

Changes in the rumen digesta kinetics of cell walls with maturity and effect of indigestible cell wall pool on the degradation of forages

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ABSTRACT

Crossbred male cattle (6) fitted with rumen cannulae in 2 groups were fed on 7 kg of *Stylosanthus hamata* and mixed grass hay in the ratio of 1 : 1 (SHMGH) along with 1 kg concentrate for 2 years. SHMGH was harvested at 45 d (CG) or 90 d (EG). DM, OM, NDF or ADF digestibility was higher in CG than EG. Rumen kinetics was studied using rumen evacuation technique. Total rumen content in EG was 9% higher than CG. Rumen NDF pool was 6.67 and 7.91 kg/d, respectively, k in CG and EG. Rate of intake (K_i) and digestibility (K_d) were reduced k on CG than EG but contrary was true with true rate of digestion (K_{dt}). Average increase in indigestible NDF pool was 80 g/d on EG when compared to CG. The degradability of 5 range grasses and 3 range legumes were evaluated *in sacco*. Lag time of degradation in grasses or legumes was increased by 1/3 and effective degradability (ED) was reduced by 10%, in EG compared to CG. Average decrease in EDDM or EDNDF (%) was 0.4/d. Correlation of ED with NDF pools indicated that the main impact was due to indigestible NDF pool present in the rumen. Buffering capacity of rumen liquor was reduced significantly on EG compared to CG. Any effect of diet on different N fractions and TVFA's in rumen was cumulative. In conclusion, major hindrance in animal performance due to feeding of mature *Stylo* and mixed grass hay was related to indigestible NDF pool and decreased buffering capacity in rumen and reduced the effective degradation of forages by $1/3$ in rumen.

Key words: Buffering, Cell walls, Digestion kinetics, Legumes, Tropical grasses

Maturity or delay in regeneration in tropical forages changes the physical properties of cell walls very rapidly and any attempt to improve their utilization may have only little benefits to livestock production (Owens *et al.* 2008). Maturity of forages may have direct impact on intake, digestion and passage of digesta from rumen (Mertens 1999). Although intake is positively correlated with dry matter digestion, it ceases when digestibility exceeds 70% (Valente *et al.* 2003). Most of the observations pertaining to digestion are based on the apparent digestibility and externally observed changes in intake and digestion are very much disproportionate (Robinson *et al.* 1987). Since different nutrients in the diet have more than one rate constant, supplementation is provided to improve degradation of the diet (Srinivas *et al.* 2003). Degradation of cell wall in the rumen is referred in relation to digestible and indigestible fractions. Studies based on the digestible pools in the rumen

reported that increase in NDF digestibility in relation to decreased intake was greater than cell contents (Robinson *et al.* 1987). However, information on quantitative impact of increasing indigestible NDF on rumen kinetics and chemical pools is scanty. This study was conducted to quantify physical changes in digesta kinetics of cell wall pools in rumen on 2 diets containing *Stylosanthus hamata* + mixed grass hay harvested at 45 and 90 d of growth. Second, the effective degradation of 5 range grasses and 3 legumes in rumen under the simulated environment of both the diets was studied.

MATERIALS AND METHODS

Crossbred adult male cattle (6) fitted with flexible rumen cannulae were randomly divided into 2 groups (age 3–4 years; 265–330 kg). Experiment was repeated in the second year with switching over the diets between 2 groups. One group was fed on 3.5 kg (DM basis) each of *Stylosanthus hamata* and mixed grass hay harvested at 45 d (control; CG). Other group was fed on same roughages harvested at 90 d (EG) of maturity. Both the groups were supplemented with 1 kg concentrate (CS) consisting of barley 32, mustard cake 25, wheat bran 40, mineral mixture 2 and common salt 1% on as

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fed basis. CS and roughage was offered at 9 AM and 10 AM, respectively.

