IN SITU RUMINAL DEGRADATION OF FOLIAGE WITH VARYING pH LEVELS FROM SELECTED TREES AND SHRUBS

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ABSTRACT

In situ **(nylon bag) technique was used to investigate the effects of forage pH level and rumen pH manipulation on rumen degradability of foliages from selected trees and shrubs. Six forages - Kakawate (*Gliricidia sepium)*, Madre de agua (*Trichanthera gigantea)*, Acacia (*Samanea saman)*, Gmelina (*Gmelina arborea)*, Robles (*Cassia siamea)* and Santol (*Sandoricum koetjape)* - were analyzed for their pH level and were categorized as low, medium and high pH; consequently, two samples in each category were used in the study. The study was set-up in a completely randomized design. The pH level of forages significantly influenced dry matter disappearance (DMD) in the first 24 hours of incubation, such that forages with medium to high pH levels generally showed higher values than those with low pH levels. The DMD after 48 hours of incubation appeared to be more affected by the characteristics of the forages influencing degradability rather than their pH levels. The addition of acetic acid to bring the rumen pH into an ideal level of 6.0-6.4 increased DMD, indicating the beneficial effects of rumen pH manipulation on forage digestibility.**

Keywords: forage, *in situ* degradability, pH level, rumen fermentation

INTRODUCTION

Tree leaves and shrub forages have always played a role in feeding livestock. Trees and shrubs are increasingly recognized as important components of animal feeding, especially as suppliers of protein and energy (FAO, Gutteridge and Ikhimioya as cited by Nahand *et al*., 2011). In difficult environmental conditions, where available grazing is not sufficient to meet the maintenance requirements of animals for a part of the year, the contribution from trees and shrubs is significant. The accepted fodder trees include *Artocarpus, Azadirachta, Calliandra*, *Canarium*, *Cocos nucifera, Desmodium, Flemingia*, *Gliricidia, Gmelina*, *Leucaena, Morus*, *Sesbania, Musa*, *Manihot, Mangifera, Psidium*, *Tamarindus*, *Terminalia, Theobroma cacao, Trichanthera, Swietenia* and *Sonneratia* (Devendra and Burns, 1983; Chen *et al.*, 1991; Moog, 1992; Gutteridge and Shelton, 1994; PCARRD, 1997; Ng'ambi, 1999; Melana, 2000; Boschini, 2002; Lejano, 2003; Suchitra, 2008).

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Madre de agua shrub, which was introduced from Columbia (Rosales, 1997), has been readily adopted by farmers in different countries for livestock feeding because of its tolerance to a wide range of ecological conditions and apparent resistance to pests and diseases (Nhan and Van Hon, 1999). Other fodder trees naturally grow or planted in pasture gardens and used as live fences. Elevitch and Wilkinson (2000) reported that *Gliricidia sepium* is the most common live-post species planted. Some are used as hedgerows of contour farms such as guava and citrus (Elevitch and Wilkinson, 2000), alley, and plantation crops or in sylvo-pastoral systems. Sylvo-pastoral systems can provide an economically-attractive timber-livestock production package for farmers by providing annual income from meat goats and sustainable browse systems and timber from trees (Nowak, 2008). Trees and shrubs are valuable sources of fuel wood, shelter, timber, herbal medicines, and food for people, particularly from fruit trees, and also help maintain soil fertility. Tree fodders contain high levels of crude protein and minerals, such as calcium and phosphorus, and many show high levels of digestibility. They are readily accepted by livestock, and presumably because of their deep-root systems, they continue to produce well into the dry season.

The productivity of ruminants is determined by many factors, but two of the most important are the quality and quantity of the feed they eat. There are many available forages; however, anti-nutritive factors can be a problem in some species (Paterson as cited by Nahand *et al*., 2011). Some forages are rejected or less accepted by animals because of high content of tannin, saponins, gossypol or mimosine. They may also be rejected because they are highly lignified, odorous, or may have high pH level (Fulgueira *et al*., 2007). As the quality of roughage exhibits a close relationship with rumen ecology, rumen microbes and fermentation patterns (Wanapat, 2000), the pH level of the forage is one of the factors that affect voluntary intake and determine forage quality (Fulgueira *et al*., 2007). It is considered to be a parameter that best determines the quality and fermentation rate and successful conservation of forages with moisture contents higher than 65% (Ferret *et al.* as cited by Fulgueira, 2007).

Ruminal pH plays an important role in rumen fermentation. Low ruminal pH, to a level below 5.5 is used to indicate subclinical ruminal acidosis (Oetzel *et al*. as cited by Beauchemin, 2011). This significantly shows negative impact on dry matter digestibility, dry matter intake, and microbial yield, thus, decreasing milk production while increasing feed costs (Allen, 1997). This is supported by Beauchemin (2011) who reported that the enzymes necessary for fiber breakdown do not function effectively at pH <6.0 and the growth rate of fibrolytic bacteria declines markedly at low pH.

