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Relationship Between Chemical Component and *In Vitro* Digestibility of Tropical Grasses

PRAPTI MAHYUDDIN

Indonesia Research Institute for Animal Production, Jalan Raya Tapos, Ciawi, P.O.Box. 221, Bogor 16002, Indonesia
Phone: +62-251-8240751, Fax: +62-251-8240754, E-mail: pmahyuddin@yahoo.com

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Fifty samples of leaf and stem of *Sorghum stipodeum, Themeda australis, Iseilema vaginiflorum, Brachyacne convergens,* and *Dicanthium fecundum* with different stage of maturity were used to study the relationship between their chemical components and *in vitro* dry matter digestibility (IVDMD). The IVDMD was performed by two stage of digestion; the first stage was digestion in rumen inoculum and the second stage was digestion of protein using neutral detergent solution. The relationship between chemical components and IVDMD was analysed using regression method. There was a negative correlation between fiber component [neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin] and a positive correlation between water soluble extract (WSE) or crude protein with IVDMD. Water soluble extract was the best predictor of IVDMD with r = 0.71 and residual standard deviation of 8.4 unit. Since the method of WSE is simple and inexpensive, it can be used as a predictor of dry matter digestibility of most forages. Although there were large variation exist among the species, however, the stage of maturity was an important factor affecting IVDMD showed in four out of five species tested. In two of the five species measured, the IVDMD were higher in leaf than that in stem.

Key words: *in vitro* dry matter digestibility (IVDMD), chemical component, forage quality, maturity

INTRODUCTION

Forage is a basic feed for ruminant animal; knowledge of forage quality is required by farmers so that they can select the best quality forage offerred to their livestock. Forage quality is directly related to the extent to which the plant provides nutrient for animals. Reid (1994) defined forage quality as the product of voluntary intake, digestibility and the efficiency of nutrient used by the animal. The efficiency of ruminant production based on forage as the main protein and energy sources are highly dependent on forage maturity, which is often considered to be the primary factor determining its nutritive value (Nelson & Moser 1994).

Assessment of forage quality which include the measurement of animal performance that have production potential, requires significant amounts of feed, is expensive, laborious, and unpractical for screening large numbers of feeds. Most methods of feed evaluation involves the determination of chemical composition and digestibility, followed by calculation of energy values. Methods of determination of chemical composition have been developed which give some reliable results. Determination of feed digestibility by animal trial also gives reliable results however, this method is expensive, time consuming and laborious. Therefore, *in vitro* digestibilty methods have been developed using small quantities of feed (< 1 g) to simulate *in vivo* digestion (Tilley & Terry 1963; Van Soest *et al.* 1967; Orskov & McDonald 1979).

Digestibility (and its depression) is a function of the competition between digestion and rate of passage. Van Soest (1994) pointed out that digestibility depression is inversely related to lignification and to the rate of digestion. Both lignification and rate of digestion are influenced by maturity and anatomy of the plant fraction, leaf, and stem (Wilson & Hatfield 1997).

This paper reports the *in vitro* dry matter digestibility (IVDMD) of some tropical grasses and its relationship with the chemical component.

MATERIALS AND METHODS

This Experiment was Part of the Experiment Previously Reported by Mahyuddin (2007). There were five species used in this experiement, *Sorghum stipodeum*, *Themeda australis*, *Iseilema vaginiflorum*, *Brachyacne convergens*, and *Dicanthium fecundum*, harvested at interval of 9, 12, 16, 23, and 36 days during the month of March to June. The forage grew under natural conditions in the Kinberley region of northern Western Australia which has an arid to semi-arid monsoonal-type climate, characteristically hot and wet in summer (wet season), warm and dry in winter (dry season). Annual rainfall ranges from 250 to 1000 mm; ninety percent of the rainfall of 750 mm falls during the four months of the wet season (mid November to early April) with January and February the wettest months. The reminder of the year is virtually dry. The coolest months are from May to August with a maximum temperature of 32 ^{o}C and minimum temperature of 16 ^{o}C. For the rest of the year the maximum temperature exceeds 35 ^{o}C and the hottest months (October to November) often exceeds 38 ^{o}C.

