IN SITU DEGRADATION OF SELECTED PROTEIN SOURCES IN RUMEN-CANNULATED BRAHMAN CATTLE (*Bos indicus* Linn.)

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ABSTRACT

This study assessed the rumen bypass potential of various protein sources at 24, 48 and 72 hr of incubation in rumen-cannulated cattle. The animal was fed with Napier and legume supplement following a ratio of 80:20. The incubation process followed the sequential addition method and digested samples were analyzed for percent dry matter and percent crude protein. Significantly lower dry matter digestibility and dry matter disappearance rate were noted using Flemingia (*Flemingia macrophylla*) leaf meal than in Calopo (*Calopogonium muconoides*), *Kakawate* (*Gliricidia sepium*), Pinto peanut (*Arachis pintoi*) and Soybean meal (SBM) in all rumen exposure times. Crude protein digestibility (CPD) and crude protein disappearance rate (CPDR) of Flemingia were significantly lower than Calopo, *Kakawate*, Pinto peanut and SBM at all periods which could be due to its high tannin content. Calopo had significant reduction in CPD and CPDR than Pinto peanut and SBM at 24 hr, SBM at 48 hr, and *Kakawate* and Pinto peanut at 72 hr and this could be due to its physical properties, maturity and source of leaves. The study suggests that Flemingia is a good source of bypass protein for ruminants.

Keywords: bypass protein, cattle, *in situ* degradation, soybean, tannin

INTRODUCTION

Chemical analysis of feedstuffs provides information concerning nutrient composition, but the critical evaluation of any feedstuff must be based on its nutritive value and its fate in the digestive system of animals. Various approaches are available to assess the degradability of protein feed sources including *in vivo*, *in sacco* or *in situ*, and *in vitro* methods (Elwakeel *et al*., 2007). The most appropriate method for assessment of ruminal degradation would involve incubating feedstuffs in nylon bags in the rumen of fistulated animals given a well-balanced diet for efficient rumen and animal function (Preston, 1986). This offers a quick and easy method (Van Dyne, 1952) to assess the rate and extent of degradation of nutritional parameters of feedstuffs (Osuji *et al.*, 1993). This is widely used for estimating ruminal protein degradation because it is relatively less expensive and simpler

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(Ørskov *et al*., 1980)

The technique has implications on bypass protein supplementation in ruminants. Such practice is grounded on the fact that apart from the need for rumen degradable nitrogen (RDN) by rumen microbes, there is a need for amino acids at the intestinal level. Such need may not be satisfied by microbial cell protein alone, so a supply of bypass protein becomes imperative especially to young, actively-growing animals, lactating and highly-productive animals, and those under stress. One way of assessing the amount of bypass protein is through the determination of the rate and extent of rumen degradation of protein supplements and leftovers that reach the small intestines for absorption as bypass protein. Hence, this study measured the dry matter degradability (DMD) and crude protein degradability (CPD) of protein feedstuffs using *in situ* technique to determine respective potential sources of bypass protein in ruminants.

MATERIALS AND METHODS

A rumen cannula-fitted (Bar Diamond Lane, Parma, ID, U.S.A.) yearling Brahman bull with good appetite and no traces of parasitism was used in the study. A month before the actual experiment, the animal was closely monitored and prepared to ensure healthy condition. During this period, the yearling bull was confined for easy handling, and was treated against external and internal parasites with Ivermectin at 1 ml/50 kg BW injected subcutaneously and Albendazole at 1 ml/10 kg body weight given orally.

The basal diet consisted of freshly chopped Napier grass (7.62-10.16 cm long) while the supplement was composed of five sundried and ground protein feedstuffs such as Soybean (*Glycine max*) meal (SBM), *Kakawate* (*Gliricidia sepium*), Flemingia (*Flemingia macrophylla*), Pinto peanut (*Arachis pintoi*), and Calopo (*Calopogonium muconoides*) leaf meals.

Legume leaf meals and SBM were assessed for their potential as bypass protein supplements based on *in situ* technique in a cannula-fitted yearling bull. Dietary treatments were: (T_1) SBM, (T_2) *Kakawate* leaf meal, (T_3) Flemingia leaf meal, (T_4) Pinto peanut leaf meal, and (T_5) Calopo leaf meal. The experiment was conducted in a Randomized Complete Block Design (RCBD) with four blocks/ periods. Blocking was based on periods (period 1 as block 1, period 2 as block 2 and so on) wherein all the treatments/samples in each period/block have 3 days of rumen exposure and each treatment/sample was replicated four times. Replication was based on nylon bags.

