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Original Research

Comparative Studies of Two In Vitro Forage Evaluation Technique

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Abstract

The study was conducted with eight indigenous forages collected from two different places namely Khanapara and Barnihat, Assam at 45 days interval for three times. The main aim is to compare in vitro nutrient digestibility of forages by using two stage Tilley and Terry method, (1963) and Enzymatic digestion using pepsin and cellulase described by De Boever et al. (1985). The mean in vitro DM and OM digestibility % decreased from 1st to the 3rd harvest in both the method. The combined analysis revealed no significant difference on In vitro digestibility values estimated by both the methods between the locations but difference between species were highly significant (P<0.01). Both dry matter and organic matter digestibility values were higher with the enzymatic method as compared to two stage Tilley and Terry method.

Keywords: Comparison, Digestibility, Dry Matter, Forage, In vitro, Organic Matter

Introduction

Forages are the single most important feed source for ruminants worldwide. Forages are edible parts of plants, other than separated grain, usually with substantial contents of cell walls. They are suited to utilisation by herbivores that have a capacity for microbial digestion of cell wall constituents (Wilkins, 2000). Forages are essential component of ruminant's diet. They provide the coarse fibers required to optimize the rumen function. Forage is defined as the edible parts of plants, other than separated grain that can provide feed for grazing animals or that can be harvested for feeding (Forage & Grazing Terminology Committee, 1991). This definition includes the classes of feed such as herbage, hay and silage, browse and straws (Wilkins, 2000). The narrower term 'forage crop' is often used to describe crops, commonly annual or biennial, which are grown to be utilized by grazing or harvested as a whole crop (for example maize and sorghum). Forage consists largely of carbohydrate in the form of fibre, and its digestion is accomplished through the enzymic action of the rumen microbes. A wide range of plant feeds have substantial cell wall content (root crops are an exception) and are suited for utilization by ruminants with their substantial capability of cell wall component digestion by rumen microbes. Quality forage production is important in livestock production. The forage quality is highly variable and therefore their quality must be assessed before formulation of the diet. Forages contribute a large part of total dietary DM and ruminant animal can convert the fibrous plant materials into energy. However, the forage quality is highly variable which depends on species, stages of maturity of forages, soil type and water level in the soil. The stage of maturity influenced the chemical composition and nutrient digestibility. Therefore, the chemical analysis and evaluation of the forage is important to know the nutrient availability of the ruminant.

The developing country like India is basically agricultural based and about 70% of its people live in villages where fodder crops are equally important as that of any of the food crops. Assam, an agrarian state with most of the population relying directly or indirectly on agriculture for their livelihood. The ruminants are normally fed on the crop residues, cultivated fodder and fodder from common resources like forests, permanent pastures and grazing lands. An accurate ruminant ration calculation needs assessment of the forage samples/batch at suitable time interval. Chemical and *in vitro* method of forage analysis are considered as the suitable method for screening a large number of samples which serve as an indirect measure of forage quality. As the nutritive value of forages varies not only between species, but also within species and time of harvest. Digestibility experiments with animals are expensive, time-consuming and require large quantities of feed, so they are unsuitable for practical feed evaluation.

Among the existing laboratory methods, those using rumen liquor appear to be the most precise for predicting the nutritive value of forages. However low reproducibility between laboratories and the need for fistulated animals are disadvantages with this technique. Thus, many attempts during the last 40 years have been made to predict the nutritive value of forages by using enzymatic preparations (De Boever *et al*., 1985) which also give good correlation with *in vivo* estimates. In the State of Assam, the fodder cultivation practices are limited and the animals mainly depend on indigenous grasses and various fodder tree leaves as the forage source. However, the information on the nutritive values of the indigenous grasses are limited. Keeping in view the above facts, an attempt will be made to study the correlation between *in vitro* digestibility of forages by using rumen liquor with *in vitro* digestibility of forages using enzymatic technique.