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 1 mm sieve in a small laboratory mill. After being ground, they were kept in closed containers, ready for chemical analysis (Mahyuddin 2007) and *in vitro* digestion.

***In Vitro* Digestibility.** The *in vitro* digestibility method used was a two main stages; the first stage is designed to simulate conditions within the reticulo-rumen, and involves the anaerobic digestion of forage at 39 °C in the dark (without light) by rumen micro-organisms. The inoculum of micro-organisms was supplied as strained rumen fluid obtained from fistulated animals. To maintain the microbial activity, the pH level in the inoculum was controlled by adding a relatively large amount of buffer solution to the inoculum. Most fiber digestion occurs in the first stage which is complete in 48 hours. The second stage is designed to simulate conditions in the abomasums and small intestine, and involves digestion of the protein in the residue left by the first stage. The residue was plant fragments mostly undigested dietary protein and the microbial protein. In this experiment the modified Tilley and Terry (1963) was used, in which removal of the undigested protein was carried out using neutral detergent solution (Van Soest *et al*. 1967).

First Stage of Digestion. Centrifuge tubes containing 0.75 g ground forage were inoculated with 75 ml rumen-buffer inoculum, and flushed with CO_2. The centrifuge tubes were capped immediately with rubber bungs fitted with gas-release valves and were placed in a constant temperature waterbath at 39 °C. The waterbath was equipped with a thermostatic stirrer which gently stirred the water. The tubes were shaken gently to enhance the sinking of floating particles. Fermentation was stopped at 48 hrs using approximately 8 ml of 95% ethanol. In each *in vitro* run, duplicate sample of leaf and stem of one species in different stage of growth plus a standard sample were tested.

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

Period of harvest	*Sorghum stipodeum, Themeda australis, Iseilema vaginiflorum*	*Brachyacne convergens, Dican fecundum*
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.

Rumen Fluid. Samples of rumen fluid were obtained from two wethers fitted with permanent fistulae. They were fed on high-quality (50:50 mixture of oaten and lucerne chaff) maintenance ration (900 g/day). The rumen fluid was taken via fistulae immediately before feeding at 8.30 a.m. Rumen samples from the two sheep were mixed then strained through a double layer of muslin cloth into thermos flasks for immediate use.

Buffer Solution. The buffer solution had a composition similar to rumen saliva with the addition of urea. Each liter contained 18.44 g of anhydrous Na_2HPO_3, 49 g $NaCO_3$, 23 g NaCl, 2.84 g KCl, 5.25 g urea, 0.2 g CaCl, and 0.3 g $MgCl_2$, with CaCl and $MgCl_2$ added at last (pH 8.2). The buffer solution was then mixed with rumen fluid in the proportion of 4 buffer:16 distilled water: 5 rumen fluid. This fluid was kept at 39 °C in a waterbath and bubbled with CO_2 for approximately 20 minutes before use.

Second Stage of Digestion. The centrifuge tubes were removed after 48 hours period of digestion and the contents were filtered on previously weighed coarse-porosity sintered glass crucibles. The digestion residues were then treated with neutral detergent solution for 1 hour to dissolve substances left from the first stage. It was considered unnecessary to use blanks in this procedure since no residues were found in the blank after being tested with neutral detergent.

Statistical Analysis. This experiment had no replication 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 was done to see if there was 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. Data on *in vitro* dry matter digestibility (IVDMD) were subjected to one-way analysis of variance using model of SAS (1988). Multiple comparison test of Duncan's multiple range was applied (Snedecor & Cochran 1980).

