Asian-Aust. J. Anim. Sci.
Vol. 22, No. 1 : 72 - 81
January 2009

www.ajas.info

Effect of Frequency of Meals on Intake and Digestion of Tropical Grass Consumed by Rams

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ABSTRACT : Eight Black Belly rams (45.2 kg) fitted with permanent ruminal cannulae were used in a 2×2 factorial design to determine the effects of feeding frequency and regrowth age on intake and digestion. Rams were fed with 21- or 35-day old fresh pangola grass offered *ad libitum* two or four times a day. Irrespective of the regrowth age, there was a tendency for intake to be positively correlated with increase in meal frequency. Differences were not significant (p>0.25). Significant effects of meal frequency were observed in NDF and ADF total tract digestibility of the 35-day grass which decreased as the number of meals increased. Meal frequency had no visible effect on feeding behaviour. Total rumen content increased when animals were fed twice a day as opposed to four times a day. Similarly, an accumulation of small and very small particles was observed in the rumen of rams fed twice a day in comparison with those fed four times a day. These results suggest that studies of digestive dynamics performed at a steady state are not representative of the rumen loading observed in farm rams which have two important peaks of meal. (**Key Words :** Tropical Forage, Intake, Digestive Dynamics, Sheep)

INTRODUCTION

Digestible organic matter intake is a major factor influencing the feed value of tropical grass (Aumont et al., 1995). To improve the nutrition of ruminants fed with tropical grass, it is important to increase their feed intake level by controlling influencing factors that could affect intake. These factors include the residence time of forage in the rumen which affects the quantity of forage consumed, particularly for low digestible forages (Poppi et al., 1980; Poppi et al., 1981a b; Poppi et al., 1985); as well as the comminution and chemical degradation which are believed to influence the fill effect of tropical grass in ruminants (Wilson, 1994).

Studies on digestive dynamics are often cumbersome and complicated; therefore most experiments are carried out with animals fed frequently at regular time intervals on a daily basis to obtain a quasi-steady state situation in the rumen. These types of studies have provided interesting insights on the digestion process in the rumen, particularly cellulolysis and physical degradation of forage. However, under farm (pasture or stall) and in wild conditions, ruminants usually have two main meals per day. Consequently, it could be expected that feed intake and digestibility in ruminants, on-farm and in the wild, may be different from those obtained with experimental steady-state type of studies. In this study, we evaluated the effect of frequency of meal on the process of intake, chewing and digestion of tropical grass in rams.

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Received January 26, 2008; Accepted July 29, 2008

MATERIALS AND METHODS

Location

The experiment was conducted in 2002 at the animal experimental station of the ‘‘National Institute of Agronomic Research” (INRA, French West Indies, Guadeloupe, latitude 16.16 N, longitude 61.30 W). The mean rainfall on the experimental site was 3,000 mm/year and the average monthly temperatures ranged from 21°C to 31°C. The entire experiment was carried out during the rainy season when rainfall and temperature remained almost constant.

Experimental design, animals, diets and feeding

Experimental treatments consisted of 21- or 35-day old *Digitaria decumbens* (pangola) grasses offered *ad libitum* (1·15 times the animals' estimated voluntary intake) two or four times a day, at 12- and 6-h intervals, respectively. Eight Black-belly rams (mean liveweight (LW): 45.2±(s.d. 1.0) kg) were used for the experiment. The rams were fitted with rumen cannulae and maintained in individual metabolism cages, with free access to water and mineral blocks. Rams were weighed at the onset of the study and for each experimental period. For the 4 months trial, the animals were placed into two blocks (A and B). Group A was offered a 21-day old grass (21-day grass) twice a day, a 21-day grass four times a day, a 35-day old grass (35-day grass) twice a day and a 35-day grass four times a day in that order. The animals in group B were offered a 35-day grass twice a day, a 35-day grass four times a day, a 21-day grass twice a day and a 21-day grass four times a day. Each period of the trial lasted 33 days which consisted of 14 days of adaptation, 5 days of intake and total tract digestibility estimation and 14 days of rumen emptying.

The harvesting of the perennial pangola pasture was planned in order to have two plots (P21) and (P35) with pastures at 21 days and 35 days regrowth stages respectively, at each harvesting period. The P21 plot was divided into 21 subplots and the P35 plot into 35 subplots respectively. The first subplots of both P21 and P35 were cut at 21 and 35 days, respectively, before the beginning of the experiment and the following subplots were cut daily in successive order. As such, the grass in subplot n was one day older than that in subplot n+1. Consequently, during each experimental period the regrowth age of the grass harvested daily on the subplots of P21 and P35 was exactly 21 and 35 days old. Mineral nitrogen fertiliser was applied every day after each harvest at 1 kg/ha/regrowth age for each plot. The harvested grass was kept overnight at 4°C in a cold chamber in preparation for feeding the following day. In the morning, the pangola grass was chopped to an average length of 4 cm just before feeding.

Measurements

Intake and apparent digestibility were determined from daily weighing of the amounts of food offered, refusals and faeces. Dried and ground samples of grass, refusals and faeces were stored for chemical analyses.

Dry matter intake (DMI2-3 h) during the morning main eating period was estimated as the amount of grass eaten during the two first hours following the morning distribution of the meal.