Total rumen contents were emptied manually to quantify the digesta kinetics (Robinson and Kennelly 1989). Animals were prevented from drinking water 4 h prior to rumen evacuation. Rumen content was emptied manually at 9 AM before feeding (0) h followed by 4, 8, 12, 16 and 20 h intervals. A minimum of 24 h gap was maintained between 2 consecutive evacuations. Total faeces voided by the animal were also measured. About 10 to 25 min was taken in emptying and refilling rumen contents depending on the postprandial time. Rumen was empty for about 2–4 min. At the end of refilling, CO₂ was flushed for 2 min and cannula was closed. Total rumen content was weighed using single beam weighing machine. At the end of evacuation, total rumen content was mixed manually and about 1/100 of the total proportion was taken and dried at 60 ± 5°C. Dried sample weights were recorded, analysed for NDF and milled in mill through 2.5 mm sieve. Indigestible NDF in the samples was estimated by incubating in Dacron bag with 50 µ for 15 d. Faecal flow of NDF was calculated by multiplying with a factor 0.85 (Robinson *et al.* 1987). The difference between total NDF and indigestible NDF pools was considered as potentially digestible NDF (PDNDF) pool. Based on the first order kinetic relationship, rate of intake (K_i), passage (K_p), digestion (K_d), true rate of digestion (K_{dt}) and true rate of passage (K_{pt}) were derived (Robinson and Kennelly 1989). Retention time of large particles (Illius and Gordon 1991) and effective digestible NDF (EDNDF) pool in rumen (Waldo *et al.* 1972) were also estimated.

Feed, faeces and ort were analyzed for proximate (AOAC 1991), cell wall (Goering and Vansoest 1975) constituents. Rumen liquor collected at each evacuation interval simultaneously and strained (SRL) through double layer cheesecloth. The pH of the SRL was estimated within 15 min of collection with digital pH meter. Buffering capacity was measured by taking 2 portions each of 25 ml of SRL. One titrated with 0.1 N HCl to pH of 5 against 0.1 N NaOH

to pH 8.0 and other part (Mohini and Arora 1984). Total nitrogen, ammonia nitrogen, TCA precipitated protein (AOAC 1991) and, total volatile fatty acids (Barnett and Reid 1977) in SRL were estimated.

Range grasses—*Cenchrus ciliaris* (CC), *Heteropogon contortus* (HC), *Chrysopogon fulvus* (CF), *Sehima nervosum* (SN), *Dicanthium annulatum* (DA) and, range legumes — *Stylosanthes hamata* (SH), *Atylosia scarbasides* (AS) and *Lablab purpureus* (LP) were collected at 45 d of growth and using *in sacco* nylon bag technique, their potential degradation in CG and EG were estimated (Orskov *et al.* 1980). Dried samples were ground using wiley mill through 2.5 mm sieve. A sample of 4–5 g was taken in a 5 × 10 cm nylon bag having a pore size of 50–60 µm and incubated in triplicate in each animal. Bags were removed after 3, 6, 12, 24, 48 and 96 h of incubation, washed with tap water and washing losses were estimated. After drying at 70°C, weights were recorded and corrected for washing losses. Samples analyzed for CP and NDF. Disappearance of DM, CP and NDF values were fitted to the non-linear equation (McDonald 1981) and effective degradability was calculated based on estimated passage rate.

Effects of digestion kinetics calculated from different time intervals for CG and EG were tested. Biochemical parameters at different time intervals and for their pooled values were also tested using paired sample t-test. Statistical analysis was done using statistical package SPSS version 10.0 and calculations were programmed on excel spreadsheet of MS-Office 2000.

RESULTS AND DISCUSSION

Intake and digestibility: CP, NDF and energy content of *Stylo* and mixed grass hay was affected with maturity (Table 1). CP content reduced by 30 and 35% and, NDF increased by 40 and 16%, respectively, in *Stylo* and mixed grass at 90 d of harvesting compared to 45 d. Sharp decline in the nutritive value between early cut to late cut in tropical temp was a well documented natural phenomena in forages

Table 1. Chemical composition of diet

Particulars	Stylo		Mixed grass		Concentrate Supplement
	Early cut	Late cut	Early cut	Late cut	
Dry matter	19.80	28.92	23.30	35.17	91.18
Organic matter	91.66	89.26	93.45	91.58	93.28
Crude protein	10.9	7.3	4.9	3.2	15.2
Ether extract	2.88	2.24	1.28	0.86	3.46
Total carbohydrates	77.88	79.72	87.27	87.52	74.62
Neutral detergent fibre	52.2	72.6	67.1	78.1	33.1
Acid detergent fibre	30.06	43.48	40.69	45.92	14.10
Cellulose	20.56	30.82	33.11	34.89	11.62
Acid detergent lignin	5.41	7.80	3.06	6.19	1.76
Acid insoluble ash	0.92	1.27	1.12	1.84	0.18

Values were average of quadruplets.