RESULTS

***In Vitro* dry Matter Digestibility (IVDMD).** Although there was a large variation among the species studied, on average the IVDMD tended to decrease with advancing maturity and the leaf had higher IVDMD than the stem fraction (Table 2). As maturity increased on average the leaf IVDMD decreased from 56.1 to 42.7% while the stem IVDMD

Table 2. *In vitro* dry matter digestibility (IVDMD) of stem and leaf in grasses at different stage of maturity

Species	EF		LF		SS		SSh		DO	
	S	L	S	L	S	L	S	L	S	L
	%									
Sorghum stipodeum	68.90	60.10	71.80	61.00	37.70	48.40	38.60	48.90	30.90	40.30
Themeda australis	45.90	36.30	38.80	38.80	38.50	43.50	34.80	41.00	36.50	29.60
Iseilema vaginiflorum	44.60	51.10	43.00	42.00	46.00	42.30	32.20	40.50	35.20	35.80
Brachyacne convergens	39.10	56.40	47.30	59.50	45.30	59.70	40.60	56.80	34.40	47.70
Dicanthium fecundum	58.70	76.60	39.10	71.50	42.80	65.20	40.30	62.70	36.20	60.30
Averange	51.40	56.10	48.00	54.60	42.10	51.80	37.30	50.00	34.60	42.70
Standard error	5.43	6.55	6.16	6.17	1.71	4.55	1.64	4.37	1.01	5.30

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; S: stem; L: leaf.

decreased from 51.4 to 34.6%. Except in *Themeda* where the effect of maturity was not significant, the other four species showed a decrease in IVDMD as maturity advanced. *Sorghum* in particular showed a sharp decrease in IVDMD from 68.9 to 30.9% in stem and from 60 to 40.2% in leaf. This marked dropped in IVDMD occurred at seed set and at dry off in both stem and leaf.

Only two (*Brahyacne* and *Dicanthium*) out of five species studied showed highly significant between leaf and stem IVDMD. Leaf had a higher IVDMD than the stem fraction with the mean difference of 15 and 23.6% unit for *Brachyacne* and *Dicanthium*, respectively. Whereas in *Sorghum*, *Themeda* and *Iseilema* there were no significant difference in IVDMD between leaf and stem (Table 3).

Table 3. Summary of statistical analysis of grass *In vitro* dry matter digestibility (IVDMD)

Species	Part	Period
Sorghum stipodeum	NS	++
Themeda australis	NS	NS
Iseilema vaginiflorum	NS	+
Brachyacne convergens	++ L > S	+
Dicanthium fecundum	++ L > S	+

S: stem; L: leaf; ++: significant at 1% level; +: significant at 5% level; NS: non significant.

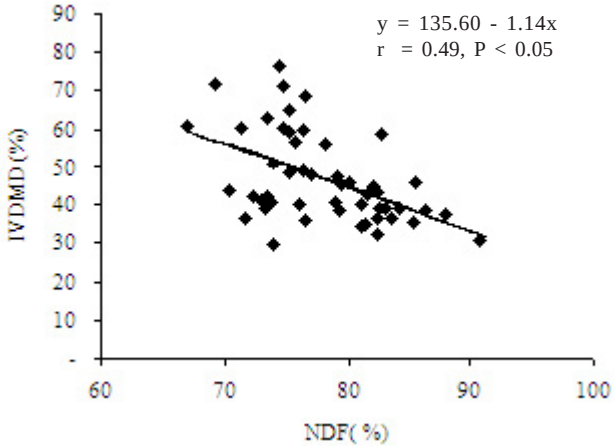

Figure 1. Realtionship between neutral detergent fiber (NDF) content and *In vitro* dry matter digestibility (IVDMD) of tropical grasses.

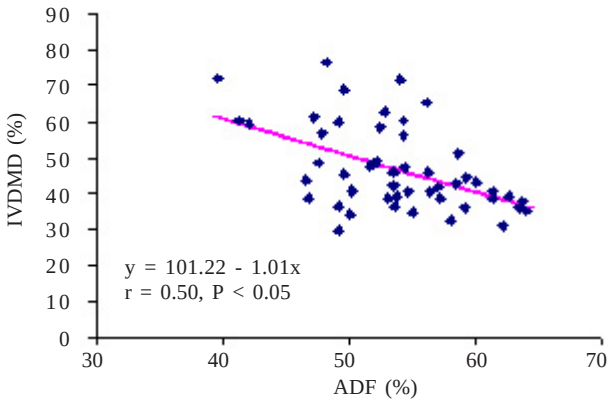

Figure 2. Relationship between acid detergent fiber (ADF) content and *In vitro* dry matter digestibility (IVDMD) of tropical grasses.