The transit time of the liquid phase was estimated using PolyEthylene Glycol (PEG) as the marker. Eight hours before the first collection of faeces, 30 g of PEG was introduced directly into each rumen. The faeces were then harvested at 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 48, 52, 56, 62, 68, 80 and 104 h after the introduction of the PEG. Transit time of indigestible fibre was estimated using lignin as the marker.

Feeding behaviour parameters (time spent eating, ruminating and idling) were determined for 24 h per measurement period by an automatic recording system (Rutter et al., 1997). Moreover, eating, ruminating and idling times were estimated during the first 6 h after the distribution of the morning meal. Eating, ruminating and chewing indexes were estimated as follow:

Eating index = (daily time spent eating)/(daily intake of dry matter or neutral detergent fibre)

Ruminating index = (daily time spent ruminating)/(daily intake of dry matter or neutral detergent fibre)

Chewing index = (daily time spent chewing)/(daily intake of dry matter or neutral detergent fibre)

Kinetics of intake were estimated by dividing daily intake by 24 based on the assumption that hourly intake rate was identical.

Rumen degradation of forage offered was measured using the nylon bag method (Michalet-Doreau et al., 1987). Nylon bags of 10 cm×5 cm, with a pore size of 50×50 μm, were filled with 15 g of fresh forage of the basic diet. The grass was cut manually into particles of a mean length of approximately 2 mm long. Incubation time in the rumen was 24 h.

During the rumen sampling period of animals consuming two meals per day, four total emptyings of the rumen were carried out every 3 days, at 0, 3, 9 and 21 h after the morning meal which was limited to 3 h. On the day of the third emptying, the second meal was distributed just after the rumen emptying, while on the day of the last emptying, the animals only received the first meal which lasted 3 h, thus fasting (without food) for 21 h until the emptying. When rams consumed four diets daily, rumen emptyings were carried out at 0, 1, 2, and 4 h after the morning meal which was limited to 2 hours. After each emptying, the rumen content was weighed and mixed thoroughly by hand and four sub-samples were taken. Two of these sub-samples were used for dry matter determination, one sub-sample was freeze-dried and preserved at (-20°C) for chemical analyses and the last was used for determination of the digesta particle size. Rumen samples were separated into Large particles (LP), Small Particles (SP) and Very Small particles (VS), by wet sieving using gradual sieves. LP were defined as those retained in

Table 1. Chemical composition (% dry matter) of a 21- and 35-day old fertilized *Digitaria decumbens* grass

Regrowth age (days)	21	35
Organic matter	87.1	89.0
Crude protein	13.0	12.3
Neutral detergent fibre	72.0	74.2
Acid detergent fibre	37.7	40.0
Acid detergent lignin	7.0	7.0

the 4 mm and 1.18 mm sieves, SP were defined as those that passed through 1.18 mm sieves but retained in 0.050 mm-sieves and particles that passed through the 0.050 mm were considered as VS.

Mean rumen load (WRC) was estimated with two different equations depending on the frequency of feeding as follow:

i) when meal was distributed twice a day

$$\text{Mean WRC} = (3\times\text{WRC 0 h}+3\times\text{WRC 3 h} +6\times\text{WRC 9 h}+ 12\times\text{WRC 21 h})/24$$

ii) when meal was distributed four times a day

$$\text{Mean WRC} = (2\times\text{WRC 0 h}+1\times\text{WRC 1 h} +1\times\text{WRC 2 h}+ 2\times\text{WRC 4 h})/6$$

where WRC (0, 1, 2, 3, 4, 9, 21 h) corresponded to rumen load collected at 0, 1, 2, 3, 4, 9 and 21 h respectively after the morning meal.

Different values of coefficients were applied (for example we used the mean time between the distribution of the meal and the rumen emptying) to calculate the mean WRC. Only minor differences were observed between the different methods of estimation and the range of effects was always the same.

The passage rate (%/h) of indigestible rumen fibre (kpADL) was estimated using the ratio : (faecal intake of lignin (g)/24)/(mean total amount of lignin in the rumen (g)).

The mean retention time (h) of indigestible rumen fibre (MRT) was estimated as the ratio : 1/kpADL (%/h).

Because there were 4 rumen emptyings for animals receiving two meals per day, it was possible to fit the corresponding data to a bicompartmental model. The first compartment was for the large particles (LP>1 mm) and the second was for the small particles (SP<1 mm). It was assumed that LP were trapped in the rumen. They were comminuted into SP by rumination. The basic differential dynamic equations of this system were:

$$dLP/dt = - kc\cdot LP$$

$$dSP/dt = kc\cdot LP-kp\cdot SP$$

In these equations kc was the fractional comminution rate of LP and kp the fractionnal outflow rate of SP. The 16 individual values of kc and kp and the initial values of LP (LPo) and SP (SPo) were obtained by fitting data with the software “Modelmaker” (version 3, Cherwell). The initial values for the flow of comminution (FLco) and transit (FLto) were calculated from LPo, Spo, kc and kp.