Table 2. Rumen digestion kinetics of cattle fed *Stylo* and mixed grass hay harvested at 45 and 90 d

Variable	CG (45 d)	EG (90 d)	SEM
Body weight	304	310	4.36
DM intake (kg/d)*	6.88 ^a	5.89 ^b	0.38
DM digestibility (%)**	74.39 ^a	63.50 ^b	3.47
Cell wall intake (kg/d)	4.81	4.33	0.16
Cell wall digestibility (%)**	68.79 ^a	57.51 ^b	3.02
<i>Rumen fill</i>			
Total dry matter content (kg/d)**	55.78 ^a	66.56 ^b	1.94
Dry matter (%) ^{NS}	13.83	14.00	0.67
Solid pool (kg/d)*	7.69 ^a	9.29 ^b	0.43
Liquid pool (l/d)*	48.09 ^a	57.27 ^b	1.88
Total NDF pool (kg/d)*	5.96 ^a	7.47 ^b	0.31
Potentially digestible NDF pool (kg/d)*	4.31 ^a	3.56 ^b	0.46
Effectively digestible NDF Pool (kg/d)*	1.90 ^a	1.53 ^b	0.32
Indigestible NDF pool (kg/d)*	1.65 ^a	3.92 ^b	0.16
<i>Digestion kinetics</i>			
Rate of intake (K_i /d)*	81.30 ^a	57.95 ^b	6.29
Rate of passage (K_p /d) ^{NS}	30.34	29.23	7.32
True rate of passage (K_{pt} /d)**	54.59 ^a	30.49 ^b	0.88
Retention time of large particles (h)*	34.69 ^a	64.85 ^b	3.12
Rate of digestion (K_s /d)*	50.96 ^a	28.72 ^b	4.90
True rate of digestion (K_{st} /d)*	62.20 ^a	73.48 ^b	5.33
<i>Rate constant of nitrogen and TVFA pools</i>			
Ammonia N (10^{-2}) ^{NS}	1.85	6.71	4.09
Total N ^{NS}	0.36	0.63	0.22
True protein N ^{NS}	0.22	0.39	0.26
Total volatile fatty acids (10^{-2})*	4.41 ^a	2.27 ^b	1.59

^{a,b}Values bearing different superscripts differ significantly; * $P < 0.01$, ** $P < 0.05$; and NS, nonsignificant.

and effected ($P < 0.01$) intake and DM digestibility in EG (Table 2). NDF digestibility was reduced significantly ($P < 0.01$) due to late harvesting although cell wall intake was statistically not significant between CG and EG. During maturity of tropical forages, lignin complexes with cellulose and hemicellulose and become a barrier in their digestion (Beever *et al.* 1999). Although CP digestibility was 68.7 ± 1.15 and $70.0 \pm 0.77\%$, respectively, in CG and EG, maturity of *Stylo* and mixed grass hay affected total CP intake. Illius and Jessop (1996) reported that decrease dry matter intake (DMI) and digestibility of NDF with maturity in tropical forages was not linear.

Rumen fill: Rumen total volume on EG was 9% more ($P < 0.05$) than CG. Both solid and liquid pools in rumen were higher ($P < 0.05$) on EG. Higher rumen volume not only limited the DMI but it may also extend all the metabolic events in rumen (Romney and Gill 2000). Diet had no affect on faecal DM in CG and EG. NDF excretion in faeces was more ($P < 0.01$) on EG. Rumen NDF pool was also significantly ($P < 0.01$) different between CG and EG. Total NDF pool of CG and EG consisted of 72 and 48% of PDNDF pool, respectively, and PDNDF pool in turn consisted, $1/3$

and $1/5$ of EDNDF pool ($P < 0.05$), respectively. Indigestible NDF pool was relatively higher ($P < 0.05$) in CG than EG. Average increase in NDF pool due to late harvesting was 41 g/d in rumen, which was due to 80 g/d increase in indigestible NDF pool ($P < 0.01$) with simultaneous decrease in 40 g/d of PDNDF pool. Accumulation of higher indigestible NDF in rumen appeared to be the major physical constraint to livestock in tropical conditions.

Rumen digestion kinetics: NDF in faeces was significantly different between CG and EG ($P < 0.05$). Late harvesting of forages had significant effect on the kinetic parameters such as K_i , K_d ($P < 0.05$) and K_{dt} ($P < 0.01$). K_p , was insignificant between 2 gps. K_{pt} , was significantly higher in CG than EG because it takes into account only indigestible pool which passes vertically down the gut. Longer retention of digesta in rumen may be due to lesser K_d (Mertens 1999). Irrespective of unchanged K_p , EDNDF pool in rumen was low ($P < 0.05$) due to decreased rate of digestion. However, K_{dt} was greater on feeding of late harvested *Stylo* and mixed grass hay. As per the estimates of K_{dt} , digestibility of PDNDF pool was 2.68 and 2.60 kg/d, respectively, in CG and EG. This indicated quantitatively K_{dt} did not increase, yet difference between both the groups was lesser than the decrease in intake. Earlier workers also opined that digestibility of reduced digestible portion would increase to compensate reduced intake but not in direct proportion (Robinson *et al.* 1987). K_{dt} was higher in EG because comparatively lesser digestible part of diet may preferentially be attacked by more number of microbes than in CG as suggested by Meng *et al.* (2000). Decrease in the K_{pt} on EG was attributed to higher mean retention time of large feed particles (Murphy and Kennedy 1999) in indigestible NDF. K_p for large particles in CG and EG was 0.029 and 0.016, respectively. Not only delay in harvesting but also delay in regrowth of some of the perennial forages also adversely affects the rumen digestion kinetics (Owens *et al.* 2008).