The inherent properties of feedstuffs such as moisture, pH, starch content and availability, crude protein and fat content, can have a pronounced effect on ruminal pH, VFA and microbial protein production, and ultimately growth of the animal. For example, feeding forage high in pre-formed acids, such as some silages, will also reduce rumen pH (Lean as cited by RAGFAR, 2007). Since rumen pH plays an important role in rumen fermentation, the pH level of the forage must be connected to its degradability as it triggers changes in ruminal pH. Nevertheless, there is only limited information about the DM degradability in the rumen of the

forages as affected by their pH content.

There are several methods that have been used in order to evaluate the digestibility of feedstuffs such as *in vivo, in situ* and *in vitro* techniques (Maheri-Sis *et al*. as cited by Nahand, 2011). The *in vitro* method is used commonly because of its convenience, or when a large-scale testing of feedstuffs is required. The *in situ* method is routinely used for studying effects of the ruminal environment on digestibility of feedstuffs (Uden and Van Soest, Nocek, Marinucci *et al*. as cited by Varel and Kreikemeier, 1995). The *in situ* method is also capable of large-scale testing, but it has a faster rate of digestion compared to *in vitro* method (Graham and Aman as cited by Varel and Kreikemeier, 1995). This study tried to: 1) find out the effects of forage pH level on rumen degradability of the foliage from different trees and shrubs; and 2) improve rumen degradability of foliage from different trees and shrubs with varying pH levels through rumen pH manipulation.

MATERIALS AND METHODS

Measurement of foliage pH

Fresh leaves from different trees and shrubs were separately placed in a blender and homogenized for easy extraction of the juice. The juice was collected and placed in a clean container for pH determination. After pH determination, the foliage samples were categorized according to the pH levels as low, medium and high (Table 1). Two samples in each category were selected for the experiment.

Preparation of the animal and adjustment period

The experiment used a young Brahman yearling bull fitted with cannulae in the rumen. The bull was adjusted to the test diets two weeks prior to the conduct of the experiment. It was also dewormed with Ivermectin, and was housed in a pen with free access to drinking water. The diet of the cannulated yearling bull was composed of 70% Napier grass and 30% of the test forages (supplement); each of the six different foliage contributed 5% to the total diet. *Ad libitum* intake was established by giving 15-20% allowance of the day's offering based on the previous day's voluntary intake.

Rumen incubation of test forages

An important tool in the measurement of the quality of ruminant feeds is the use of the *in situ* Dacron bags feed evaluation method by Orskov *et al*. (1980). Aside from the fact that it provides a reliable means of predicting the digestibility of feedstuffs in the rumen, it further provides information on their degradation kinetics (Arieli *et al*. and Dhanoa *et al.* as cited by Ikhimioya *et al*., 2005).

The preparation of the samples for incubation was critical as they should represent, as much as possible, the materials as they would appear in the rumen had they been consumed by the animal (Orskov *et al.*, 1980). The sample was dried and hammer milled using a 2.5-3.0 mm screen. The milled leaves were oven dried at 100ºC overnight for 24 hr to determine the dry matter content of the leaves. The nylon bags were also oven dried at 60-65ºC for 30 min, and weighed right after

Table 1. The pH levels of different trees and shrubs tested.

Categories	Tree leaves	Scientific name	pH levels
Low (pH 3.25-4.50)	Robles	*Cassia siamea*	3.73
	Santol	*Sandoricum koetjape*	3.45
	Mango	*Mangifera indica*	4.38
	Talisay	*Terminalia catappa*	4.38
Medium (pH 4.51-5.76)	Kalumpit	*Terminalia microcarpa*	4.89
	Pili	*Canarium ovatum*	4.94
	Mahogany	*Swietenia mahagoni*	5.18
	Molave	*Vitex parviflora*	5.19
	Alagaw	*Premna odorata*	5.24
	Caimito	*Chrysophyllum cainito*	5.45
	Acacia	*Samanea saman*	5.43
	Pagatpat	*Sonneratia alba*	5.50
	Duranta	*Duranta erecta*	5.56
	Gmelina	*Gmelina arborea*	5.69
	Jackfruit	*Artocarpus heterophyllus*	5.72
High (pH 5.76- up)	Gumamela	*Hibiscus rosa-sinensis*	5.80
	Kakawate	*Gliricidia sepium*	7.06
	Mulberry	*Morus sp.*	7.16
	Thrichanthera	*Trichanthera gigantea*	7.47

cooling at room temperature. The forage sample was weighed and placed inside the individual nylon bags and sealed using an electric heat sealer to prevent the escape of samples during rumen incubation. Each sample was numbered corresponding to the feedstuff tested, and all the nylon bags with feed samples were placed in one lingerie bag and placed inside the rumen. The lingerie bag was weighted with stainless metal to prevent the nylon bag from floating on top of the rumen fluid which would result to uneven exposure of the test forages to the rumen microbes. Incubation of test forages in the rumen of the young bull at 24 and 48 hr was conducted without manipulation. This was followed by a 24-hour incubation of test forages with acetic acid addition to achieve ideal rumen pH.

