

The Neutral Detergent Fiber Digestibility of Some Tropical Grasses at Different Stage of Maturity

(Kecernaan Serat Detergen Netral Beberapa Rumput Tropis pada Tahap Penuaan yang Berbeda)

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Abstract. The digestibility of neutral detergent fiber (IVNDFD) was determined by Telly and Terry methods *in vitro* on 5 tropical grasses species, *Sorghum*, *Themeda*, *Iseilema*, *Brachyacne* and *Dicanthium*. Stem and leaf samples were harvested at different maturity stages, started from early flowering stage to the stage when the grasses were dried. In general, IVNDFD ranged from 22% to 41%. Stages of maturity affected IVNDFD in 4 species; IVNDFD was higher in the stems than in the leaves for 2 species out of 5 species of grasses; the rest was similar. There was no correlation between NDF and IVNDFD, showing that NDF degradability in the rumen was vary. Digestibility potential of NDF (PDNDF) varied from 21% to 44% and has negative correlation with IVNDFD ($r=0.75$). Growth affected PDNDF in 2 species; and 3 out 5 species observed showed PDNDF in the leaves was higher than that in the stems. Negative correlation was exist between dry matter digestibility (IVDMD), water soluble extract (WSE) and protein with PDNDF. Grasses with stated PDNDF values have relatively high NDF retention in the rumen, which will cause low NDF or dry matter consumption.

Key Words: NDF digestibility, tropical grasses, stem, leaves, maturity stage

Introduction

Ruminants require sufficient neutral detergent fiber (NDF) in their diets to maintain rumen function and maximize production. A large proportion of dietary NDF come from forages. The NDF content of forage however varies widely, depending on species, maturity, and growing environment (Nelson and Moser, 1994; Buxton and Fales, 2004; Mahyuddin, 2007).

The digestibility of NDF is an important parameter of forage quality because NDF comprise the largest amount of nutrient in ruminant diet and varies widely in its degradability in the rumen (Nocek and Russell, 1988; Allen and Oba, 1996). Forage with similar fiber (NDF) levels may have very different NDF digestibility (Grant *et al.*, 1995; Dado and Allen, 1996). Data of legumes with an NDF level of 40% showed a variation in NDF digestibility ranging from 38% to 57% (Ward, 2001). A number of factors influence NDFD levels, includes forage species, maturity, genetics and environment (Ariel and Adin, 1994; Llamas-Lamas and Comb,

1990; Jensen *et al.*, 2005; Robinson *et al.*, 2004). Several experiments show the impact of NDF digestibility (NDFD) on dry matter intake (DMI) and milk yield. An increased in dry matter intake (DMI) and milk yield were observed when animals were fed forage with a higher NDF digestibility (Dado and Allen, 1996; Llamas-lamas and Comb, 1990; Aydin *et al.*, 1999, Grant *et al.*, 1995; Oliver *et al.*, 2004; Mertens *et al.*, 2005; Raeth-Knight *et al.*, 2005). Oba and Allen (1999) have calculated that a 1 unit rise in NDFD in diet results in a 0.17 kg/day rise in dry matter intake and 0.25 kg increase in 4% fat-corrected milk. Thus changing NDFD in the forage base diets results in a dual mechanism whereby energy intake is increased. Simply lactating dairy cows will consume more forage which is of a higher energy content when forages are high in NDFD. The NDFD of forage can have a large impact on energy value of the diet.

The determination of NDFD is also important to use in the summative equation to predict energy content in forage and mixed ration. Lunberg *et al.* (2004) evaluated the utility of summative energy equations using 48 h *in vitro*

