solution of TCA were immediately added to the tubes to stop enzymatic action and to precipitate undigested proteins. All tubes were vortexed and allowed to stand for 15 min. Sample were centrifuged at 10,000×g for 15 min and the supernatant analysed for soluble N by the Kjeldahl method (AOAC, 1990). Pepsin-pancreatin digestion of protein was calculated as TCA-soluble N divided by the amount of sample N (dacron bag residue) used in the assay (Calsamiglia and Stern, 1995).

Statistical analyses

All data were statistically analyzed by SAS (1996) (Proc. GLM). Statistical analyses of *in situ* data and three-step *in vitro* procedure were done according to the following model:

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

Y_{ij} = the criteria under study, μ = overall mean, δ_i = feed source effect and ε_{ij} = residual.

Treatment means were determined by using Duncan's New Multiple Range Test.

RESULTS AND DISCUSSIONS

Chemical composition

The chemical composition of the feeds is presented in Table 2. With the exception of soybean meal and palm kernel meal, all the six feeds had similar dry matter (DM), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Palm kernel meal contained the highest NDF and ADF (70 and 48.5%, respectively), while soybean meal had the lowest (12.3 and 8.4% NDF, ADF, respectively). The CP

Table 2. Chemical composition of protein sources used for *in situ* trial (DM basis)

Items	DM[1]	Ash	CP	NDF	ADF
	--- % --	----- % DM -----			
DBG[2]	95.3	4.8	27.0	56.1	23.2
CH	93.7	6.6	24.8	41.9	27.3
CSM	93.2	7	40.2	30.5	21.3
LLM	95.1	8.4	22.3	48.5	24.5
PSM	97.3	4.5	12.4	70	48.5
SBM	92.6	6.6	44.1	12.3	8.4

[1] DM = Dry matter. CP = Crude protein. OM = Organic matter. NDF = Neutral detergent fiber. ADF = Acid detergent fiber.
[2] DBG = Dried brewers grains; CH = Cassava hay; CSM = Cottonseed meal.
LLM = Leucena leaf meal; PSM = Palm kernel meal.
SBM = Soy bean meal.

Table 3. Ruminal NH_3-N (mg%) and ruminal pH in crossbred dairy cows fed on untreated rice straw

h post feeding	Untreated rice straw	
	pH	NH_3-N
0	6.8	5.3
2	6.7	5.2
4	6.5	5.7
6	6.6	6.3
8	6.7	5.4
16	6.5	5.6
24	6.7	5.7
48	6.5	5.6
Mean	6.6	5.5

content of DBG, SBM, CSM and PSM (27, 44.1, 40.2 and 12.4%, respectively) obtained in this study were slightly lower than those reported by NRC (2001). However, they were similar to reports by Gohl (1998) and Bairagi et al. (2004). The CP content of CH in this study was similar to that reported by Wanapat et al. (2000), while that of LLM was in the range reported by Bairagi et al. (2004). The variations could possibly be attributed by conditions of soil and stage of growth etc. (Ravindran and Ravindran, 1988).

Ruminal ecology

The average ruminal pH (Table 3) was 6.6 which have been reported as optimal for microbial digestion of fiber (Hoover, 1986; Firkins, 1996). The values for low ruminal ammonia-nitrogen concentration of 5.5% obtained in the present study were quite low for cows fed rice straw.

In situ DM, CP degradabilities of feed

The lowest (p<0.05) rates of DM and CP degradability (c) (Tables 4) were found for PSM. This was similar to the report by Gralak et al. (1997) who found that both palm kernel DM and CP degradabilities were characterized by slow rates of degradation. The highest potential (a+b) (p<0.05) and rate (c) of DM and CP degradabilties were found in SBM. High levels and rates of degradability of CP in SBM could be due to the structure and solubility characteristics of protien in SBM which could be easily attached by microorganisms in the rumen (Mahadevan et al., 1980). Rate of CP and DM degradability of LLM was similar with SBM while LLM had high fiber. It is clear from a consideration of the rumen bag technique that the absolute value of the results depends on the way in which the sample is prepared, and the fineness of the material from which the bag is made. These fine particles would be rapidly fermented or else washed out of the nylon bag unfermented (Ørskov, 1992). In this experiment leucena