Chemical analyses

The DM content of the fresh forage and refusals was obtained daily by drying to a constant weight at 60°C in a forced-draught oven. Samples were then ground to 1 mm prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated using the methods of Van Soest et al. (1991). The nitrogen content was determined from a representative sample of dry forage using the Dumas method (AFNOR, 1988).

Statistical analyses

Data were analysed using the General Linear Model procedure of SAS (1987) including forage (D.F. 1), meal frequency (D.F. 1), animal (D.F. 7) and the interaction forage×meal frequency effects. The effect of periods was tested on the residues of the preceding model. It was not significant. Regressions between intake and the digestive parameters were then calculated based on the REG procedure and using the MAXR (Maximum r^2 improvement).

RESULTS

Chemical composition and intake

Chemical composition of grass is presented in Table 1. Minor differences were observed between the roughages despite the difference in regrowth age. Values for dietary intake are reported in Table 2. Irrespective of the component, intakes were significantly higher in the 21 than in the 35-day grass ($p<0.0001$). Daily intake tended to be higher as meal frequency increased but the differences were not significant ($p>0.1$).

Dry matter intake recorded just before rumen emptying (DMI 2-3 h) was significantly higher when animals fed with the 21-day-grass received two instead of four meals per day ($p = 0.000$) (Table 2). Moreover, when animals received meals twice a day, DMI2-3h was significantly higher with the 21-day than the 35-day grass (Table 2). It also appeared that this quantity was positively related to daily dry matter intake (DMI):

$$\text{DMI 2-3 h} = 135+0.098\ \text{DMI}$$
$$(n = 120,\ R^2 = 11.4\%,\ rsd = 65,\ p = 0.000)$$

Table 2. Daily intake (dry matter, neutral detergent fibre, acid detergent fibre), dry matter intake during the principal morning meal of Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at two or four meals per day

Regrowth age (days) / Frequency of meal	21		35		s.e.	Main effects		
	4	2	4	2		Age	Freq	Age×Freq
Intake								
Dry matter (g/kg $W^{0.75}$)	81.8[a]	79.3[a]	67.2[b]	61.6[b]	9.2	***	NS	NS
Dry matter (g/d)	1,475.1[a]	1,371.6[a]	1,173.8[b]	1,093.9[b]	151.9	***	NS	NS
Neutral detergent fibre (g/d)	1,102.2[a]	986.1[a]	831.2[b]	837.1[b]	114.1	***	NS	NS
Acid detergent fibre (g/d)	586.5[a]	558.6[a]	426.1[b]	442.6[b]	63.0	***	NS	NS
DMI 2-3 h (g)[1]	243.7[a]	328.4[b]	232.0[a]	238.7[a]	7.79	***	***	***

[1] DMI 2-3 h = Dry matter intake during the principal morning meal.

[a, b, c] Values within rows with different superscripts are different (p<0.05). NS = Not significant, * p<0.05; ** p<0.01; *** p<0.001.

Feeding behaviour

Intake pattern is illustrated in Figures 1a and 1b. The number of main peaks in intake was equal to the number of meals. This was consistent with the above mentioned data on DMI 2-3 h; feeding frequency had an impact on the pattern of the first meal. Eating time 6h after the first distribution of meal was more significant when two meals were offered (Table 3 and Figures 1a, 1b).

Forage and frequency of diet had no significant effect on the daily time spent eating, ruminating, chewing and idling (p>0.15; Table 3). The indexes of intake, ruminating and chewing increased with the 35-day grass in comparison with the 21-day grass (p<0.006).

Dry matter intake (DMI) was positively related with chewing time (ChewT, minute) and the regression slope obtained with the 21-day grass was much more significant (Figure 2) as indicated with the following equations:

i) 21-day grass

$DMIW^{0.75}$ (21 d) = 20.6+0.07 chewt (21d)
(n = 15; R^2 = 63.4; rsd = 9.05; p = 0.000)

ii) 35-day grass

$DMIW^{0.75}$ (35 d) = 45.2+0.03 chewt (35 d)
(n = 15; R^2 = 18.1; rsd = 5.06; p = 0.113)