NDFD as the digestion coefficient for NDF by comparing predicted total digestible nutrient (TDN) to *in vitro* digestible organic matter (IVDOM) which is a reasonable assessment of *in vivo* digestibility. Using ADF as predictor of IVDOM accounted for 0.61 of the variance of IVDOM in corn silage as compared to 0.98 coefficient of determination between summative predicted TDN and IVDOM when NDFD was used as the digestion coefficient for NDF. These data clearly demonstrate the improved capability of summative equation using NDFD as an energy predictor. Robinson *et al.* (2004) found a superior relationship using *in vitro* NDFD measurement as compared to using lignin to estimate NDFD in prediction systems to estimate *in vivo* digestibility in sheep. A relatively weak relationship between acid detergent lignin and *in vivo* NDFD in sheep for C₄ grasses was also found by Jung *et al.* (1997). At present *in vitro* NDF digestibility (IVNDFD) determination is playing a larger role in estimating NDF digestion coefficient for forages and diet energy predictions as compared to using ADF or lignin. This paper reports the IVNDFD and the potential NDF digestibility (PNDFD) of stem and leaf of some tropical grasses with different stage of maturity.

Research Methods

This study was part of that previously reported by Mahyuddin (2007, 2008). Five species were used, *Sorghum stipodeum*, *Themeda australis*, *Iseilema vaginiflorum*, *Brachyacne convergens*, and *Dicanthium fecundum*, which were harvested at interval of 9, 12, 16, 23 and 36 days during the month of March to June.

Samples of grasses were separated into their botanical component, stem and leaf. Since the separation is time consuming, being done by hand, the petiole and the leaf were not separated but mixed together as one sample. The stage of maturity of the grasses at each time of harvest are presented in Table 1.

Prior to grinding they were oven dried at 80°C over night. Samples were ground to pass through a 1mm sieve in a small laboratory mill. After being ground, they were kept in closed containers, ready for chemical analysis (see Mahyuddin, 2007) and *in vitro* digestion (see Mahyuddin, 2008).

In vitro NDF digestibility (IVNDFD)

The IVNDFD was measured by *in vitro* digestion procedure as described in the previous experiment (Mahyuddin, 2008). A buffer and rumen fluid was added to a forage sample, and the mixture was allowed to ferment for several hours. The digestibility of NDF is then measured as the difference between the amount of NDF in the forage before and after the *in vitro* fermentation.

Thus the 48 hr IVNDFD

$$= \frac{\% \text{ NDF sample} - \% \text{ NDF after 48 hr digestion}}{\% \text{ NDF sample}}$$

Potential Digestibility of NDF (PNDFD)

The PNDFD was calculated as the amount of NDF in the grass sample minus the amount of NDF residue in *in vitro* digestion. Such that the 48 hr PNDFD =

$$\frac{72 \text{ hr PNDFD} - 48 \text{ hr PNDFD}}{72 \text{ hr PNDFD}}$$

Statistical Analysis

This experiment had no replication because it was not a field trial. Therefore to obtain an estimate of error, assumption of a linear relationship between the response and the stage of maturity must be established.

The analysis fitted Part as a qualitative factor, enable to asses whether leaf and stem have different IVDMD. Regression analysis on Period is done to see if there is a change over time. Interaction between Part and Period was also fitted to test for different responses between stem and leaf over stage of maturity.

Table 1. Plant material used in the experiment and stage of maturity at each harvest

Period of harvest	<i>Sorghum s.</i> <i>Themeda a.</i> <i>Iseilema v.</i>	<i>Brahyaacne c.</i> <i>Dicanthium f.</i>
EF	Early flowering	Late flowering
LF	Late flowering	Setting seed
SS	Setting seed	Late seed set
SSh	Seed shedding	Seed shedding
DO	Drying off	Drying off

EF = early flowering to late flowering;

LF = late flowering to setting seed;

SS = setting seed to late seed set;

SSh = seed shedding; DO = drying off

Data on in vitro dry matter digestibility (IVDMD) were subjected to one-way analysis of variance using model of SAS (1988). Multiple comparison test using Duncan's multiple range test (Snedecor and Cochran, 1980).

Results and Discussion

48 hr Invitro NDF Digestibility

The values of IVNDFD in this study are highly variable and the mean values ranging from 22% to 41% (Table 2). These values are considered to be low to medium range (Hoffman, 2003 ; Chase, 2003) because the plant materials were in mature stage.

