

***In Situ* Digestion Kinetics of Mottgrass (*Pennisetum Purpureum*) With or Without Supplemental Legume at Two Levels by Buffalo Calves**

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ABSTRACT : The present study was conducted to evaluate the mottgrass alone and in combination with berseem (*Trifolium alexandrinum*) at different intake levels to determine the lag time, rate and extent of digestion of DM and NDF of mottgrass. Four ruminally cannulated buffalo calves were used in a 4×4 Latin Square Design with 2×2 factorial arrangement of treatments. The two factors were forage intake levels (*ad libitum* versus restricted) and forage source (mottgrass versus mottgrass plus 25% berseem). Four dietary treatment consisted of 1) *ad libitum* mottgrass, 2) restricted feeding of mottgrass, 3) *ad libitum* mottgrass plus berseem with a ratio of 3:1 and 4) restricted mottgrass plus berseem in a ratio of 3:1. Calves fed *ad libitum* mottgrass supplemented with 25% berseem consumed 25% more DM and 15 % more NDF than those fed mottgrass only. The *in situ* DM digestibility, the lag time and extent of digestion were not affected by intake level. However, rate of disappearance was greater in restricted fed animals than those fed *ad libitum*. This increased rate could be due to greater concentration of fibrolytic bacteria in restrict fed animals. The DM digestibility was greater (64.1%) in calves fed mottgrass supplemented with 25% berseem than those fed mottgrass only (57.7%). The reduced mottgrass DM digestion may be due to its higher NDF contents. The NDF digestibility, the lag and extent of NDF digestion were not affected by varying intake levels. However, rate of digestion of NDF was higher in restricted fed animals than those of *ad libitum* fed animals. The NDF digestibility was greater (58.4%) in calves fed mottgrass supplemented with 25% berseem than those fed mottgrass (48.7%) only. (*Asian-Aus. J. Anim. Sci.* 1999. Vol. 12, No. 3 : 371-375)

Key Words : Mottgrass, Digestion, Kinetics, Buffalo, *In Situ*

INTRODUCTION

Efficient ruminant production is dependent on good quality forage in sufficient amounts because they are well adapted to utilize forages as their principle feed. Even in countries where ruminants are fattened by feeding liberal grain supplements, forage still contributes about 75% of the dietary needs of the animal and in region where grain feeding is not common, ruminants obtain about 95% of their nutrient requirements from forages (Bula et al., 1977). In future, it is expected that ruminant will become increasingly dependent on forages because a rapidly expanding human population will force man to compete more actively with Livestock for edible grains. This situation will offer less grain and more forages to the Livestock, indicating that fiber will remain a major fraction in the diet of ruminants and thus their performance is dependent on their ability to consume and digest the fibrous portion of the diet.

Increased DMI by ruminants results in improved performance and consequently high profitability. Increased intake and animal performance by sheep has been reported with the addition of a legume to grass diet (Bowmen and Asplund, 1988). This has been attributed to the positive associative effects of legumes, which have a higher digestion when compared with grasses (Sarwar et al., 1996). Digestion of forage is influenced by species, maturity and morphological characteristics (Bowman et al., 1991 and Cherney et al., 1993). Grasses and legumes are degraded at different rates in the cow rumen (Sarwar et al., 1995). This difference stems from the varying concentration of

the cell wall material in these forages.

Digestion of forage by ruminants is limited primarily by concentration of forage NDF and its ruminal degradability. On the basis of digestibility, NDF can be divided into two fractions: potentially digestible NDF and indigestible NDF; which is not digested regardless of length of fermentation. Digestion of NDF can be characterized by a two stage process involving a lag followed by microbial degradation. Both duration of lag and digestion rate of potentially digestible NDF determine the time required for potentially digestible NDF digestion. The digestion lag is probably related to hydration rate of NDF and/or time needed for microbial association with NDF. Digestion rate of potentially digestible NDF seems to be related to intrinsic chemical characteristics of the NDF.

The scientific evidence regarding the effects of berseem supplementation to a low-quality mottgrass on its nutritive value is limited. The present study has therefore, been planned to determine the *in situ* digestion kinetics of mottgrass alone and in combination with legume at different intake levels.

MATERIALS AND METHODS

Animals and diets

Four ruminally cannulated buffalo calves, average weight 250 kg, were used in a 4×4 Latin Square Design with 2×2 factorial arrangement. The two factors were forage intake level (*ad libitum* versus restricted) and forage source (mottgrass versus mottgrass plus 25% berseem). The four dietary treatments were 1) *ad libitum* feeding of mottgrass, 2) restricted feeding of mottgrass, 3) *ad libitum* mottgrass plus berseem with a ratio of 3:1

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Received July 15, 1998; Accepted September 30, 1998

and 4) restricted mottgrass plus berseem with a ratio of 3:1. Animals were housed on a concrete floor in separate pens. Each period of Latin Square Design consisted of 15 days. Ten days in each period were given as adaptation time to the new feed followed by 5 days of collection period.