All feed samples were replicated four times, incubated simultaneously in the rumen of the young bull and were removed after 24 hr of incubation according to the method used by Suchitra (2008). Immediately after removing the samples from the rumen, the bags were placed in a bucket of cold water to prevent further fermentation, and washed in running water with frequent rubbing using forefinger and thumb until the water runs clear. The samples were then dried in forced draft oven at 60-65ºC for about 48 hr, and weighed. The dry matter degradability of the residue was determined by subtracting the weight of the oven-dried residue plus

nylon bag after incubation from the weight of the oven-dried sample before incubation divided by the original DM content before incubation and then multiplied by 100.

Treatments and experimental design

The study used the Completely Randomized Design (CRD) with four replicates for each treatment forages placed in the rumen at one time. The dietary treatments were as follows:

Forage species 1 - Kakawate (high pH)
Forage species 2 - *Trichanthera* (high pH)
Forage species 3 - Acacia (medium pH)
Forage species 4 - Gmelina (medium pH)
Forage species 5 - Robles (low pH)
Forage species 6 - Santol (low pH)

Rumen pH manipulation

Within the 24-hour incubation period of the feed samples, measurement of the rumen pH was done one hour before and after feeding, using digital pH meter to determine the type of pH manipulation to be instituted (addition of either $NaCO_3$ or acetic acid). The addition of acetic acid was done to bring rumen pH to an optimum level of 6.0-6.4 for greater fiber digestion (Mutsvangwa and Wright, 2003) in two runs. The amount added was dependent on the pH level of the rumen based on the average of the various measurement periods. The acetic acid was gradually added into the rumen (gradual addition by 25 ml) and stirred to mix the solid and liquid phase of the digesta until the desired rumen pH level was reached (Table 2).

Table 2. Rumen pH manipulation through the addition of acetic acid to maintain a rumen pH of 6.0-6.4.

Day/Time	Without acetic acid addition	With acetic acid			
		First run		Second run	
Day 1, 1:30 PM (incubation time)	7.5	7.8	+ 450 ml to lower pH to 6.0-6.4	7.6	+ 250 ml to lower pH to 6.0-6.4
1:35 PM	7.5	6.0		6.2	
4:30 PM	7.8	6.2		6.4	
7:00 PM	6.5	6.4		6.6	
Day 2, 6:00 AM	7.9	7.0		7.5	
9:00 AM	6.5	6.2	+ 100 ml to lower pH to 6.0-6.4	6.7	+ 150 ml to lower pH to 6.0-6.4
12:00 noon	6.7	6.7		6.4	
1:30 PM	6.9	6.7		6.6	

Laboratory analyses

Samples of the foliages (leaves) were analyzed according to the established protocol of AOAC (1990). Cell wall components (NDF) were determined according to the method of Van Soest *et al*. (1991). DM content was analyzed in the laboratory using a forced draft oven set at 100ºC for 24 hr (Undersander *et al.,* 1993). Organic matter (OM) was determined by ashing leaves in a muffle furnace at 550ºC for 6 hr. Crude protein was analyzed by micro-Kjeldahl method and calculated as % N x 6.25 by AOAC (1990).

Data gathered

The data gathered include the pH level of the foliage/forage and the ruminal pH of the bull with or without manipulation. The pH is the acidity and alkalinity measurement of the sample. The ruminal dry matter disappearance (DMD) of the forage was also obtained; it is the disappearance or partial digestibility of the forage in the digestive tract because it is the measurement of digestibility obtained only in the rumen. The DMD was obtained using the following formula:

$$\text{Dry matter disappearance} = \frac{SW^a - SW^b}{\text{SWa - weight of nylon bag}} \times 100$$

Where:

SW^a = Weight of dried sample + nylon bag before incubation
SW^b = Weight of dried sample + nylon bag after incubation

The crude protein (CP) and neutral detergent fiber (NDF) contents of the forages tested were also analyzed to extrapolate their possible effects on rumen dry matter degradability in tandem with forage pH levels.

Analysis of data

The data were analyzed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) ver. 15. Treatment means were compared using Least Significant Difference test.