The effect of plant parts and period (maturity) on IVNDFD (Table 4) is also similar to that for IVDMD (Mahyuddin, 2008). Maturity affected IVNDFD as shown by 4 out of 5 species; as maturity advanced, IVNDFD declined (Table 4). If the theory of Oba and Allen (1997) can also be applied vice versa, in this study approximately 19% units decline in IVNDFD with advancing maturity (Table 2) may reduce dry matter intake and milk yield by approximately 3.2 kg/d and 4.7 kg respectively. From the previous report (Mahyuddin, 2007), these tropical grasses had NDF content ranging from 73.1% to 86.3%. If dry

matter intake (DMI) is calculated based on the formula of $120/\%NDF$, then these grasses would have DMI ranging from 1.64% BW to 1.39% BW. All these calculations reflect that a high proportion of concentrate will be needed to satisfy the animal requirement.

With advancing maturity, plants develop xylem tissue for water transport, accumulate cellulose and other complex carbohydrates; these tissues become bound together by a process known as lignification (Buxton *et al.*, 1997). Lignin in plant cell wall (NDF) is more difficult for rumen bacteria to digest than cellulose or hemicellulose shown by the work of Claessens *et al.* (2005). As maturity proceeds, leaf to stem ratio declines (more stems, fewer leaves) and as a result NDF digestibility declines because a greater portion of total NDF is NDF in stem tissue (see Jung and Casler, 1990; Love *et al.*, 1998; Markovic *et al.*, 2007). However, in this study only 2 out of the 5 species showed the IVNDFD of leaf was higher than that of stem (Table 4). A higher digestible fiber is less filling because it is retained in the rumen for a shorter period of time. Since it is less filling in the rumen, diets containing highly digestible fiber allow greater dry matter intake (see Kendall and Combs, 2004; Ivan *et al.*, 2005).

Table 2. The 48 hr in vitro NDF digestibility (IVNDFD)

Species	EF		LF		SS		SSh		DO	
	S	L	S	L	S	L	S	L	S	L
Sorghum	59.4	42.8	59.2	41.9	29.2	35.2	28.7	32.8	24.0	21.6
Themeda	36.8	11.1	26.4	16.5	22.4	23.8	19.8	18.8	22.9	14.2
Iseilema	32.6	33.9	29.8	31.7	32.6	21.3	17.7	19.3	23.7	16.1
Brachyacne	26.2	44.3	32.9	46.1	30.5	47.2	24.7	43.7	19.1	30.4
Dicanthium	50.0	68.5	27.7	61.2	29.9	53.8	26.3	49.3	23.7	46.8
Average	41.0	40.1	35.2	39.5	28.9	36.3	22.3	32.7	22.7	23.7
SE	6.02	9.23	6.09	7.77	1.72	6.34	2.04	6.14	0.91	7.12

See Table 1 for EF, LF, SS, SSh and DO. S = Stem; L = Leaf

Table 3. The amount of PDNDF after 48 hr digestion

Species	EF		LF		SS		SSh		DO	
	S	L	S	L	S	L	S	L	S	L
Sorghum	12.9	15.8	12.9	30.4	22.7	15.8	34.7	29.9	19.1	52.3
Themeda	25.4	43.5	24.8	35.1	31.1	48.9	34.6	37.7	24.6	68.1
Iseilema	27.2	27.3	25.1	35.4	13.5	42.2	37.5	496.3	26.7	54.9
Brachyacne	20	20.9	29.1	20.1	19.2	11.7	23.0	18.3	43.7	27.6
Dicanthium	20.5	13.3	29.5	13.3	28.4	14.3	24.7	18.2	25.6	17.9
Average	21.2	24.2	24.3	26.9	23.0	26.6	30.9	30.1	27.9	44.2
SE	2.49	5.58	3.0	4.37	3.15	7.83	2.93	5.47	4.14	9.25

See Table 1 for EF, LF, SS, SSh and DO. S = stem; L = Leaf

Table 4. Summary of statistical analysis of grasses NDFD

Species	Part	Period
Sorghum	N.S	++
Themeda	N.S.	N.S
Iseilema	N.S	+
Brachyacne	++ L > S	+
Dicanthium	++ L > S	+

NS = non significant; ++ = significant at 1 %; + = significant at 5 % level.