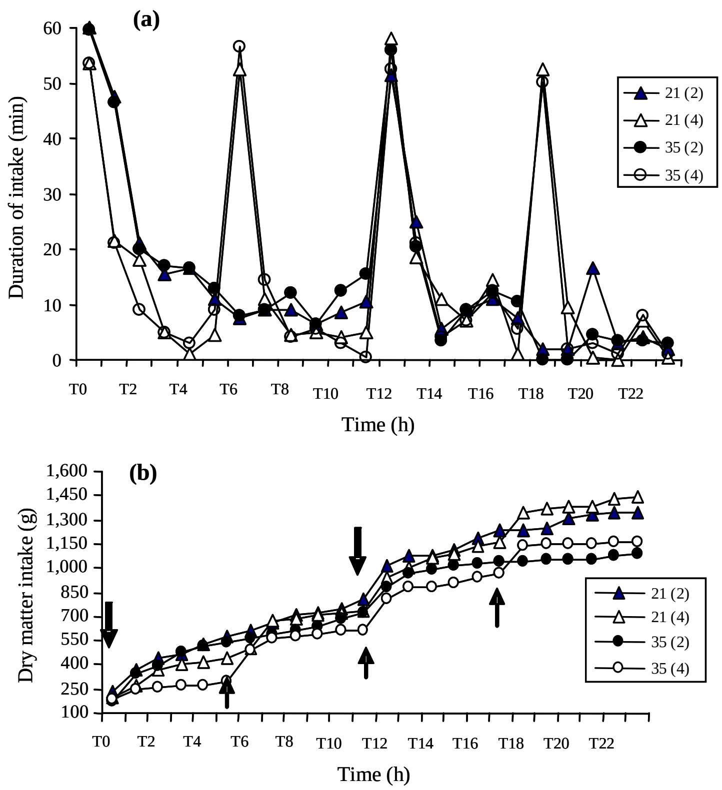

Figure 1. (a) Effect of feeding frequency on the feeding pattern of Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day. 21(2): 21-day old, 2 meals; 21(4): 21-day old, 4 meals; 35(2): 35-day old, 2 meals; 35(4): 35-day old, 4 meals. (b) Effect of feeding frequency on the dry matter intake of Black-belly rams given a 21- or a 35-old *Digitaria decumbens* at two or four meals per day. 21(2): 21-day old, 2 meals; 21(4): 21-day old, 4 meals; 35(2): 35-day old, 2 meals; 35(4): 35-day old, 4 meals.

Table 3. Ingestive behaviour parameters (idling, eating, ruminating and chewing time; eating, ruminating, and chewing index) in Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at two or four meals per day

Regrowth age (days)	21		35		s.e.	Main effects		
Frequency of meal	4	2	4	2		Age	Freq	Age×Freq
Eating time (min)	371.9	372.0	337.0	367.5	42.8	NS	NS	NS
Ruminating time (min)	431.9	414.0	423.5	364.4	76.2	NS	NS	NS
Chewing time (min)	803.7	786.1	760.4	731.9	102.4	NS	NS	NS
Idling time (min)	636.2	653.9	679.6	708.1	102.4	NS	NS	NS
Eating time 6 h[1]	103.7[a]	171.2[b]	111.2[a]	170.0[b]	24.5	NS	***	NS
Ruminating time 6 h[2]	80.0	67.6	96.6	63.7	33.9	NS	NS	NS
Idling time 6 h[3]	176.3[a]	121.2[b]	152.2[ab]	126.3[b]	40.1	NS	**	NS
Eating index DM (min/g DMI)	0.25[a]	0.27[a]	0.29[a]	0.34[b]	0.04	***	*	NS
Ruminating index DM (min/g DMI)	0.29[a]	0.31[ac]	0.36[b]	0.34[bc]	0.04	**	NS	NS
Chewing index DM (min/g DMI)	0.54[a]	0.58[a]	0.65[b]	0.67[b]	0.06	***	NS	NS
Eating index NDF (min/g NDFI)	0.34[a]	0.38[ab]	0.41[bc]	0.44[c]	0.05	**	NS	NS
Ruminating index NDF (min/g NDFI)	0.39[a]	0.43[a]	0.51[b]	0.44[a]	0.06	**	NS	*
Chewing index NDF (min/g NDFI)	0.73[a]	0.81[ac]	0.92[b]	0.88[bc]	0.09	***	NS	NS

[a, b, c] Values within rows with different superscripts are different ($p<0.05$). NS = Not significant, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

[1] Eating time during the first 6 hours after the distribution of the morning meal.

[2] Ruminating time during the first 6 hours after the distribution of the morning meal.

[3] Idling time during the first 6 hours after the distribution of the morning meal.

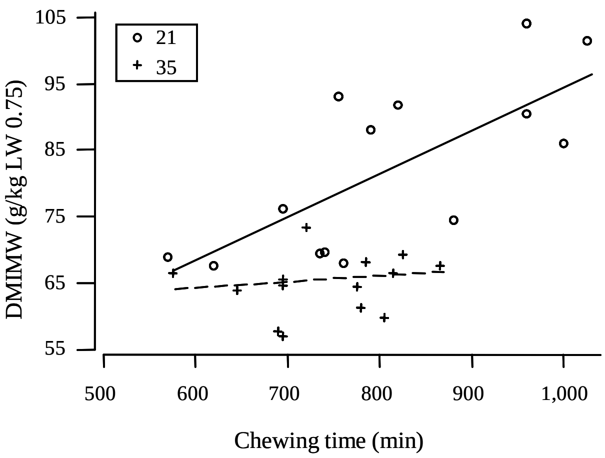

Figure 2. Changes in dry matter intake (g DM/g $LW^{0.75}$) with the chewing time (min) in Black-belly rams given a 21- or a 35-old *Digitaria decumbens* at two or four meals per day.

Thus, efficiency of chewing, which corresponds to the regressions in Figure 2, was higher at 21-day old than at 35-day old. Overall, the eating index DM decreased when meal frequency increased but no difference was recorded for the 21-day grass ($p>0.3$). No effect of meal frequency was recorded for the indexes of ruminating and chewing ($p>0.3$).