The feeding ratio of Napier grass and supplement was 80:20 during the experiment. The supplement was divided equally into its component legumes (4% each) on DM basis to complete the 20% of the diet. This was done to ensure the build-up of rumen microbes responsible for fermentation of the feedstuffs tested at a set level (20%) to avoid any deficiency of fermentable nitrogen (Preston, 1986). The daily DM intake requirement was computed following the requirement of 5.4 kg DM for 200-kg BW cattle (Kearl, 1982). Furthermore, the animal was adapted to eat the diet under test for two weeks. To allow easy access of the nylon bags into the

ventral sac of the reticulo-rumen, the feed was gradually reduced into half (Playne *et al.,* 1978). Feeding was done daily at 7:00 AM and 4:00 PM. Chopped Napier grass was fed *ad libitum* and clean potable water was made available at all times for better rumen function. A two-week dietary adjustment period was followed by a three-day incubation period following the procedures of Nishimuta *et al*. (1974).

Nylon bags measuring 5 x 10 cm with a pore size of ±53 µm (Bar Diamond Lane, Parma, ID, USA) were oven-dried for 30 min at 65ºC and then weighed immediately (Osuji *et al.,* 1993). Each sample weighing 4 g DM was placed into a nylon bag and heat-sealed before placing inside a lingerie bag weighed with stainless steel pieces to allow it to settle at the bottom of the rumen. This was done to prevent the nylon bags from floating on top of the solid phase of the rumen digesta and giving very variable degradation rates (Preston, 1986). The order of incubation was based on the "sequential addition method" incubating first the 72 hr samples, then 48 hr the next day, and 24 hr on the last day and were recovered at the same time for lesser disturbance to the rumen environment (Osuji *et al*., 1993). The recovered nylon bags were washed for 30 min in running tap water by gentle manual scrubbing until water ran clear. The washed nylon bags were then oven-dried at 65ºC for 48 hr and weighed immediately to get the DM weight. The percent crude protein (CP) content of the incubated samples after oven-drying was analyzed using Macro Kjeldahl digestion and distillation unit at the Department of Animal Science Nutrition Laboratory, Visayas State University, Visca, Baybay City, Leyte, Philippines and calculated as %N x 6.25 (AOAC, 1993).

Data were analyzed using two-way analysis of variance (ANOVA) in RCBD. Differences among treatment means were analyzed using Honest Significant Difference (HSD) test using the Statistical Package for Social Sciences (SPSS) version 15 computer program.

RESULTS AND DISCUSSION

Dry matter degradability

Among the samples tested after 24 hr, Flemingia had the lowest DMD ($P<0.01$) (Table 1) and this was due to high level of tannin in the leaves, classified as condensed tannin (CT) (FFTC and PCARRD, 2004). Calopo leaf meal had lower DMD than Pinto peanut and SBM (24 hr). Although Calopo has no reported tannin content, it has significantly lower DMD compared to other tannin-containing legumes such as *Kakawate* and Pinto peanut (48-72 hr). This could be due to several factors such as physical properties, age (maturity), and source of the leaf meal used (Osakwe and Okorie, 2007). A reported neutral detergent fiber (NDF) content of 56.8% (Serra *et al*., 1996) comprising 20-80% of its dry weight (Wilson, 1994) contributed to its resistance against rumen microbial digestion. Since Calopo is a creeping legume, Serra *et al.* (1996) found that it has higher amounts of various fiber fractions compared to tree legumes like *Gliricidia* (*Kakawate*). Cook *et al.* (2005) reported that Calopo has an *in sacco* DMD of 48-55%, which was lower than the findings in this study. Secondary compounds of *Kakawate* like the tannins significantly protected the leaf meal from excessive rumen degradation compared to

Table 1. Dry matter degradation and disappearance rate analysis of protein meals at different incubation periods.

Treatments	Dry matter degradation (%)			Dry matter disappearance rate (%/hr)		
	24-hr	48-hr	72-hr	24-hr	48-hr	72-hr
SBM	84.53^{d}	99.39^{e}	99.56^{e}	3.52^{d}	2.07^{e}	1.38^{e}
Kakawate	71.71^{bc}	76.52^{c}	77.08^{c}	2.99^{bc}	1.59^{c}	1.07^{c}
Flemingia	31.17^{a}	33.71^{a}	34.67^{a}	1.30^{a}	0.70^{a}	0.48^{a}
Pinto peanut	76.78^{cd}	81.85^{d}	82.70^{d}	3.20^{cd}	1.71^{d}	1.15^{d}
Calopo	60.95^{b}	67.80^{b}	69.15^{b}	2.54^{b}	1.41^{b}	0.96^{b}
p-value	0.001	0.001	0.001	0.001	0.001	0.001

Means with the same superscripts within a column are not different (P>0.01).