High producing herds, herds that maximize forage feeding, and high-group cows will benefit most from forages with high NDF digestibility. Among the 5 grasses measured, *Dicanthium* had the highest IVNDFD followed by *Sorghum*, *Brachyacne*, *Iseilema* and *Themeda* (Table 2).

In this study the 48 hr IVNDFD and *in vitro* dry matter digestibility (IVDMD) were calculated on the basis of undigested NDF residue, therefore it was anticipated that they were highly correlated. Since IVDMD has been found to be highly correlated with *in vivo* DMD, it is expected that IVNDFD will also be correlated with *in vivo* DMD, a similar result found with sheep experiment by Robinson *et al.* (2004).

The relationship between IVNDFD and IVDMD with chemical component is presented in Table 6. There was no significant correlation between IVNDFD and NDF content suggesting that there were highly variable in the degradability of NDF in the rumen. This is consistent with the results found by Nocek and Russell (1988) Allen and Oba (1996) and Spanghero *et al.* (2009).

A higher negative correlation coefficient between IVNDFD and lignin content may explain the interaction of lignin and other fiber components. Lignin impacts the digestibility of the forage directly as indigestible material and indirectly as it inhibits digestion of chemically associated fiber. In evaluating forage quality using lignin, it is important to view in the context of the NDF value of the forage since digestibility is a function of the lignin-fiber interaction. Although there was suggestion to be more descriptive to look at lignin in terms of lignin/NDF ratio, in this study however, when lignin/NDF ratio was used to correlate with IVNDFD there was only little

improvement in the coefficient correlation (0.52 vs 0.55).

Overall, the correlation coefficient of chemical component with IVDMD was higher than that with IVNDFD (Table 6) suggesting that chemical component is better used to predict IVDMD than to predict IVNDFD.

Unfortunately, prediction of energy content (TDN) of these grasses using summative model adopted by NRC (2001) which include coefficient digestibility of NDF were not possible since other nutrients such as NDF CP, ADF CP and Fat were not determined. Similarly calculated metabolizable energy (ME) were also not possible as organic matter digestibility were not determined.

48 hr Potential Digestible NDF (PDNDF)

Dietary NDF is partly digestible and partly indigestible. The digestible NDF is digested at a constant rate (fraction of NDF digested per unit time) (Mertens 1977), and this fraction is usually termed potentially digestible NDF (PDNDF). The PDNDF therefore, the amount of digestible NDF in the DM (dry matter) of the sample and is a measure of the amount of fiber available for microbial digestion in the rumen. The PDNDF is therefore negatively correlated with the NDFD. In this experiment, as both PDNDF and DM digestibility were measured on the same basis (based on undigested residue of NDF), they must be correlated with each other (see Table 6).

A high value of PDNDFD means that there are more fiber to be digested, indicating slow retention time in the rumen, and low dry matter intake.

Table 5. Summary of statistical analysis of grasses PDNDF

Species	Part	Period
Sorghum	N.S	+
Themeda	+ L > S	N.S
Iseilema	+ L > S	+
Brachyacne	N.S	N.S
Dicanthium	+ L > S	N.S

NS = non significant; ++ = significant at 1 %; + = significant at 5 % level

Table 6 . Correlation coefficient (r) of regression between in vitro NDF digestibility (IVNDFD), potential digestible NDF (PDNDF) and in vitro dry matter digestibility (IVDMD) and chemical component

	IVDMD	NDF	Lignin	ADF	WSE	WSC	CP
IVNDFD	0.96 **	-0.26 NS	-0.52 **	-0.35 **	0.61 **	0.14 NS	0.47 **
PDNDF	-0.75 **	-0.02 NS	0.26 NS	0.23 NS	-0.51 **	-0.12 NS	-0.34 **
IVDMD@		- 0.49 **	- 0.64 **	- 0.50 **	0.71 **	0.21 NS	0.59 **

The mean values of PDNDF in this study ranged from 21% to 44% and the highest PDNDF was found in *Themeda* followed by *Iseilema*, *Dicanthium*, *Brachyacne* and *Sorghum*. Differences among species in the amount of PDNDF residues after 48 hours, may be associated with differences in morphological and physical characteristic of the plant material (see Brink *et al.*, 2007). They may be related to factors that inhibit or stimulate microbial growth and the production of their fiber-degrading enzymes.