Relationship Between Chemical Component and IVDMD. The relationship between chemical component and IVDMD taken from 50 samples of leaf and stem of five grasses studied are shown in Figures 1-6. The individual species relationship between chemical component and IVDMD are shown in Table 4.

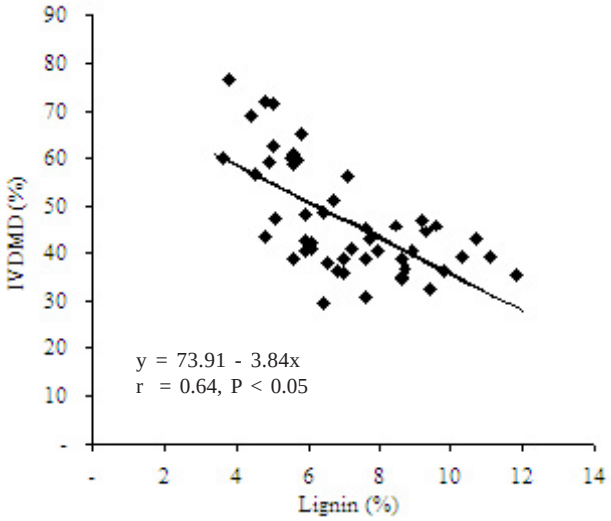

Figure 3. Relationship between lignin content and *In vitro* dry matter digestibility (IVDMD) of tropical grasses.

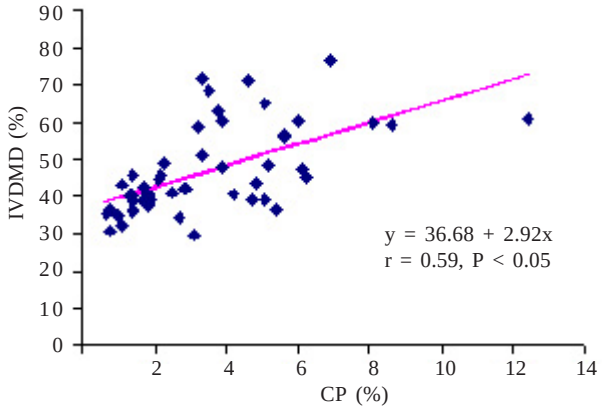

Figure 4. Relationship between crude protein (CP) content and *In vitro* dry matter digestibility (IVDMD) of tropical grasses.

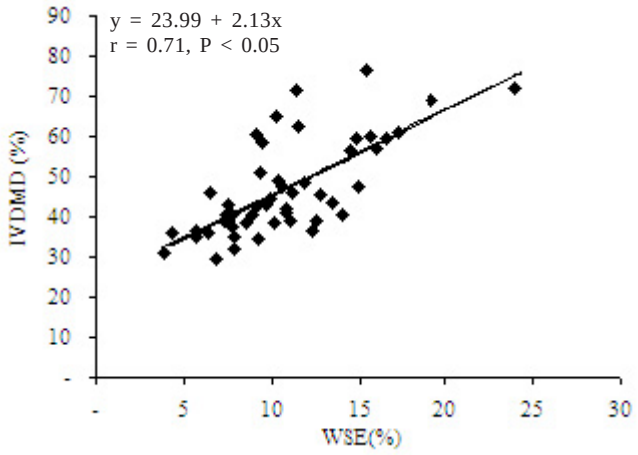

Figure 5. Relationship between water soluble extract (WSE) and *In vitro* dry matter digestibility (IVDMD) of tropical grasses.