Pinto peanut and SBM. This was due to the reported CT content in the leaves with 40.7 g/kg DM (4.07%) (Cornell University, 2009), an attribute that protected the leaf meal from excessive microbial digestion in the rumen. The same findings were noted on the significance of Pinto peanut compared to SBM which was due to the CT content that was classified by Cook *et al.* (2005) as low with high leaf meal digestibility (60-70%). Although the analysis by Nieves (2004) revealed that Pinto peanut foliage has 26.7% crude fiber (CF) and 43.8% NDF, these values were insufficient to effectively protect the leaf meal from rumen microbial digestion; on the other hand, they significantly reduced its DMD better than SBM.

It can be noted that Flemingia leaf meal was the most resistant at 72 hr of rumen exposure. This finding was due to the high content of tannin in the leaves (Lanting, 2004) with 8% that rendered the leaf meal undegraded in the rumen, but still digestible and absorbable upon reaching the small intestines of ruminants as bypass protein as observed by Jayanegara and Palupi (2010). Cortes *et al*. (2009) found that increasing CT concentration significantly decreased apparent ruminal degradation of DM (P<0.01) by inhibiting the effects of microbial growth and activity in the rumen (Akin, 1982; Akin and Rigsby, 1985; Makkar *et al*., 1988). Frutos *et al.* (2004) reported that the ability of tannins to reduce the digestibility of the diet is well documented. The DMD of Flemingia leaf meal in this study was in accordance with the findings of Gutteridge and Shelton (1994) on *in vitro* experiment with less than 40%. Furthermore, this result was consistent with the findings of Asare (1985) and Budelman and Siregar (1997) on *in vitro* DMD of Flemingia by 18-40% but higher at 48 and 72 hr than the findings of Cook *et al*. (2005) with 17.1-32.8% at three month old regrowth. Such variations could be due to the maturity of the leaves and techniques used. Aside from the capacity of tannin to reduce feed digestibility (Feeney, 1969) and increase protein in ruminants (Min and Hart, 2003), an added benefit of preventing infection in livestock and controlling some internal parasites was reported by Butter *et al*. (2000).

Among the protein supplements tested, SBM recorded the highest DMD and this was due to the lack of protection against rumen degradation which was in

agreement with the findings of Crooker *et al*. (1986). For dry matter disappearance rate (DMDR), the same level and pattern of significance was noted from the DMD showing its direct relevance.

Crude protein digestibility

The resistance of Flemingia to excessive microbial rumen digestion protected its protein more than the other samples tested in all periods (Table 2). This highest reduction on CPD shown in Flemingia leaf meal in the rumen was due to the significant resistance of tannins against microbial digestion. McLeod (1974), Mangan (1988), Hagerman *et al. (*1992) and Mueller-Harvey and McAllan (1992) reported that reduction of protein degradation in the rumen may be the most well-known effect of tannins. The significantly reduced CPD of Calopo leaf meal over Pinto peanut and SBM (24 hr), SBM (48 hr), and *Kakawate* and Pinto peanut (72 hr) could be due to its physical and chemical components. Osakwe and Okorie (2007) reported that Calopo contains 21.6% CF which is higher compared to the reported 15% CF content of *Kakawate* (Göhl, 1981; Adejumo and Ademosun, 1985). The special feature of having high densities of epidermal hairs (34 hairs/mm^2) on the surface was mentioned as having decreasing effect on *in vitro* DMD (Pizarro, 2001; Cook *et al*., 2005; Heuze and Baumont, 2011).The degree of hairiness is, therefore,

Table 2. Crude protein digestibility and crude protein disappearance rate of feedstuffs at different incubation periods.

Treatments	Crude protein digestibility (%)			Crude protein disappearance rate (%/hr)		
	24-hr	48-hr	72-hr	24-hr	48-hr	72-hr
SBM	89.28^{c}	100^{c}	-	3.72^{c}	2.08^{c}	-
Kakawate	82.89^{bc}	88.25^{b}	89.67^{c}	3.45^{bc}	1.84^{b}	1.25^{c}
Flemingia	40.40^{a}	41.40^{a}	41.44^{a}	1.68^{a}	0.86^{a}	0.58^{a}
Pinto peanut	85.69^{c}	89.65^{b}	90.91^{c}	3.57^{c}	1.87^{b}	1.26^{c}
Calopo	75.98^{b}	84.21^{b}	86.21^{b}	3.17^{b}	1.75^{b}	1.20^{b}
p-value	0.001	0.001	0.001	0.001	0.001	0.001

Means with the same superscripts within a column are not different (P>0.01).
-: Complete digestion.

important other than the age of regrowth. Although tannins were present in *Kakawate* (Cornell University, 2009) and Pinto peanut (Nieves, 2004), they were insufficient to protect their protein from massive microbial digestion.