Total tract digestibility

Total tract digestibilities are reported on Table 4. Values were highest with the youngest forage ($p<0.0001$). The meal frequency had no effect on the total tract digestibility of OM and CP ($p>0.1$). However, the NDF and ADF total tract digestibility of the 35-days old grass significantly decreased with the increase in number of meals ($p<0.009$). This latter one has no effect on the total tract digestibility of the 21-day grass ($p>0.09$).

Table 4. Total tract digestibility (dry matter, organic matter, crude protein, neutral detergent fibre, acid detergent fibre), *in sacco* DM degradability of a 21- or a 35-day old Digitaria decumbens (Deg 21, Deg 35) and rumen turnover of liquid (kl) and fibrous particle (kpADL) in Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day

Regrowth age (days)	21		35		s.e.	Main effects		
Frequency of meal	4	2	4	2		Age	Freq	Age×Freq
Total tract digestibility (%)								
Organic matter	76.0[a]	75.6[a]	66.7[b]	65.3[b]	1.6	***	NS	NS
Neutral detergent fibre	79.2[a]	79.6[a]	72.5[b]	75.3[c]	1.8	***	*	NS
Acid detergent fibre	79.2[a]	81.0[a]	70.2[b]	74.6[c]	1.9	***	***	NS
Crude protein	73.3[a]	74.3[a]	61.1[b]	61.9[b]	2.3	***	NS	NS
In sacco DM degradability (%)								
Deg 21	-	68.4[a]	-	60.4[b]	1.4	***		
Deg 35	-	67.0[a]	-	62.7[b]	2.4	***		
kpADL (%/h)	3.9[a]	3.3[a]	4.3[a]	2.9[b]	1.0	NS	*	NS
kl (%/h)	8.4[a]	7.1[ab]	6.3[b]	6.9[ab]	1.42	*	NS	NS
Flow of liquid (g/h)	1,301	1,181	1,354	1,210	288	NS	NS	NS

[a, b, c] Values within rows with different superscripts are different ($p<0.05$). NS = Not significant, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

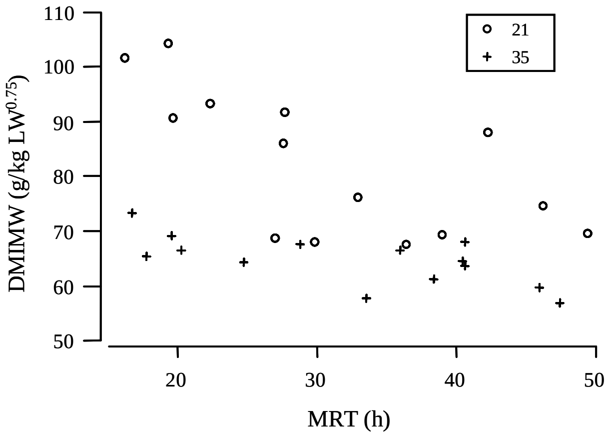

Figure 3. Changes in dry matter intake (g DM/g $LW^{0.75}$) with the mean retention time (h) in Black-belly rams given a 21- or a 35-old *Digitaria decumbens* at a frequency of two or four meals.

Nylon bag degradation

Nylon bag DM degradation (24 h) of 21-day grass was significantly higher than the 35-day grass, incubated in the rumen of animals respectively fed with 21-day and 35-day grasses ($p<0.05$; Table 4). Furthermore, the levels of degradation of the 21- and 35-day grasses were higher when the nylon bags were incubated in the rumen of animals fed the 21-day diet ($p<0.02$).

Rumen turnover

Generally, the turnover of Acid Detergent Lignin (kpADL) increased positively with diet frequency ($p<0.0005$) but the only notable difference was observed in animals offered two meals of a 35-day grass ($p<0.0005$) (Table 4). Mean retention time (MRT, hour) was negatively related with dry matter intake as illustrated in Figure 3 and the following equations:

i) 21-day grass

DM intake (g/$LW^{0.75}$) = 109-0.87 MRT
(n = 15; R^2 = 58.4; rsd = 9.31 g; p = 0.001)

ii) 35-day grass

DM intake (g/$LW^{0.75}$) = 74.4-0.31 MRT
(n = 14; R^2 = 62.5; rsd = 3.42 g; p = 0.000)

For the fractional outflow rate of liquid phase (kl) and the flow of liquid, no significant difference was reported with the meal frequency ($p>0.5$; Table 4; Figure 4). In contrast, the turnover of the liquid phase decreased with the maturity of the forage whereas fibrous particle and flow of liquid did not vary ($p>0.05$).

Rumen load

The mean rumen load of DM (g/g intake) varied significantly with respect to regrowth age of the forages ($0.03<p<0.02$) (Table 5). The pool of LP significantly increased ($p<0.05$) with the regrowth age when the rams had two meals a day whereas no significant changes were observed with the four meals. The pool of SP significantly decreased ($p<0.05$) with the regrowth age when the rams had four meals a day whereas no significant changes were observed with the two meals. The pool of VS significantly increased ($p<0.05$) with the regrowth age when the rams had two meals a day whereas no significant changes were observed with the four meals.