Table 4. Dry matter (DM) and crud protein (CP) disappearance in the rumen and lower gut digestion of DBG, CH,CSM, LLM, PSM and SBM at various incubation times in crossbred dairy cows fed on untreated rice straw

Items	DBG[1]	CH	CSM	LLM	PSM	SBM	CV (%)
DM disappearance (%)							
a	10.9[e]	18.7[d]	22.8[b]	21.0[c]	34.4[a]	24.2[b]	1.9
b	51.9[b]	58.2[a]	39.7[e]	48.3[c]	41.7[d]	60.6[a]	5.4
c	0.031[c]	0.031[c]	0.058[b]	0.061[ab]	0.016[d]	0.068[a]	7.1
a+b	62.8[c]	76.9[b]	62.4[c]	69.3[c]	75.1[b]	84.7[a]	3.6
Effective degradability (%)[2]	30.8[d]	41.1[c]	41.9[c]	47.5[b]	47.0[b]	56.12[a]	0.8
In vitro pepsin -pancreatin digestibility (%)[3]	52.3[c]	50.2[d]	58.5[b]	49.8[e]	48.9[f]	60.7[a]	0.2
Total tract digestibilty (%)	67.0[e]	70.6[d]	75.9[b]	73.6[c]	72.9[d]	82.7[a]	0.2
CP disappearance (%)							
a	13.8[d]	26.3[c]	31.0[b]	38.11[a]	11.9[e]	15.1[d]	2.2
b	53.9[c]	43.2[d]	40.5[e]	44.0[d]	63.9[b]	72.7[a]	1.9
c	0.043[c]	0.052[b]	0.043[c]	0.079[a]	0.025[d]	0.078[a]	3.0
a+b	75.8[c]	69.6[d]	71.5[d]	82.1[b]	75.9[c]	87.7[a]	1.2
Effective degradability (%)[2]	40.9[e]	47.3[d]	49.6[c]	65.0[a]	33.5[f]	59.2[b]	0.8
In vitro pepsin -pancreatin digestibility (%)[3]	71.7[c]	70.4[d]	77.4[b]	68.5[e]	67.9[f]	79.8[a]	0.7
Total tract digestibility (%)	83.3[d]	84.4[c]	88.6[b]	89.0[b]	78.0[e]	91.7[a]	0.1

[a-f] Within rows not sharing common superscripts are significantly different (p<0.05). CV = Covariance.
[1] DBG = Dried brewers grains; CH = Cassava hay; CSM = Cottonseed meal; LLM = Leucena leaf meal.
PSM = Palm kernel meal; SBM = Soy bean meal.
[2] Effective degradability in the rumen (assuming rate of passage of 0.05 h^{-1}).
[3] *In vitro* pepsin -pancreatin digestibility. % of rumen residual.

leaf meal was very fine and dusty particles. which would easily be lost in the rumen. Lower (p<0.05) potential CP degradabilities (a+b) than the other feeds were found in BDG, CSM and CH. These similar results have also been reported in previous work (Wohlt et al., 1973; Armentano, 1986; Gohl, 1998; Wanapat et al., 2000). BDG has both low protein solubility and degradability (Wohlt et al., 1973; Armentano, 1986). Cottonseed meal has relatively low rumen degradability and is therefore a good source of by-pass protein (Gohl, 1998). The formation of tannin-protein complexes in CH could result in higher by-pass protein reaching the lower gut (Wanapat et al., 2000).