Materials and Methods

The study was undertaken on eight indigenous forages namely- Ulu (*Imperata cylindrica),* Arali (*Leersia hexandra*), Kuchi *(Thysanolaena maxima),* Bahpotia *(Ophiuros megaphyllus),* Aruna (*Setaria palmifolia),* Dub (*Cynodon dactylon),* Dol (*Hymenachne amplexicaulis*) and Tora *(Alpinia officinarum)* which were collected from in and around Khanapara, AAU campus and from Goat Research Station Barnihat at a time. Total three collections were done at 45 days interval. After collection, the individual grass sample was chaffed, dried by using hot air oven and then grind using a suitable grinder and were passed through 1 mm screen.

In vitro digestibility trial by using rumen liquor (Tilley and Terry, 1963) and enzymatic digestibility by using pepsin-cellulase enzyme (De Boever *et al*., 1985) was carried out to estimate the Dry matter and Organic matter digestibility. It has to be mentioned that the Dry matter digestibility by Tilley and Terry method has been written as DMD and Organic matter digestibility as OMD. For the pepsin-cellulase method these have been marked as CDMD and COMD respectively.

Analysis of Data and Statistical Test Applied

The experimental data were analyzed by using Statistical Package for Social Science (SPSS) version 20.0 and SAS 9.3 available at Biostatistics unit, Department of LPM, CVSc, Khanapara according to the method of Snedecor and Cochran (1994). The means were compared according to Duncan Multiple range test (Duncan, 1995) at 5 % level ($P<0.05$).

Results and Discussion

The *in vitro* DMD, OMD% and CDMD, COMD% decreased with the advancement of time which was in close agreement with the McCawley and Dahl (1980) and Bora *et al*. (2012) and higher DMD at early stage of growth was also in agreement with the finding of Kamalak (2005). From the present experiment it was found that *Tora* had significantly ($P<0.01$) lower amount of *in vitro* DMD than other grasses. However, both Ulu and Tora had significantly ($P<0.05$) lower OMD than other grasses. Whereas *Aruna* had the highest *in vitro* DMD and OMD%. By using pepsin-cellulase technique we have found that *Ulu* had significantly ($P<0.01$) lower amount of CDMD and COMD than other grasses. Whereas *Aruna* had the highest CDMD and COMD%. However, the enzymatic digestion method values increasingly showed about 5% higher both for DMD and OMD.

The mean DMD and OMD value estimated by two stage Tilley and Terry method (1963) and the mean CDMD and COMD value estimated by the Enzymatic method using pepsin-cellulase De Boever *et al*. (1985) revealed that both CDMD and COMD were higher with the Enzymatic method as compared to two stage Tilley and Terry method. Overall mean DMD estimated by Tilley and Terry were 47.44% and by Enzymatic method the CDMD value was 54.69%. The OMD also showed the similar trend i.e., 56.43 % (OMD) and 63.69 % (COMD). Analysis of variance revealed that the CDMD and COMD values were significantly ($P<0.01$) higher than the DMD and OMD estimated by Tilley and Terry method (1963). To evaluate the two-method paired 't' test was conducted and it was described that for both DMD and OMD the t value was not significant.

Table 1: *In vitro* dry matter and organic matter digestibility estimated by two methods at three stages of harvesting from two locations