The meal frequency had a significant effect ($0.06<p<0.0001$) on the rumen load of DM (g/g intake). There was a significant interaction between the forage age and the meal frequency. The amount of LP (g/g intake) in the rumen content increased with the meal frequency ($p<0.01$). The

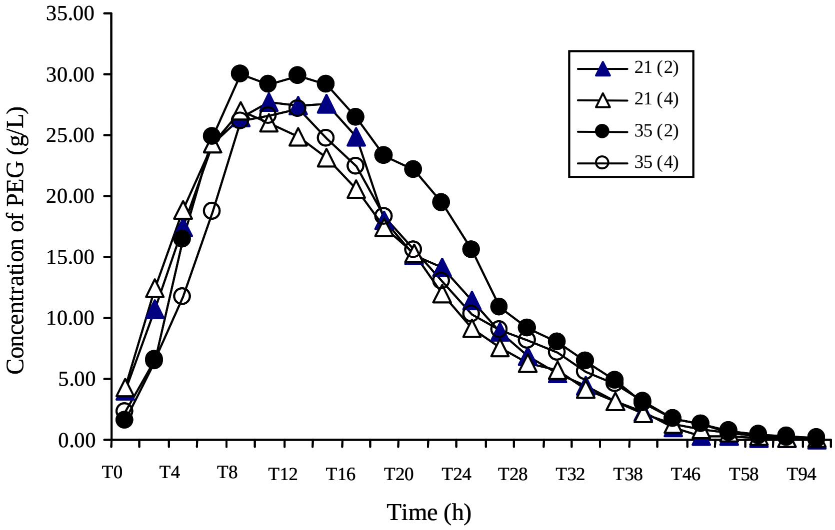

Figure 4. Effect of frequency of meals on transit time of the liquid phase of rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day. 21(2): 21-day of regrowth, 2 meals; 21(4): 21-day of regrowth, 4 meals; 35(2): 35-day of regrowth, 2 meals; 35(4): 35-day of regrowth, 4 meals.

Table 5. Whole rumen content (WRC) of dry matter (DM), neutral detergent fibre (NDF) and granulometry in Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day

Regrowth age (days) / Frequency of meal	21		35		s.e.	Main effects		
	4	2	4	2		Age	Freq	Age×Freq
DM WRC (g)	710.0[a]	858.1[b]	665.0[a]	1,011.6[c]	110.8	*	***	***
NDF WRC (g)	425.6[a]	490.8[a]	358.2[b]	611.8[c]	73.4	NS	***	***
Dry matter WRC (g/g DM intake)	1.01[a]	0.77[b]	0.96[a]	1.03[a]	0.10	*	NS	***
NDF WRC (g/g NDF intake)	0.81[a]	0.58[b]	0.77[a]	0.80[a]	0.09	*	*	***
Large particle (g/g DM intake)	0.44[a]	0.21[b]	0.37[ac]	0.32[c]	0.04	NS	***	***
Large particle (g/g NDF intake)	0.49[a]	0.25[b]	0.43[ac]	0.36[c]	0.05	NS	***	***
Small particle (g/g DM intake)	0.23[a]	0.19[b]	0.18[b]	0.20[ab]	0.02	*	NS	***
Small particle (g/g NDF intake)	0.25[a]	0.21[b]	0.20[b]	0.22[b]	0.02	*	NS	***
Very small particle (g/g DM intake)	0.34[a]	0.37[a]	0.41[a]	0.51[b]	0.05	***	**	NS

[a, b, c] Values within rows with different superscripts are different (p<0.05).
NS = Not significant, * p<0.05; ** p<0.01; *** p<0.001.

amount of SP (g/g intake) in the rumen content increased with the frequency of distribution of the 21-day grass (p<0.01) whereas no significant effect was observed with the 35-day grass. With respect to the pool of VS, its amount decreased with the frequency of distribution of the 35-day grass (p<0.05) whereas no significant effect was observed with the 21-day grass.

Assuming that hourly intake rate was constant, intake rate recorded 6 hours after the distribution of the morning meal was positively correlated with the pool of LP. Moreover, it appeared that, at the same intake rate there were more LP with the 35-day grass (Figure 5).

Figure 6, which results from kinetic adjustments, clearly shows that the regrowth age mainly influences the LP stasis in the rumen and that comminution is not able to compensate the higher LP fill with the 35-day grass.

Intake and digestive parameters correlations

Correlation analyses indicate that DMI (%LW) was mostly related with dry matter intake rate (0.82). It was also linked with organic matter total tract digestibility (R = 0.68) and chewing index (R = -0.64). In addition, intake rate was, linked with chewing index (R = -0.80) and organic matter total tract digestibility (R = 0.61) at a constant intake. Organic matter total tract digestibility was limited with the chewing index (R = -0.66). All these coefficients of correlation were significantly high. In contrast, age response of DMI (%LW) showed no significant link to total chewing duration, rumen load and lignin MRT.

Individual variations of intake

The major relationship among animals was that individual DMI (%LW) was negatively related with lignin MRT (R = -0.87) and with organic matter total tract digestibility (R = -0.68), whereas DMI (%LW) was positively related with total chewing time (R = 0.84). Consistent relationships associated lignin MRT and organic matter total tract digestibility (R = 0.65) and chewing time (R = -0.79). The latter was negatively related with organic matter total tract digestibility (R = -0.44). All the coefficients of correlation, except the last one, were significant.