Effective DM and CP degradability of CH

Effective DM degradability of CH was lower (p<0.05) than the other feeds except for DBG (Table 4). This could be due to CH containing high level of undegradable protein as it contains quite a high CP content. As determined in this study, the effective CP degradability of CH was 47.5% which was lower (p<0.05) than in the other feeds except for DBG and PSM. The effective CP degradability of CH in the present study was similar to that found by Wanapat et al. (2000) who stated that protein degradability 'a', 'b' and 'c' of CH were 28.4, 47.9% and 0.037% h^{-1}, resulting in an effective protein degradability at 48.8%. As reported by Wanapat et al. (2000) that the formation of tannin-protein complex of CH could result in higher rumen by-pass protein in the lower gut. Cassava hay was used as an ingredient in cassoy-urea pellet with SBM or urea and the pellet was very good source of protein in cattle due to its high rumen by-pass protein (Wanapat et al., 2006). The studies conducted using feeding trials had presented additional useful results by using of CH as a protein supplement in ruminant rations (Hong et al., 2003; Kiyothong and Wanapat, 2004; Chantaprasarn and Wanapat, 2007; Granum et al., 2007).

Effective DM and CP degradability of CSM

The *in situ* predicted ruminal degradation of DM for rate of passage at 0.05 h^{-1} was lower than the value reported by Gohl (1998) (45.6%). Values were similar among feeds, with the effective CP degradability of CH, being lower (p<0.05) than in other feeds except in DBG. This pattern was also supported by Gohl (1998). Efective CP degradability of CSM were 49.6. This value was slightly lower than found by Erdman et al. (1987) who stated that mean fractional turnover (per hour) rate, measured by labeling CSM with cerium, samarium or lanthanum was.049 and *in situ* predicted ruminal degradation of N. for rate of passage. was 54.2%. higher (p<0.05) than in DBG but lower (p<0.05) than in SBM. Undegradability of 50.5% was reported for CSM protein by Krishnamoorthy et al. (1982) which is also higher than the current study.

The lower effective CP degradability of CSM in the current study work could be due to the effect of heat treatment during the oil extraction process (Mahadevan et al., 1980) and NRC (2001). CSM prepared in Thailand is mechanically extracted while CSM reported by NRC (2001) was solvent extracted. The lower rumen ammonia-N might be another reason for the reduced degradability. Mehrez et al. (1977) studied rate of rumen fermentation in relation to ammonia concentration by varying the amount of urea added to a whole barley diet (from 0 to 10 g/kg diet) and then incubating in bags in the rumen of animals given these diets. They found that the disappearance of dry matter from the bag was positively correlated with increasing level of rumen ammonia nitrogen from below 10 to 24 mg% at which point degradability had plateaued. Erdman et al. (1986) also found that estimated effective dry matter degradation based on *in situ* rates of digestion were increased from 77.5 to 80.3% for soybean meal with increasing rumen fluid ammonia-N from 4.3 to 25.0 mg/dl. respectively. However rumen ammonia-N levels in this experiment, were in range of ammonia-N reported by Satter and Slyter (1997) in mixture culture batch that ammonia-N lower than 2.0 mg% of rumen fluid can be limiting for microbial growth.

Effective DM and CP degradability of DBG

Effective DM degradbility of DBG was lowest (p<0.05) at 30.8% the lowest of the feedstuffs investigated in the current study. This was similar or a bit lower, than that reported by Gohl (1998) (33.5%). The effective CP degradability for DBG was 40%. One of the reasons why effective DM degradability of DBG was lowest when compared with other feeds was that effective CP degradability of DBG was lowest than of other feeds since almost 50% of DM containing in DBG was CP. This value was also lower than previous work (Stern et al., 1983; Armentano et al., 1986) (42%) and (47.01%) (NRC, 2001). Effective CP degradability of DBG was lower (p<0.05) than other feeds except PSM. However, degradation rate constant (c) was higher than PSM. while potential degradability of PSM was higher (p<0.05) than DBG. It could be concluded that CP of DBG was low in rumen degradable protein. This agrees with previous work (Armentano et al., 1986; NRC, 2001). Blethen et al. (1990) stated that among the feeds that were evaluated, those with the highest percentage of insoluble protein (>40% of CP) were DBG, fish meal, meat and bone meal, forages, and soy hulls. High amounts of insoluble protein could provide high levels of rumen by pass-protein for the host ruminants.