The mottgrass was first chopped in a locally manufactured chopper and then dried in the forced air oven at 55 °C. These feed sample were ground through a Wiley mill (2 mm) and analyzed for Nitrogen (N), DM (AOAC, 1990), NDF and ADF (Van Soest et al., 1991).

Nylon bag experiment

Nylon bags measuring 10×23 cm with an average pore size of 50 µm were used for determination of rate and extent of NDF and DM disappearance in situ. Incubations were duplicate with a blank. The 2 sample bags were used for determination of NDF and DM digestion and the third as a blank for DM and NDF disappearance.

On day 9 of each period, the bags containing mottgrass were placed in the rumen of animals being fed different dietary treatments after being soaked in warm (39°C) water for 15 minutes (Anderson et al., 1988) and were exposed to ruminal fermentation for 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hours. After removal from the rumen, the bags were washed in running tap water until the rinse was clear. These bags were dried in a forced air oven at 55 °C for 48 hours. After equilibration with air for 48 hours, the bags were weighed and the residues transferred to containers for later analysis.

In situ digestion kinetic parameters i.e., extent of digestion, rate of digestion and lag time were determined for each incubation individually. Degradation rates were determined by subtracting the indigestible residue i.e., the 96 hours residue from the amount in the bag at each time point and then regressing the natural log (Ln) of that value against time (Sarwar et al., 1991). The slope of the regression is equivalent to the rate of digestion while the lag time was calculated using the following equation.

$$\text{Lag time (\%/hour)} = \frac{(\text{Ln} \times 100) - \text{Intercept}}{\text{Rate of digestion}}$$

Statistical analyses

Data were analyzed as a 4×4 Latin Square Design with a 2×2 factorial arrangement of treatments using the General Linear Model procedure of SAS (1988). The two factors were forage source and intake level. The sum of squares of the model were separated into animal, period, treatments and interaction between forage source and intake level. When an interaction occurred means were separated by Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 shows the differences in composition between the two forages. The berseem hay was higher in CP and had lesser cell wall contents than mottgrass. Similarly ADF and cellulose were lower in berseem than mottgrass but lignin was 33% higher in berseem. The lignification on either a NDF or ADF basis (Van Soest, 1994) was higher in berseem than mottgrass.

Table 1. Composition of forages

Item	BERSEEM	MOTTGRASS
	(% of DM)	
OM	89.7	94.1
CP	16.7	7.6
NDF	53.4	74.6
ADF	36.7	45.8
ADL	9.9	7.4
Cellulose	26.8	38.4
Hemicellulose	16.7	28.8

Feed intake

The DM intake by buffalo calves fed *ad libitum* was higher ($p < 0.01$) than that of those fed restricted diets (table 2). Calves fed *ad libitum* mottgrass supplement with 25% berseem consumed 25% more DM ($p < 0.01$) than those offered mottgrass only. This effect of berseem on intake of low-quality forage supports the findings of previous workers in which addition of legumes to grass forages increased DM intake by steers (DelCurto et al., 1990). Various theories have been proposed to explain the mechanism. Moseley and Jones, (1989) attributed this increased DM intake of legume to its higher flow rate and post ruminal organic matter digestion in sheep fed rye grass. DelCurto et al. (1990) reported that higher DM intake was due to the provision of supplemental CP supplied from the alfalfa hay. The CP supplied from alfalfa hay probably was sufficient to alleviate the decrease in intake often noted with forage of low CP.

Cell wall concentration is negatively related to intake of ruminants consuming high forage diets because cell wall can affect intake by contributing to ruminal fill (Jung and Allen, 1995). Bowman and Asplund, (1988) concluded that increased DM intake in sheep fed grass supplemented with alfalfa was because of an increased amount of nitrogen and cell solubles available for microbial growth and fiber digestion. In a later study, Bowman et al. (1991) reported that legume supplementation may boost DM intake by increasing the particle size reduction and passage rate of large particle. Van Soest (1994) evaluated data on the effect of dietary CP content on DM intake. A depression in DM intake was associated with CP concentration below 8%. In the present study, the CP of the mottgrass was 7.8%, which is below the threshold level of protein content described in the preceding study. This may explain why the DM

Table 2. Effect of forage level and source on the nutrient intake by buffalo calves