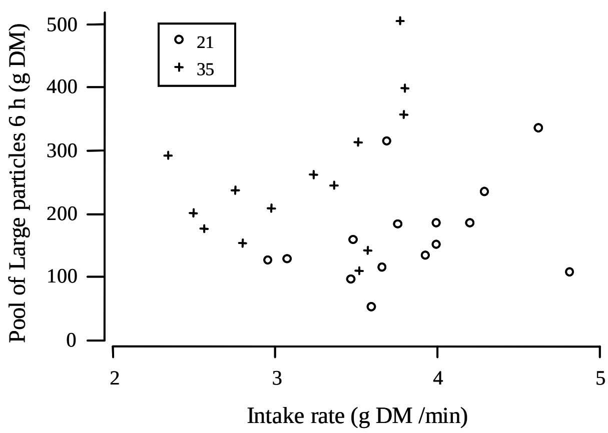

Figure 5. Changes in the pool of large particles (g DM) registered 6 hours after meal distribution with rate intake (g DM/min) in Black-belly rams given a 21- or a 35-day old *Digitaria decumbens* at a frequency of two or four meals per day.

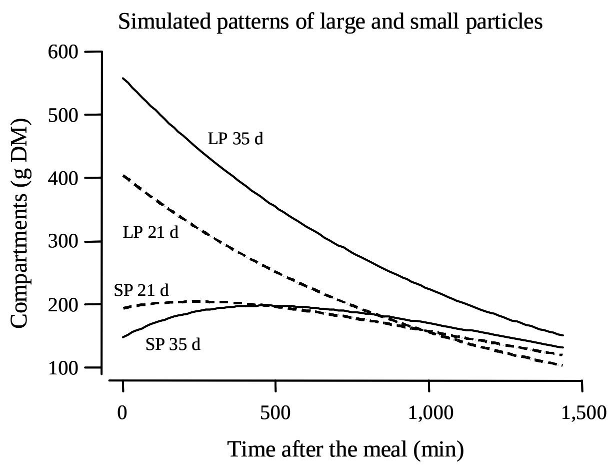

Figure 6. Simulated patterns of large (LP) and small particles (SP) compartments, with time, in the rumen of Black-belly rams given a 21- (21 d) or a 35-day old (35 d) *Digitaria decumbens*.

Table 6. Predictive equations of fractional comminution rate (kc), initial flow of transit (FLto), initial values of total large particle compartment (LPo), LPo with a 21-day old (LPo21) and 35-day old *Digitaria decumbens* (LPo35) from initial values of small particle compartment (SPo), LPo, dry matter intake during the main morning meal (DMI 2-3 h) and chewing index (ChewId) respectively

	s.e	p
kc = 0.075-0.00011×SPo	0.0079	***
FLto = 0.049×LPo	6.1	***
LPo = 229+0.479×DMI 2-3 h	95	*
LPo21 = 980-0.82×ChewId+354	98	**
LPo35 = 980-0.82×ChewId+608	98	**

* p<0.05; ** p<0.01; *** p<0.001.

Modelling particle kinetics with two meals

The initial LP compartment (LP_0) increased significantly at 35-day grass vs. 21-day grass (558 vs. 404 g, rsd = 100 g, p<0.02). In contrast, the SP_0 compartment decreased at 35-day grass vs. 21-day grass, though not significantly (148 vs. 194 g, rsd = 62 g, p<0.18). The parameters kc (0.056±0.011 L/h) and kp (0.096±0.034 L/h) were not altered by any of the regrowth age. The initial flow of comminution decreased between the 35-day grass and 21-day grass, though not significantly (15.6 vs. 23.4 g/h, rsd = 11.3 g/h, p<0.21). Meanwhile, there was a significant increase in the flow of transit at 35-day grass vs. 21-day grass (30.3 vs. 16.1 g/h, rsd = 6.0 g/h, p<0.002). There was no significant influence of rams on these elements.

Although, the parameters kp and kc were closely linked (R = 0.63, p<0.009), the relationship was not influenced by either the ages of regrowth or the rams. The SPo compartment was negatively related to kc (Table 6). Another major relationship was the positive influence of LPo on flow of transit (Table 6).

The initial value of LP was significantly and positively influenced by DM intake before the first rumen emptying (DMI 2-3 h, Table 6). DMI 2-3 h was negatively linked with the daily level of DMI (see part of the Results-Diet composition and intake).

Table 6 shows that the chewing index (min/kg DMI), which was largely explained by the ram effect, was negatively related to LPo (p = 0.005). However, with a single ram, the influence of regrowth age was significantly positive (p = 0.001), thus inducing a larger LPo compartment for the same chewing index value. The two equations of this model of adjustment are presented in Table 6.