Effective DM and CP degradability of LLM

Effective DM degradability of LLM was similar to PSM

and higher (p<0.05) than in other feeds except for SBM. Mbanzamihigo et al. (1996) reported that the *in sacco* degradability of hay (dry matter) was not affected by monensin administration. Effective DM degradability of LLM was 47.5%. The result was similar to Tolera et al. (1998) who reported that effective DM degradability of leucaena leaf was 50.5%. However, effective CP degradability of LLM was the highest (p<0.05) of the feeds under investigation in the current study. High effective CP degradability of LLM, could be due to the fact that LLM contained the highest level of rapidly soluble fraction 'a'. It has been reported by NRC (2001) that grasses and legume forages (hay or silage) contained the higest concentration of nonprotein N (NPN) compounds which are degraded quickly in the rumen. There was higher effective DM degradability of LLM as compared to CH, CSM and DBG. The remarkable high rate of CP of LLM could partly attribute to high effective DM degradability.

Effective DM and CP degradability of PSM

Effective DM degradability of PSM was similar to LLM and higher (p<0.05) than in other feeds except for SBM. Gohl (1998) showed that the soluble faction (a), potential degradation constant (b) and rate of degradation (c) of DM in PSM were 48.4, 15.9% and 0.042%/h, respectively, with an effective DM degradability at a rate of passage of 0.05 h⁻¹, was 55%. While effective DM degradability of BDG CSM. SBM in the same report were 33.5, 45.6 and 67.9%, respectively. Although, ADF content of PSM was very high and effective CP degradability was very low as compared to other feeds but effective DM degradability of PSM was higher (p<0.05) than other feeds except for SBM. The reason could be due to PSM was very fine and might be more rapidly escaped from the bag as the soluble fraction (a) value of DM was very high.

However, the value for effective CP degradability of 33.5% was the lowest (p<0.05) of the feeds in the current study. This level was higher than given by NRC (2001) of 29.2%. In this study, the CP 'a' was higher (11.9%). Fraction 'a' is the percentage of total CP that is NPN and a small amount of true protein that may rapidly escape from the *in situ* bag and feedstuffs that contained high concentrations of NPN in the CP contribute little RUP to the host animal. Therefore, higher effective CP degradability of PSM in this study, might be due to the PSM used in this study containing a higher amount of the high solubility fraction (i.e., NPN) or being a very small particle size which might more rapidly escape from the *in situ* bag than the PSM that was reported by NRC (2001).

Effective DM and CP degradability of SBM

Effective DM and CP degradability of SBM (for rate of passage of 0.05 h⁻¹) used in the present study was higher (p<0.05) than the other feeds, except for LLM in CP degradability. One reason for the highest effective DM degradability was found in SBM was that there was highest (p<0.05) effective CP degradability as compared to other feeds, since almost 50% of DM of SBM was CP.

Effective CP degradability of SBM in the present study was lower than those reported by NRC (2001), Gralak et al. (1997), Erdman et al. (1987) and Armentano et al. (1986) but was higher than reported by Islam (2002). Soybean mean (SBM) in the report of NRC was prepared by solvent extract, while SBM for feeding ruminants in Thailand was prepared by heating and mechanical extraction. Presumably, some of the protein is denatured by the heating and passed through the rumen without being utilized by the rumen microbes which may have resulted in the lower effective CP degradability of SBM in this study. The effect of heat treatment on decreasing ruminal protein degradability was also reported by Mahadevan et al. (1980) and NRC (2001).