Degradation of grasses and legumes: Lag time of DM and NDF was increased ($P < 0.05$ to 0.001) in EG compared to CG for all grasses and legumes (Table 3). Initiation of degradation of DM and NDF in EG was 25 and 35% more in grasses and, 65 and 12% more in legumes, respectively. Rate constant of degradation (RCD) was higher for DM than NDF in EG than CG. EDDM and EDNDF of early cut range grasses and legumes were diminished in the rumen environment of EG ($P < 0.05$ to 0.001). EDDM in forages was reduced by 30% in EG. EDNDF was reduced by 30% in grasses and only 20% in legumes due to higher levels of cellulose in grasses while hemicellulose reported to be equal or greater than cellulose (Bailey 1973). The rate of decline in degradability of similar samples of forages was 0.4% per d in the EG compared to CG. Deterioration in the digestion kinetics of digestible and indigestible NDF pools in rumen on feeding of late harvested *Stylo* and mixed grass prevented inherent potential degradation of tropical grasses and legumes

Table 3. Effective degradability of grasses and legumes in the rumen of cattle fed *Stylo* and mixed grass hay harvested at 45 and 90 d

Species	Diet	Lag time (hr)		Rate constant(×10 ⁻²)		Effective degradability (%)	
		DM	NDF	DM	NDF	DM	NDF
Grasses							
<i>Cenchrus ciliaris</i>	CG	7.47	5.70	2.37	3.92	35.97	42.43
	EG	9.10	7.67	3.53	3.20	27.93	31.33
	SEM	0.17**	0.15*	0.05**	0.24*	1.04*	0.26***
<i>Heteropogon contortous</i>	CG	5.40	6.17	2.64	3.16	41.17	43.23
	EG	8.47	8.57	3.14	2.79	27.23	30.21
	SEM	0.08***	0.01***	0.03**	0.06*	0.06***	0.01***
<i>Crysopogon fulvus</i>	CG	5.80	5.80	2.64	3.56	40.07	43.90
	EG	6.67	7.47	3.32	2.93	29.42	30.33
	SEM	0.07**	0.24*	0.01**	0.08*	0.19***	0.06***
<i>Sehima nervosum</i>	CG	6.83	5.73	2.41	3.15	38.90	43.83
	EG	7.85	7.77	3.45	2.80	28.40	30.33
	SEM	0.03***	0.03***	0.02**	0.01*	0.11***	0.05***
<i>Dicanthium annulatum</i>	CG	6.00	5.30	2.62	3.73	42.00	43.97
	EG	7.27	7.47	3.58	3.17	27.73	29.63
	SEM	0.25*	0.07***	0.06**	0.04*	0.62**	0.04***
Mean	CG	6.30	5.74	2.56	3.51	39.62	43.47
	EG	7.87	7.79	3.41	2.98	28.14	30.37
	SEM	0.22***	0.08***	0.07***	0.07***	0.66***	0.29***
Legumes							
<i>Stylosanthes hamata</i>	CG	4.90	8.23	5.55	3.23	43.93	34.17
	EG	6.87	9.33	3.06	3.37	26.67	26.93
	SEM	0.02***	0.03***	0.02***	0.11 ^{NS}	0.16***	0.03***
<i>Atylosia scarboides</i>	CG	3.77	7.53	4.93	3.59	44.23	35.47
	EG	6.60	7.80	4.12	3.32	32.83	28.91
	SEM	0.08***	0.13 ^{NS}	0.01**	0.11 ^{NS}	0.01***	0.01***
<i>Lablab purpureus</i>	CG	4.57	6.93	4.53	4.61	47.60	40.37
	EG	8.36	8.23	3.67	3.98	35.55	30.67
	SEM	0.04***	0.02***	0.03**	0.04**	0.25***	0.01***
Mean	CG	4.41	7.56	5.01	3.81	45.25	36.67
	EG	7.28	8.45	3.62	3.56	31.68	28.84
	SEM	0.26***	0.38**	0.28**	0.12*	0.93**	0.48***