DISCUSSION

Effect of regrowth age

The effect of regrowth age on intake and total tract digestibility in this study are consistent with previous studies (Chenost, 1975; Aumont et al., 1995; Archimède et al., 2000; Assoumaya et al., 2007b), illustrating negative correlations between forage age and the two main nutritive parameters (intake and total tract digestibility) of forage. Archimède et al. (2000) reported a mean daily decrease in intake and total tract digestibility with increasing regrowth age of pangola (14, 28, 42 and 56 days) at an average rate of 0.66 g/kg $LW^{0.75}$ and 0.52% respectively. For this current study, with respect to the regrowth age of pangola at 21 and 35 days, the corresponding values for mean daily decrease in intake and total tract digestibility as regrowth age increases are 1.26 g/kg $W^{0.75}$ and 0.74% respectively. The low range between the two regrowth ages (14 days) and the younger stages of the grass in this study could explain the observed higher values for mean daily decrease in comparison to those observed by Archimede et al. (2000). These results could be closely linked with the physiology of pangola grass. Wilson (1994) reported that C4 grasses have faster maturation compared to C3 grasses. Considering the low chemical differences that we recorded between the two regrowth stages, it can be argued that chemical composition alone may not necessarily be sufficient criterion for explaining all the effects of maturation. Rather structural changes in the cell wall characteristics could be better indicators.

The improvement observed in rumen degradation, with the nylon bag method, may strongly suggest that enzymatic microbial activity could also be a limiting factor of the forage digestion at 35-days grass vs. 21-day grass.

On the one hand, for rumen fractional turnover where rams were offered two meals, results obtained in our study were generally similar to those cited in previous studies (Poppi et al., 1981a; Ichinohe et al., 1995; Archimède et al., 2000; Assoumaya et al., 2007a). On the other hand, where rams were offered four meals our results showed little difference, which differs from previous studies. This showed that rumen fractional turnover decreased significantly with the maturity of the forage. Lower density of large particles being more important in the 35-day grass could explain this decrease.

With respect to the pool of rumen content, our results are quite similar to studies reported in the literature. Rumen content increased with the maturity of the forage. The amount of digesta trapped in the rumen as LP largely increased with regrowth age as illustrated by several authors (Poppi et al., 1981b; Ichinohe et al., 1995; Assoumaya et al., 2007a). Modelling the rumen emptying data of the two meal frequency clearly emphasized the increase of LP load throughout the day and the limited influence of chewing efficiency on rumen fill for aged forage.

Furthermore, the correlations between DMI (% LW) and the other parameters suggested that the limitation of intake capacity due to age increase was, firstly, chewing efficiency, appreciated with values of intake rate and chewing index. The second factor seemed to be the amount of indigestible matter contained in the forage which could limit the reticulo-omasal orifice capacity to treat larger flows of matter.

The correlations between individual variates revealed that animals having a higher capacity of intake also presented a shorter transit time and a lower digestibility capacity. Moreover, they also had longer mastication time per day. More work is required to assess the extent to which these individual variations and relationships are repeatable and linked to genotypic influence.

Effect of diet frequency

Few studies have dealt with the effect of meal frequency on intake and digestion with forage being the sole ingredient of the ration (Burt and Dunton, 1967; Faichney, 1968; Dulphy et al., 1980; Bunting et al., 1987). Moreover, none of these studies have dealt exclusively with fresh grass and tropical grass.

In our study, although it was not significant, an increase in intake was recorded with an increase in feeding frequency, with a higher incidence on the older forage. This tendency is the same as that found by Burt and Dunton (1967) and Bunting et al. (1987), who worked with high quality forage. These authors have explained this increase of intake by an increasing rate of DM outflow from the rumen, as recorded in our experiment. In addition, similarly to Ulyatt et al. (1984), we observed that the dynamics of digesta in the rumen was altered by feeding frequency. The highest reticulo-rumen pool sizes were recorded when feeding frequency decreased and occured above all with the older forage (35 days). This variation could be explained by the lower ruminal turnover. The highest frequency of meals could accelerate ruminal motility and stimulate transit. Consequently, an increased intake was recorded.

The negative effect of the increased rate of DM turnover from the rumen is the escape of potentially degradable fibre from the rumen of animals fed with low digestible grass. This phenomenon could explain the depressive effect of meal frequency on digestibility. Our results for impact of feeding frequency on total digestibility of DM, OM and cell wall constituents do not differ from previous studies, where differential effects were reported according to forage quality. Bunting et al. (1987), when studying a high quality forage, noted that apparent tract digestibilities of DM, OM and cell wall constituents were not significantly affected by frequency (2, 4, 8 times daily), which was also observed with our 21-day pangola grass. These authors concluded that the response of animals to frequent feeding would be highly dependent on forage quality. Our study confirms this hypothesis. Negative effect of increasing meal frequency on the total tract digestibility of NDF (and ADF) was recorded for the 35-day grass whereas no effect was recorded for the 21-day grass.

CONCLUSIONS

This experiment was performed to evaluate the pertinence of working in non-steady state conditions when studying intake and digestion of tropical forage. Increasing frequency of meals tended to increase intake whereas significant decrease of fibre digestion was recorded with the older forage. This result shows a faster evacuation of the rumen content mainly with older forage. These results underline the need to conduct studies with animals fed according to a pattern closely related to field conditions.

ACKNOWLEDGMENTS

The authors would like to thank F. Pommier, T. Etienne, B. Calif, L. Philibert, P. Despois, F. Périacarpin, F. Nipeau and M. Arjounin for their technical assistance. This work has been partly supported by the European Union (FEOGA) and Region Guadeloupe.

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