Lower gut and total digestibility of feeds

Comparison between feeds of intestinal digestibility (*in vitro* pepsin -pancreatin digestion) of DM and CP are shown in Table 4. Expressed in % of the rumen residues DM and CP intestinal digestibility, as ranked from the highest to the lowest were: SBM, CSM. DBG, CH. LLM, and PSM, respectively. In SBM, CSM. DBG, LLM, and PSM were slightly lower than those given by NRC (2001) at 93, 92, 80, 75 and 75% of rumen undegradable protein for SBM, CSM, DBG, PSM and legumes forage hay (NDF 40-46%), respectively. However, the estimates of pepsin-pancreatin digestion of SBM using the three-step procedure was 89.8±2.6 by Calsamiglia and Stern, (1995). Grings et al. (1991) reported that the lower rate and extent of CP disappearance from CSM would allow 57% of the CP supplied from this feed to pass to the small intestine which would render 87.6% of the CP available for absorption. The intestinal digested crude protein of DBG (dried at 50°C to 175°C) ranged from 51.6 to 84.3% of the rumen residue, depending upon the level of heating. Drying at 50°C and 100°C had no adverse effects on the intestinal digested crude protein but drying at 135 and 175°C decreased it to values of 80.1 and 51.6%, respectively. Post ruminal digestion of leucaena forage was reported by Garcia et al. (1996) at 58.3% of rumen undegradable protein which was lower than in this report. The low level of rumen CP degradability of CH, probably resulted from the formation of tannin-protein complex (TPC), but was still higher than in PSM and LLM. Intestinal digestibility was 73% of the rumen residue. This could be due to dissociation of the TPC to free protein which was then digested by the enzymes. Jones and Mangan (1977) reported that a TPC will maintain

its complex at pH 3.5-7.0, but will dissociate at pH lower than 3 or higher than 8. The low CP intestinal digestibility for PSM in this study may be due to the high level of NDF.

Ammonia-N in cattle fed rice straw

For all the protein sources in the current study, the values for rumen degradable DM and CP (% effective degradability, assuming rate of passage of 0.05/h[1]) were lower than previous work. Mehrez et al. (1977) studied rate of rumen fermentation in relation to ammonia concentration by varying the amount of urea added to whole barley diets (from 0 to 10 g/kg diet), and then incubated in bags in the rumen of animals given these diets. They found that the disappearance of dry matter from the bag was positively correlated with increasing level of ruminal ammonia nitrogen from below 10 to 24 mg% with dry matter degradability detaining at the higher level. In the present study, ammonia-N in cows fed rice straw was 4.5 mg%. Perdok and Leng (1989) showed that higher level of rumen NH_3-N (15-30 mg%) improved digestibility and feed intakes, while ammonia nitrogen lower than 2.0 mg% of rumen fluid can be limiting for microbial growth (Satter and Slyter, 1974).

Wanapat et al. (2003) reported that higher bacteria counts (cellulolytic, proteolytic and amylolytic bacteria) were found in cows fed with urea-treated rice straw than untreated rice straw and this resulted in higher degradability. Erdman et al. (1986) also showed that increasing ruminal NH_3-N from 4.3 to 17.2 mg% by urea infusion could increase DM and CP degradabilty in SBM and CSM. Reduced ruminal ammonia-N concentrations appeared to be the cause of reduced microbial CP production (microbial growth efficiency) and hence degradability and reduced fiber digestion at levels of peptides greater than 10% of total N (NRC, 2001). In addition, Erdman et al. (1986) also found that estimated effective dry matter degradation based on *in situ* rates of digestion were increased from 67.9 to 74.4% for corn and 77.5 to 80.3% for soybean meal with increasing rumen fluid ammonia nitrogen from 4.3 to 25.0 mg/dl, respectively. As there was lower DM and CP degradation in the rumen, degradability of fiber (NDF and ADF) were also lower resulting in high levels of fiber passing to the lower gut. In this case, lower rumen degradable CP and higher by-pass protein reaching the lower gut of feedstuffs were found in current study, it was possible that rumen undegradable protein may have high levels partition of CP recovered with NDF and result in lower gut protein digestibility.

CONCLUSIONS

Lower rumen ammonia-N may inhibit degradation rates when cows are fed with low quality roughages such as rice

straw. When using reference values regarding DM and CP degradation and intestinal digestion of feedstuffs for feed formulation in tropical ruminant production systems based on low quality roughage one should be aware that the values may be an overestimation. Based on this research, the conclusion can be made that SBM and LLM were highly degraded in the rumen, while CH, CSM and DBG were less degraded and hence resulted in higher rumen undegradable protein.These protein sources can be to used to improve rumen ecology (SBM and LLM) and rumen by-pass protein for ruminant feedings (CH, CSM, BDG).

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