Place	Name of grass	Tilley & Terry						Enzymatic					
		DMD (%)			OMD (%)			CDMD (%)			COMD (%)		
		First	Second	Third	First	Second	Third	First	Second	Third	First	Second	Third
Khanapara	*Ulu*	38	38	28	43.11	43	35	43.76	36.51	33.49	51.26	47.11	43.44
	Arali	54	52	50	67.44	63.27	58.93	64.03	64	60	74.96	72.56	69.58
	Kuchi	50	46	36	60	55.33	47.14	56.69	50.74	42.85	66	60.25	54.15
	Bahpotia	56	56	40	63	62.89	48	62.38	60.15	56.5	67.2	65.89	60.03
	Aruna	70	64	50	75	70	61.66	76	68.25	55.55	81.55	75.23	69.13
	Dub	64	54	46	68	66	61	68.73	65.55	52.85	79.65	76.84	67
	Dol	56	48	44	62	60.67	59.11	61.9	58.73	46.5	69.35	66.41	64.87
	Tora	40	32	30	48.12	40.99	39.36	49.68	46.5	40.15	59.43	52.56	49
SEM		3.85	3.6	3.02	3.72	3.75	3.67	3.63	3.84	3.26	3.59	3.78	3.48
Mean		53.50^{a}	48.75^{ab}	40.50^{a}	60.83	57.77	51.27	60.39^{b}	56.30^{ab}	48.49^{a}	68.67	64.61	59.66
Barnihat	*Ulu*	42	33	30	45.19	43	41	45.88	35.99	33.33	55.46	49.11	45.63
	Arali	56	53	50	68.23	60	60	64.03	63	60	73	71.23	69.26
	Kuchi	50	38	32	61	48.57	43.84	54.97	44.44	39.42	65	55.29	49.45
	Bahpotia	54	52	48	60.76	59	56.34	59.2	58.2	50.03	62.39	61.95	58
	Aruna	59	64	56	72	69.89	63	76.85	69.21	59.2	83.11	76.67	65.56
	Dub	58	52	44	65	61.9	56.66	69.2	59.2	56.5	80	71.2	62.25
	Dol	58	56	44	64	62	60.15	62.38	58.73	46.03	72	66.78	63.76
	Tora	36	30	30	46.19	41.02	40.85	51.7	46.98	38.98	60.11	53.46	43.11
SEM		3.62	4.27	3.51	3.44	3.63	3.27	3.49	3.87	3.38	3.45	3.48	3.48
Mean		52.88	47.25	41.75	60.29	55.67	52.73	60.53^{b}	54.47^{ab}	47.94^{a}	68.88^{b}	63.21^{ab}	56.13^{a}

Table 2: Mean dry matter and organic matter digestibility (%) of indigenous grasses estimated by two methods

Grass name	DMD (%)	CDMD (%)	OMD (%)	COMD (%)
Ulu	34.83^{ab}± 2.2	38.16^{a}± 2.19	41.72^{a}± 1.45	48.67^{a}± 1.75
Arali	52.50^{c}± 0.96	62.51^{de}± 0.81	62.98^{b}± 1.65	71.78^{ef}± 0.88
Kuchi	42.00^{b}± 3.14	48.19^{bc}± 2.85	52.65^{cd}± 2.92	58.36^{bc}± 2.66
Bahpotia	51.00^{c}± 2.52	57.74^{d}± 1.74	58.33^{bc}± 2.31	62.58^{cd}± 1.42
Aruna	62.17^{d}± 3.17	67.51^{e}± 3.33	68.59^{d}± 2.31	75.21^{f}± 2.8
Dub	53.00^{c}± 3.04	62.01^{de}± 2.79	63.09^{cd}± 1.67	72.82^{ef}± 2.96
Dol	51.00^{c}± 2.62	55.71^{cd}± 3.05	61.32^{c}± 0.70	67.20^{de}± 1.23
Tora	33.00^{a}± 1.69	45.67^{ab}± 2.08	42.76^{a}± 1.44	52.95^{ab}± 2.62
Overall mean	47.44± 1.59	54.69± 1.58	56.43± 1.48	63.69 ±1.51
P-value				
Grass	0	0	0	0
Place	0.882	0.703	0.788	0.462
Grass× place	0.979	0.994	0.957	0.974

The correlation coefficient between the two methods was 0.93 and 0.95 for DMD and OMD respectively. Goto and Minson (1977) obtained correlation coefficient 0.94 for DMD estimated by the two methods which were in close agreement with the value estimated in the present study. Dickerson *et al.* (1988) calculated correlation between *in vitro* Tilley and Terry method and pepsin-cellulase method and the R^2 value ranged between 0.91 to 0.94 for cool season grasses. The R^2 value for warm season species ranged between 0.81 to 0.86.