Items	Treatments					Probability		
	AM	AMB	RM	RMB	SE	IL	FS	ILXFS
Intake (kg/d)								
DM	5.0	6.25	2.5	2.5	0.21	0.01	0.02	0.02
OM	4.7	5.81	2.4	2.3	0.18	0.01	0.03	0.03
NDF	3.6	4.16	1.8	1.7	0.14	0.01	0.14	0.03
ADF	2.3	2.70	1.2	1.1	0.12	0.01	0.08	0.03
Cellulose	1.9	2.20	1.0	0.9	0.12	0.01	0.14	0.04
Hemicellulose	1.3	1.50	0.6	0.6	0.01	0.01	0.28	0.04
CP	0.5	0.70	0.2	0.3	0.07	0.01	0.01	0.01
DM intake, % of body weight	2.0	2.50	1.0	1.0	0.08	0.01	0.02	0.01

AM=*ad libitum* mottgrass, AMB= *ad libitum* mottgrass +25 % berseem, RM=restricted mottgrass and RMB =restricted mottgrass +25 % berseem, IL= intake level, FS= forage source and ILXFS= interaction between intake level and forage source.

intake by calves fed mottgrass was less than that of those fed mottgrass in combination with berseem.

Intake of NDF, ADF, hemicellulose, cellulose and CP were altered significantly in calves fed *ad libitum* mottgrass supplemented with 25% berseem compared to those fed mottgrass only. At *ad libitum* feeding, calves offered mottgrass supplemented with 25% berseem in these diets ate 15% more ($p<0.01$) NDF than those offered mottgrass only. This was probably due to an increase in the rate of ruminal digestion of mottgrass DM and cell wall material, as shown by the *in sacco* degradability results (table 3). The results of the present study did not support the findings of Reid et al. (1990) who reported minimal difference in intake of NDF by sheep consuming various combination of grass and legume forages. Our study supported the concept advanced by Van Soest (1994) that cell wall (structural volume) may limit forage intake in situations in which nitrogen is not limiting.

DM digestion kinetics

1. Effect of intake level

The DM digestibility was not affected by intake level (*ad libitum* versus restricted feeding). The lag time and extent followed a similar pattern (table 3). However rate of disappearance was significantly greater ($p<0.05$) in restricted fed animals. This increased rate from restricted fed animals could be due to greater concentration of fibrolytic bacteria in the animals fed at

restricted than *ad libitum* feeding levels (Dearth et al., 1974). These results were in agreement with previous studies in which the DM digestibility was not affected by DM intake level (Firkins et al., 1986; Cherney et al., 1990).

2. Effect of legume

The forage source significantly altered the DM digestibility, lag time, rate of disappearance and extent of digestion (table 4). The *in situ* DM digestibility was greater (64.1%) in calves fed mottgrass supplemented with 25% berseem than those fed mottgrass only (51.7%). The reduced DM digestibility by calves fed only mottgrass may be due to the lower levels of certain nutrients (N and cell solubles) in mottgrass than berseem and this might have adversely affected the fermentation in the rumen. Earlier studies (Mertens, 1985; Varga and Hoover, 1983) had shown that the NDF contents were negatively correlated with the apparent digestibility of the forages. The higher DM digestibility in calves fed mottgrass supplemented with 25% berseem was associated with a shorter lag time and faster rate of disappearance. Glenn et al. (1989) reported a higher ($p<0.05$) DM digestibility of alfalfa hay than orchardgrass hay fed to holstein steers which was due to higher digestion rate of the former. Extent of digestion (calculated at 96 hours) of the feedstuff did not follow the same trend as DM digestibility. Slabbert et al. (1992) reported reduced extent of DM digestibility by steers fed low-quality diets.

Table 3. Effect of forage level and source on *in situ* DM digestion kinetics of mottgrass in male buffalo calves

Items	Feed intake		Forage source		SE	Probability		
	AL	R	MG	MG+Ber		IL	FS	ILXFS
DMD, %	55.7	56.1	51.7	64.1	1.7	0.54	0.05	0.05
Lag, h	1.5	1.7	3.0	1.0	1.1	0.61	0.01	0.05
Rate, %/h	0.0451	0.0491	0.0421	0.0517	0.06	0.05	0.01	0.81
Extent, %	64.7	64.9	67.9	66.8	1.9	0.81	0.21	0.70

AL= *Ad libitum*, R= restricted, MG= Mottgrass, MG+Ber= Mottgrass plus 25 % berseem, IL= Intake level, FS= Forage source and ILXFS=interaction between intake level and forage source.