Table 4. Correlation coefficients between chemical components and *In vitro* dry matter digestibility (IVDMD) in each species

Species	NDF	ADF	Lignin	CP	WSC	WSE
Sorghum stipodeum	-0.82**	-0.86**	-0.85**	0.52NS	0.91**	0.98**
Themeda australis	0.15NS	-0.02NS	-0.1NS	0.11NS	-0.22NS	0.29NS
Iseilema vaginiflorum	-0.37NS	-0.40NS	-0.26NS	0.66**	0.37NS	0.69**
Brachyacne convergens	-0.86**	-0.42NS	-0.71**	0.79**	0.16NS	0.77**
Dicanthium fecundum	-0.85**	-0.89**	-0.95**	0.96**	-0.63**	0.89**

NDF: neutral detergent fiber, ADF: acid detergent fiber, CP: crude protein, WSC: water soluble carbohydrate, WSE: water soluble extract, **: highly significant; NS: non significan.

Table 5. Correlation coefficient (R) and residual standard deviation (RSD) of regression between chemical component and *In vitro* dry matter digestibility (IVDMD)

		NDF	ADF	Lignin	CP	WSE	WSC
IVDMD	R	0.49	0.50	0.64	0.59	0.71	0.21
	RSD	10.39	10.36	9.16	9.67	8.41	11.70

NDF: neutral detergent fiber, ADF: acid detergent fiber, CP: crude protein, WSC: water soluble carbohydrate, WSE: water soluble extract.

There were negative correlations between fiber component, neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin with IVDMD. While NDF and ADF had similar (r = 0.5, Figure 1 & 2) lignin had higher (r = 0.64) coefficient correlation with IVDMD (Figure 3). A positive correlation of crude protein (CP, r = 0.59) and water soluble extract (WSE, r = 0.71) with IVDMD existed (Figures 4 & 5), whereas there is no significant correlation of water soluble carbohydrate (WSC) with IVDMD (Table 5).

Water soluble extract was important as a predictor of digestibility appeared in four of five species (Table 4). Among the chemical components WSE was the best predictor of digestibility with r = 0.71 and residual standard deviation (RSD) of 8.41 unit (Table 5).

DISCUSSION

***In Vitro* dry Matter Digestibility (IVDMD).** It has been accepted that the *in vitro* digestion technique is the most accurate method available at present to predict digestibility. Since the standard forage available in this laboratory did not have a digestibility within the range of the samples being tested, and also because they came from a different environment, it was not valid to extrapolate from the standard sample data to predict *in vivo* digestibility. However, the preliminary test to predict *in vivo* digestibility from the *in vitro* data of standard samples was successful (r = 0.92). This suggests that the *in vitro* digestion system was reliable in this work and therefore, it is assumed that the *in vitro* digestibility of the tropical grasses tested would have had a high correlation with their *in vivo* digestibility.

The IVDMD Value Varied Among the Species Studied. However, on average the highest value of IVDMD was 56.1% suggesting that rumen distention could be a primary factor in regulating voluntary intake (Minson 1990). Furthermore, since the nitrogen level was low (less than 7%) in these plant material (Mahyuddin 2007), voluntary intake may be reduced below that limited by rumen distention. Moran (2005) related dry matter (DM) digestibility of some tropical grasses with metabolisable energy (ME) content; based on his finding, the ME content of the species tested in this experiment might fall with in the range of 7.2 MJ/kg to 7.9MJ/kg DM.

The low values of IVDMD was anticipated as the plant materials were already in advanced stage of growth, the values might be higher at immature stage. The stage of maturity has proven to be an important factor affecting IVDMD showed in four out of five species tested.

Leafiness seems to be another factor that should be considered when evaluating forage quality. From the five species measured, three species showed no difference and two species showed the higher IVDMD for the leaf than for the stem. Laredo and Minson (1973) found that the voluntary intake of leaf was always higher than that of the stem, even though the leaf digestibility might be lower, this was because of the shorter retention time of leaf compared to that of the stem.