RESULTS AND DISCUSSION

***In situ* dry matter disappearance**

Presented in Table 3 is the percent ruminal DMD of foliages from different trees and shrubs with varying pH levels. The percent DMD after 24 hr incubation in the rumen showed significant differences among forages of varying pH levels; highest DMD values were observed from forages with medium to high pH levels falling within the optimum pH of 5.5-7.0 (RAGFAR, 2007) in the order of Kakawate, *Trichanthera* and Gmelina. Although Acacia has medium pH level of 5.43, ruminal DMD appeared to be limited by plant factors other than pH, most likely its high NDF content (37.79%), and perhaps its high tannin content (Silanikove as cited by Nahand, 2011; Cheema *et al.,* 2011). After 48 hr of incubation, there appeared to be no definite pattern of differences in DMD among forages tested relative to pH level

Table 3. *In situ* dry matter disappearance after 24 and 48 hr of incubation of forages from trees and shrubs with varying pH levels.

Treatments	Forage pH	Crude protein %	NDF %	% DMD		% DMD Increase
				After 24 hrs	After 48 hrs	
Kakawate	7.06	19.76	21.11	67.62[a]	75.78[c]	12.07
Trichanthera	7.47	18.95	23.26	65.82[ab]	85.30[a]	29.60
Acacia	5.43	26.71	37.79	37.79[d]	47.20[e]	24.90
Gmelina	5.69	16.74	20.77	64.79[bc]	85.13[a]	31.39
Robles	3.73	16.83	30.02	62.65[c]	72.76[d]	16.14
Santol	3.45	8.07	26.46	63.41[c]	83.41[b]	31.54
p-value				0.000	0.000	

Means with the same superscripts within a column are not different (P>0.05).

effects; thus, it appeared to be influenced greatly by other foliage characteristics and less by their pH contents. Highest DMD values were obtained from *Trichanthera* (high pH) and Gmelina (medium pH) followed by Santol (low pH). While Acacia appeared to be least digested after 24 hr of incubation, it had an increase in ruminal DMD comparable to that of *Trichanthera*, Gmelina and Santol after 48 hr of incubation, and with its high CP content of 26.71%, it can be a good source of bypass protein for ruminants, together with Kakawate and Robles.

The pH level of the forage affects ruminal pH and, thus, rumen degradability of the forage. This is because rumen pH plays an important role in maintaining ideal microbial ecology and providing a healthy rumen environment for achieving a good fermentation pattern (Beauchemin, 2011), eventually improving digestibility and animal production (Weimer, 1998). As expected, rumen pH rises following morning feeding as fermentation speeds up, and later stabilizes at a level characteristic of the type of feed despite variations in the pH of forages being fed; rumen degradation may now depend on diet factors other than forage pH. Thus, it is recommended to monitor in further studies rumen pH changes after 24 hr of feeding forages with varying pH levels.

***In situ* dry matter disappearance with acetic acid addition**

A similar pattern of differences, but of higher values, in ruminal DMD of foliages of varying pH levels after 24 hours of incubation can be observed with acetic acid addition as that of without (Table 4), indicating beneficial effects of rumen pH manipulation. Ruminal DMD values in the first run showed significantly high values for Gmelina, *Trichanthera*, Santol and Kakawate, with Acacia having the lowest. Practically all forages improved in ruminal DMD in the second run, except for Acacia which remained the lowest, indicating once again the beneficial effects of

Table 4. *In sit*u dry matter disappearance (DMD) as affected by acetic acid addition.

Treatments	Forage pH	DMD		
		Without acetic acid Addition	With acetic acid addition	
			First run	Second run
Kakawate	7.06	67.62[a]	72.07[b]	68.56[a]
Trichanthera	7.47	65.82[ab]	73.63[ab]	66.95[a]
Acacia	5.43	37.79[d]	38.71[d]	39.00[d]
Gmelina	5.69	64.79[bc]	75.24[a]	65.18[b]
Robles	3.73	62.65[c]	68.28[c]	63.54[b]
Santol	3.45	63.41[c]	73.30[ab]	59.61[c]
p-value		0.000**	0.000**	0.000**

Means with the same superscripts within a column are not different (P>0.05).

acetic addition to maintain rumen pH at optimum level. Mutsvangwa and Wright (2003) reported that for optimum rumen fermentation and fiber digestion, the ruminal pH should lie between 6.0 to 6.4, while Pitt *et al*. and Kolverver *et al*. as cited by Mourino *et al.* (2001) stated a rumen pH of 6.0 to 6.9 for optimal fiber digestion. However, the level of ruminal pH is not the only factor that needs consideration, but the rumen pH depression below 6.0 and the duration thereof as this could also affect overall fiber digestion (Orskov and Istasse as cited by Lehmann *et al.*, 2007). The amount of acetic acid to be added should, therefore, be based on the present level, and fluctuations thereafter, of rumen pH.

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