Table 4. Effect of forage level and source on *in situ* NDF digestion kinetics of mottgrass in buffalo male calves

Items	Feed intake		Forage source			Probability		
	AL	R	MG	MG+Ber	SE	IL	FS	ILXFS
DMD, %	51.8	52.7	48.7	58.4	1.94	0.8	0.01	0.07
Lag, h	2.1	2.3	3.5	1.2	1.21	0.61	0.01	0.05
Rate, %/h	0.0401	0.0430	0.0401	0.0499	0.07	0.05	0.01	0.71
Extent, %	60.1	59.8	62.1	59.7	2.1	0.71	0.01	0.70

AL= *Ad libitum*, R= restricted, MG= Mottgrass, MG+Ber= Mottgrass plus 25 % berseem, IL= Intake level, FS= Forage source and ILXFS= interaction between intake level and forage source.

The rate of DM disappearance was higher ($p < 0.01$) in animals fed mottgrass supplemented with 25% berseem. The higher rate of disappearance of mottgrass supplemented with 25% berseem may be due to leguminous portion as legume (alfalfa 7.8%/h; clover 6.2%/h) had a higher rate of DM disappearance than timothy grass (5.8%/h) and orchardgrass (5.8%/h) (Varga and Hoover, 1983).

NDF digestion kinetics

1. Effect of intake level

NDF digestibility, lag time and extent of NDF digestion were not affected by varying intake levels (*ad libitum* versus restricted feeding). However, rate of digestion of NDF was greater ($p < 0.05$) in restricted fed animals than those of *ad libitum* fed animals. This increased rate from restricted fed animals could be due to a greater concentration of fibrolytic bacteria in the animals fed at restricted than *ad libitum* feeding level (Dearth et al., 1974).

2. Effect of legume

The forage source significantly altered the NDF digestibility, lag time, rate of disappearance and extent of digestion (table 4). The *in situ* NDF digestibility was greater (58.4%) in calves fed mottgrass supplemented with 25% berseem than those fed mottgrass only (48.7%). The low N content of mottgrass may have restricted microbial activity, and the addition of the higher N berseem may have corrected that limitation. Earlier studies (Mertens, 1985; Varga and Hoover, 1983) had shown that the NDF contents were negatively correlated to the apparent digestibility of the forages. Another possible explanation for the higher NDF digestibility by calves fed mottgrass plus 25% berseem can be attributed to their higher ratio of rapidly degradable cell wall sugar (arabinose) to slowly degradable cell wall sugar (xylose) of grasses (Ben-Ghedalia and Miron, 1984). The forages with the lowest cell wall contents had the highest ruminal degradability for NDF and organic matter (Sarwar et al., 1996). The animals fed mottgrass plus 25% berseem had higher rates and lower extent of NDF digestion of mottgrass than animals fed mottgrass only. The results supported the findings of Sarwar et al. (1995), Sarwar et al. (1996) and Varga and Hoover (1983). They reported higher rate and lower extents of NDF digestion

of legumes than grasses. Shaver et al. (1988) evaluated bromegrass and alfalfa and reported that rate of NDF digestion *in situ* was higher (7.5%/h) and extent was lower (43.3%) in alfalfa than bromegrass (4.3%/h; 61.7%). Schofield and Pell, (1995) reported higher (5.2%/h) rates of digestion of neutral detergent solubles of legumes than that of grasses (1.9%/h) and they concluded that legumes had faster digesting material compared to grasses.

Generally the extent of NDF digestion of leguminous forages is lower than that of grasses because of lower cell wall content and higher lignification of the former (Van Soest, 1994). Rate of cell wall digestion seems to be related to the anatomical and histological structure of plant tissues (Akin, 1989) or greater microbial colonization of legumes versus grasses (Bowman and Firkins, 1993). Firkins et al. (1991) evaluated carboxymethylcellulase (CMCase) activity as a marker of cellulolytic bacterial colonization and concluded that CMCase activity gave a qualitative assessment of the mass of cellulolytic bacteria colonizing plant fiber. Using CMCase as an indicator of cellulolytic bacteria, it has been shown that cellulolytic as a proportion of total particle associated bacteria, were attached in higher concentrations to red clover than grasses (Gammagrass and orchardgrass) during early (3–18 hour) incubation (Bowman and Firkins, 1993). This difference in colonization rate of forage particles by cellulolytic bacteria was implicated as a reason for more rapid rate of fiber degradation for legumes than in grasses. Huhtanen and Khalili (1992) also reported that CMCase activity was highly correlated with disappearance rate of NDF *in situ*. This could be one possible explanation of why the grasses-legume combination have a higher NDF degradation rate than grasses alone.

CONCLUSION

Increased intake and performance by ruminants can be obtained with the addition of berseem to a grass-based diet. The forage source significantly altered the DM digestibility, lag time, rate of disappearance and extent of digestion of mottgrass *in situ*. The lower DM and NDF digestibilities of mottgrass compared to a mixture of mottgrass and berseem may be due to its higher NDF contents and a longer lag and a slower rate of disappearance.

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