Relationship between IVDMD and chemical component. Except for water soluble carbohydrate (WSC), the NDF, ADF, lignin, protein, and WSE were all significantly correlated with IVDMD. The fiber component (NDF,ADF, and lignin) was negatively correlated with IVDMD because those components were less digestible than the non-fibrous components (protein, WSE, and WSC).

Many forage testing programs use simple regression equations to predict dry matter intake (DMI) from NDF and digestible dry matter (DDM) from ADF. Coleman and Moore (2002) pointed out that published r values had ranged from -0.03 to -0.90 between DMI and NDF and from -0.39 to -0.93 between DDM and ADF. In the present study, the r value was -0.5 between ADF and IVDMD was within that range.

Compared to other chemical components, WSE was the best predictor of IVDMD with coefficient correlation of 0.71 and residual standard deviation (RSD) of 8.4 unit (Table 6). This results was consistent with the result found by Lai and Huong (1999) and Buntha and Ty (2006) where both *in vitro* and *in vivo* DM digestibility of various plant species was highly correlated with WSE. This is true since WSE was found to be readily fermentable fraction of roughages in ruminants (Orskov & Shand 1997). Furthermore, it has been suggested that WSE is related to the cell content of plant materials (Chermiti *et al.* 1996). Since the method of WSE is simple and inexpensive, it can be used as a predictor of the DM digestibility of most forages.

Barton *et al.* (1976) found that protein was the best predictor of digestibility for tropical (r = -0.90), but not for temperate species (r = -0.17). In this study the correlation coefficient between protein and IVDMD was only 0.59 similar to that found by Nasrullah *et al.* 2003 (r = 0.54) who tested 121 sample of tropical grasses grown in South Sulawesi.

The non significant correlation between WSC and IVDMD was also detected by the work of Michell (1973) with temperate pasture. Water soluble carbohydrate however, is often related with dry matter intake (Michell 1973; Huntington & Burns 2007). The low correlation between IVDMD and chemical component in this study was partly due to large variation among the species. *Themeda* for example, had no significant correlation between all chemical components and IVDMD while for *Dicanthium*, all chemical components were highly correlated with IVDMD (Table 5).

At present, the *in vitro* digestion technique is the only accurate laboratory method for predicting *in vivo* digestibility. A good relationship between *in vivo* and *in vitro* digestibility can be obtained if the procedure is well standardized. The determination of forage quality at this level should be considered as the first stage of a screening programs, but the final stage of a program should include the actual animal production potential. The screening procedures using laboratory, animal house, and grazing trials although are accepted at present, must continually be improved. Particularly in the laboratory stage, an improvement is required to obtain an accurate laboratory method for estimation of the voluntary intake of animals. So far, it has not been possible to predict accurately the voluntary intake from *in vitro* digestibility data. In tropical grasses, the relationship between digestibility and intake is not good (Minson 1990). In all cases, the coefficient of correlation is too low to suggest that digestibility is the only factor controlling intake. Therefore, a complete understanding of intake control in tropical grasses, can only be achieved after a more thorough examination of the chemical and physical characteristics of the material, a description of their structure and an examination of the cause and effect relationship with intake, digestibility, rate of digestion, and rate of passage. If there was a low correlation between intake and digestibility within species, it is essential that laboratory quality estimations are interpreted very carefully since they would only estimate digestibility.

The quality of tropical grasses is quite variable as it can be seen from the chemical composition (Mahyuddin 2007) and IVDMD data from this experiment. In general, tropical grasses have a lower digestibility and intake compared to temperate grasses. This may be reflected in a low production level of the animal. Under practical farming conditions, insufficient intake of digestible energy from feed is a factor responsible for limiting production. This problem may be solved by: (i) selection and breeding of better cultivars with high digestibility and intake, (ii) grazing or harvesting at immature stage to avoid the decrease in digestibility and intake, (iii) inclusion of legumes and the application of fertilizer to increase the protein content in the plant, (iv) feeding concentrates and pelleting forages to increase feed consumption.

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