*P< 0.05; **P< 0.01; ***P< 0.001.

by $1/3$. Effect was more conspicuous with grasses than legumes because of variability in the degradation of different nutrients in the feed (Srinivas *et al.* 2003). A highly significant negative correlation was observed between EDDM ($R^2 = -0.73$, $P<0.001$) or EDNDF ($R^2 = -0.85$, $P<0.001$) with indigestible NDF pool. Correlation of EDDM or EDNDF of different forage samples was significant with indigestible NDF rather than PDNDF. Indigestible NDF pool not only induced effect on the physical kinetics but also was responsible for the decrease in the degradation of feed because the quantitative changes took place in tropical roughages was twice faster for indigestible NDF pool than the PDNDF pool in rumen.

Rumen buffering capacity and chemical pools: The pH of SRL was higher ($P<0.01$) at all intervals in EG than CG (Table 4) and lower pH in CG may be due to more cell contents. b-Value was not changed at zero or 4 h of interval, however, significantly ($P<0.001$) reduced thereafter probably due to inadequate buffering of released TVFA. Rumen pH in either CG or EG might not affect NDF digestion as it was more than its threshold level of 6.2 (Argyle and Baldwin

1988). Buffering capacity of SRL in EG was lesser than CG with maturity of *Stylo* and mixed grass hay may be due to decreased rate of digestion (Erdman 1988). Diurnal variation in total-N and ammonia-N in CG and EG was not significant at different fermentation intervals true protein-N and TVFA were significantly low at zero and 16 h ($P<0.05$) of intervals in EG than CG. The impact of different N fractions and TVFA in rumen between CG and EG was cumulative effect of the diets. Different biochemical parameters indicated that the decrease in the buffering capacity of SRL was major hindrance on feeding of late harvested *Stylo* and mixed grass hay.

In conclusion, the feeding of late harvested *Stylo* and mixed grass hay resulted in the deterioration of digestible pool in rumen. The major effect hindering the animal performance was more related to accumulation of indigestible NDF @ 80 g/d and it affected potential degradation of forages. Indigestible NDF pool in rumen increased at 2-folds faster rate with maturity of roughage and affected feed-system physically more than decrease in potential digestible NDF pool. Decreased buffering capacity also affected the rumen

Table 4. pH, buffering capacity, different nitrogen fractions and TVFA concentration in the rumen of cattle fed *Stylo* and mixed grass hay harvested at 45 and 90 d

Rumen parameter	Diet	Fermentation interval (FI), h							SEM	
		Zero	Four	Eight	Twelve	Sixteen	Twenty	Mean	FI	Diet
Rumen pH	CG	6.62	6.28*	6.48*	6.28**	6.34*	6.22*	6.37***	0.05	0.26
	EG	7.26	7.13*	6.97*	7.04**	7.03*	7.06*	7.08***		
Buffering capacity (meq/100 g of rumen DM)	CG	44.89	44.39	41.57*	46.00**	49.63***	51.62***	46.35***	1.44	0.77
	EG	40.90	39.60	28.97*	36.82**	37.39***	36.66***	36.72***		
Total pool size of nitrogen and TVFA in rumen										
Ammonia N (g)	CG	4.85	5.61	6.05	6.46	5.17	5.32	5.58***	0.80	0.46
	EG	7.16	6.999	5.84	8.10	8.54	6.92	7.26***		
Total N (g)	CG	38.89	45.06	52.63	49.50	46.81	39.86	45.46	4.34	2.51
	EG	36.55	52.94	46.98	51.40	45.04	36.37	44.88		
True protein N (g)	CG	21.10	26.29	30.39	28.61	27.43	23.39	26.20	2.67	1.54
	EG	20.81	30.94	27.45	28.83	26.28	21.20	25.92		
TVFA (moles)	CG	3.29	5.62	5.60	5.21	3.90	3.16	4.46**	0.32	0.18
	EG	3.31	4.08	4.67	5.01	3.63	2.82	3.92**		
ATP (moles)	CG	8.23	14.04	13.96	13.02	9.74	7.90	11.15**	0.80	0.46
	EG	8.27	10.19	11.68	12.53	9.08	7.05	9.80**		

*P<0.05; **P<0.01; ***P<0.001.

environment. Hence, breeding the forages for decreased rate of increase in indigestible NDF with maturity may be taken as criteria and care should be taken to improve the buffering capacity in rumen on such diet.

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