Two regression equations were developed to predict the DMD and OMD of Tilley and Terry method from the results of enzymatic digestion i.e., CDMD and COMD. The two equations with respective R^2 has been given below-

$$DMD\ (\%) = -3.944 + 0.940 \times CDMD\ (\%)\ (R^2 = 0.872)$$

$$OMD\ (\%) = -2.824 + 0.930 \times COMD\ (\%)\ (R^2 = 0.898)$$

So, it can be concluded that the pepsin-cellulase method can be effectively used instead of the two stage Tilley and Terry method.

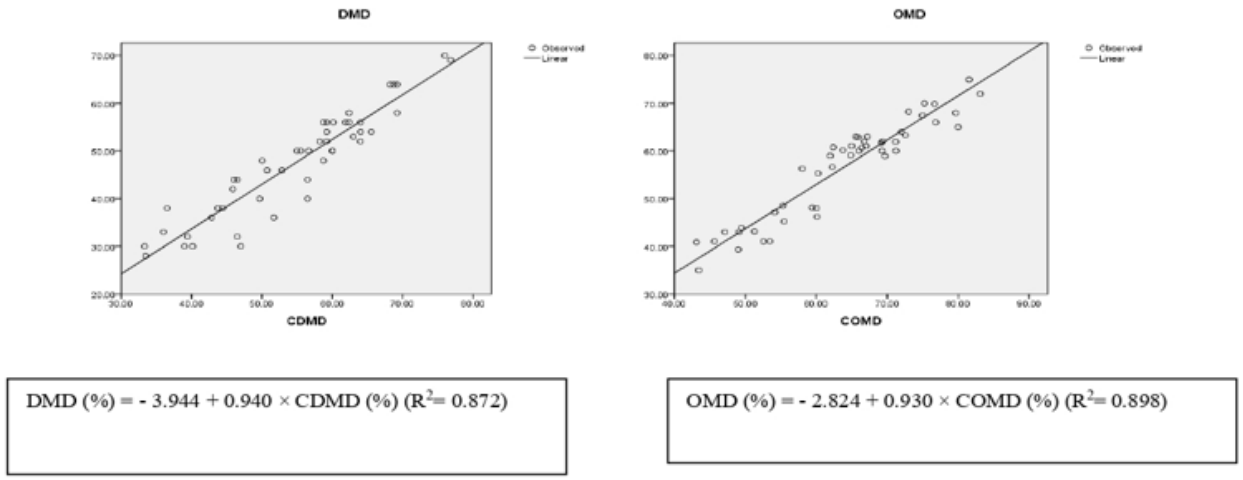

Fig. 1 Regression Equations For Both The Digestibility Method

Correlation Coefficient of DMD and OMD of Two Methods

Parameter	DMD	OMD
CDMD	0.93**	-74
COMD	-	0.95**

***P<0.01*

Conclusion

The mean DMD and OMD value estimated by two stage Tilley and Terry method (1963) and the Enzymatic method, Pepsin-cellulase technique (De Boever *et al*., 1985) revealed that both DMD and OMD were higher with the enzymatic method as compared to two stage Tilley and Terry method. Overall, mean DMD estimated by Tilley and Terry method were 47.44% and by Enzymatic method the CDMD value was 54.69%. The OMD also showed the similar trend i.e., 56.43 Vs 63.69% for two stage Tilley and Terry method and the pepsin cellulase method, respectively. Analysis of variance revealed that the DMD and OMD were significantly (P<0.01) higher when estimated by enzymatic method. There is strong and positive correlation established between the two methods. The correlation coefficient of DMD between the methods was 0.93 and for OMD correlation was 0.95 which was highly significant (P<0.01). From the *in vitro* DMD and OMD estimated by both method it was clear that *Aruna* had the highest dry matter and organic matter digestibility followed by *Dub, Arali, Dol* and *Bahpotia*. So, this grass can be considered as good fodder from nutritional point of view. From the present study it can be concluded that the pepsin-cellulase method can be effectively used instead of the two stage Tilley and Terry method.

Conflict of Interests

There is no conflict of interest.

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