The greatest protection of CT in Flemingia was tested at 72 hr when it decreased its CPD compared to other treatments (P<0.01). Aharoni *et al*. (1998), Frutos *et al.* (2000) and Hervás *et al.* (2000) concluded that a reduction in the immediately degradable fraction and the fractional rate of degradation are the protective effects of tannins against protein degradation. Tannins have the capability

of inhibiting the activity of bacteria (Chesson *et al*., 1982) through precipitating the bacterial enzymes in the rumen, thus, preventing the degradation of plant cell walls (Reed, 1995). Although, tannin binds with proteins and decreases the nutritive value of plants (Cornell University, 2009), increasing levels of CT in both *in vivo* and *in vitro* studies clearly hampered nutrient digestibility (Min and Hart, 2003). Jayanegara and Palupi (2010) stated that tannins increase nutritional protein postruminally. Although tannins are known to reduce fractions of proteins digested in the rumen, a reversed reaction of increasing availability as bypass protein in the small intestines is expected (Min and Hart, 2003). Thus, retaining undegraded protein which is made available upon reaching postruminal digestion and absorption as bypass protein due to the change in pH is possible. This mechanism was best explained by Barahona *et al.* (1997), Carulla *et al.* (2001) and Lascano *et al.* (2003) wherein tannin-protein complexes would split from the acidic pH in the true stomach (abomasum) which would result to a high availability of protein at the intestinal level for digestion. This was supported by Frutos *et al.* (2004) who found that tannins have the ability to form stable hydrogen bonds at approximately 3.5 and 8 pH levels with proteins. Moreover, McLeod (1974), Mangan (1988), Hagerman *et al*. (1992) and Mueller-Harvey and McAllan (1992) observed that when pH falls below 3.5 such as in abomasum (pH 2.5-3) or greater than 8 at the duodenum (pH 8) they dissociate, which explains much of the tannins activity in the digestive tract.

Regardless of the negative effect of tannin on binding protein and altering its digestion in the rumen, proteins bound by tannins are made available at the intestinal level (Min and Hart, 2003). Lanting (2004) explained that the effect of low CP digestibility in the rumen of cattle was due to the tannin content, but which is well compensated by high bypass protein values for ruminants. This means that when more rumen undegradable protein (RUP) is left after ruminal fermentation, the ruminant is benefited by the amino acids postruminally. Min and Hart (2003) explained that aside from the protection provided by plant proteins, they can increase invasion of essential amino acids in the small intestines. Therefore, when protein bypasses ruminal digestion it will lead to improved production performance which is expressed as better weight gain and increased milk yield (Lanting, 2004). This complies with the conclusion of Wina *et al.* (2008) that overall efficiency of animal performance is improved if the readily degradable protein is protected from degradation in the rumen, while still digested in the intestines. Bateman (2005) and Ipharraguerre and Clark (2005) concluded that increase in milk yield is expected when level of RUP increases. At longer incubation period (72-hr), still high fractions of RUP could be expected to be digested due to the pH factor postruminally. This means that among the legumes tested, Flemingia is one of the most effective sources of bypass protein in ruminants and this was in accordance with the report of Lanting (2004). The effective protection provided by tannins in blocking/ counteracting the digestion effect of rumen microbes on the leaf meal and becoming inactive postruminally when pH changes as it passes through the abomasum and duodenum will allow more bypass protein reaching the small intestines.

With regards to CPD of SBM, Netemeyer *et al.* (1982) explained that enzymatic digestion increases when protein dispersibility index of SBM decreases. Furthermore, CP of SBM had high solubility in the rumen fluid due to the low amount of protein left at the earliest period (24 hr) which was relatively dependent on the

DMD. This was confirmed by Crooker *et al*. (1986) that SBM is extensively susceptible to ruminal degradation. The order and level of significance of crude protein disappearance rate (CPDR) was completely similar with CPD.

Among the protein feed sources tested, Flemingia had the lowest DM and CP degradation, indicating a greater potential as bypass protein source in ruminants. Moreover, Flemingia will provide the greatest amount of bypass protein than other legumes tested and is, therefore, recommended for ruminants that are in high level of production especially when diets are nutritionally deficient and concentrates are unavailable or expensive.

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