The effect of plant fraction and maturity on the amount of potential digestible NDF (PDNDF) after 48 hr of digestion vary with plant species (Table 5). The PDNDF leaf of *Themeda*, *Iseilema* and *Dicanthium* were higher than stem whereas, no significant difference between PDNDF leaf and stem in *Sorghum* or *Brachyacne*. Two (*Sorghum* and *Iseilema*) out of 5 species showed that maturity had an effect on PDNDF.

There was a strong negative correlation between IVDMD, WSE and protein with PDNDF (Table 6) meaning that the lower IVDMD, WSE and protein content of a grass the higher the NDF retain in the rumen. Since the values of PDNDF in this study were considered high and thus NDF retention were high, it is anticipated that animals consume these grasses will have a lower intake of NDF or dry matter (see Nousiainen *et al.*, 2003).

Application

The measurement of NDF digestibility accurately and precisely is important in generating a quantitative summative energy prediction. Unfortunately most laboratory cannot conduct the assay because an *in vitro* NDFD procedure requires rumen fluid from a live cannulated animal. In addition to that, energy prediction using summative equation become expensive since it also requires some chemical analysis.

However, *in vitro* NDF digestibility can be useful as a troubleshooting tool when considering

ration performance problem. It is useful for ranking forage quality on the farm for allocation to various animal groups. Those that have significant better or poorer digestibility values may be allocated to animal groups on the farm where they may be best utilized. Forages with low NDFD are mainly of concern in diets of lactating dairy cows. Other classes of dairy cattle such as dry cows and heifers can utilize forages with lower NDFD. Similarly beef cows and ewes which do not have high energy demand may do well with lower NDFD forages. Since high producing dairy cows are often in negative energy balance, forages and diets low in NDFD that have the potential to limit feed or energy intake are of a great concern.

The NDFD although can have an impact on ration formulation and animal performance, NDF concentration in feeds and diet has much greater impact. The impact of changing NDFD among sources is much less than the impact of differences in NDF and NDS (Neutral Detergent Soluble) among sources of feeds. Thus, it is most important to ensure that the NDF and energy density of the ration are suitable for the animal. Variation of NDFD in forages is of secondary importance and should be used to fine-tune rations. If rations are formulated to have high energy density (low fiber content) in relation to animal requirements and forages contribute a small portion of the NDF in the ration, then the primary impact of NDFD is on the energy value of the forage. Various summative equations can be adjusted the energy value of the forage for changes in NDFD. If ration are formulated to maximize the proportion of forage in the ration, then the impact of NDFD on intake as well as energy should be considered.

The evaluation of NDF digestibility provides more information for assessing quality when making forage purchasing decisions and provide quality information for characterizing forage materials and anticipating animal performance. It is important for the user of this information to

have an understanding of the average and ranges for NDF digestibility values for various forage types from the laboratory used. Comparison can be adequately made only as this information is available and understood by those using NDF digestibility information. A user of this information should have a good understanding of the basis and quality of the information coming from the forage laboratory. It should be recognized that significant differences exist between laboratories in NDF digestibility procedures and that average values and ranges will differ.

Conclusions

The IVNDFD is affected by stage of maturity more than by plant fraction. There was no correlation between IVNDFD and NDF content suggesting that NDF varies in degradability. The potential digestibility of NDF (PDNDF) was negatively correlated with IVDMD, WSE and protein. Since PDNDF values in these tropical grasses were high, thus NDF retention was high, it is anticipated that intake of NDF or dry mater will be reduced.

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