METHODOLOGICAL ASPECTS IN RUMINAL DIGESTION STUDIES.
I. EFFECT OF FEEDING FREQUENCY[1]

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ABSTRACT - Two experiments were conducted to compare different feeding frequencies with regard to dry matter (DM) intake, rumen contents, apparent ruminal retention time of the neutral detergent fiber (rNDF) and 48-h *in situ* DM digestibility (ISDMD). In experiment 1 three rumen-fistulated steers were fed a ration of brachiaria (*Brachiaria decumbens*) hay, freshly cut pigeon pea (*Cajanus cajan*) forage and ground sorghum; six treatments were applied in an unbalanced design: a) Hourly feeding, b) Feeding every 3 h, c) Feeding every 6 h, d) Feeding every 12 h, e) Feeding every 3 h from 6:00 AM to 9:00 PM at which time the rest of the daily ration was given, and f) Feeding every 3 h from 6:00 AM to 6:00 PM when the rest of the daily ration was offered. In experiment 2, six animals were used to compare hourly feeding vs. feeding every 6 h in a switchback design. The ration consisted of sugarcane tops, pigeon pea forage and ground corn ears. Means for net DM intake (kg/100 kg LW/day), rumen contents (kg DM/100 kg LW), rNDF (h), brachiaria ISDMD (%) and pigeon pea forage ISDMD (%) for experiment 1 were: 1.93, 1.76, 23.8, 66.0 and 63.1, respectively, with no significant differences. In experiment 2, the means for DM intake, rumen contents, rNDF and pigeon pea ISDMD were: 1.76, 1.79, 37.6 and 53.4, respectively, again with no significant treatment differences. It was concluded that feeding frequency is not a factor of importance in the methodology for rumen digestion studies.

Index terms: steers, feed intake, rumen contents, *in situ* digestibility, ruminal retention time.

ASPECTOS METODOLÓGICOS EM ESTUDOS DE DIGESTÃO RUMINAL.
I. EFEITO DA FREQÜÊNCIA DE ALIMENTAÇÃO

RESUMO - Realizaram-se dois experimentos para verificar o efeito de várias freqüências de alimentação no consumo de matéria seca (MS), conteúdo ruminal, tempo aparente de retenção ruminal da fibra detergente neutro (rFDN) e na digestibilidade *in situ* em 48 h da MS (DISMS). Experimento 1: 3 novilhos fistulados no rumen receberam uma ração de feno de braquiária (*Brachiaria decumbens*), guandu fresco (*Cajanus cajan*) e panícula de sorgo moído; foram testados 6 tratamentos em um delineamento não balanceado: a) Alimentação a cada hora, b) a cada 3 h, c) a cada 6 h, d) a cada 12 h, e) a cada 3 h, das 6:00 horas até as 21:00 horas, quando receberam o restante da ração, f) a cada 3 h, das 6:00 horas até as 18:00, quando receberam o restante da ração. Experimento 2: utilizaram-se 6 novilhos e 2 tratamentos: Alimentação a cada hora vs. alimentação a cada 6 h, num delineamento de reversão dupla. A ração consistiu de ponta de cana, guandu e milho com palha e sabugo. As médias para consumo de MS (kg/100 kg PV/dia), conteúdo ruminal (kg MS/100 kg PV), rFDN (h), DISMS do feno de braquiária (%) e DISMS do guandu (%) foram: 1,93; 1,76; 23,8; 66,0 e 63,1, respectivamente. No experimento 2, as médias para consumo de MS, conteúdo ruminal, rFDN e DISMS do guandu, foram: 1,76; 1,79; 37,6 e 53,4; respectivamente. Não se apresentaram diferenças significativas devidas aos tratamentos. Concluiu-se que a freqüência de alimentação não é de importância para o estudo da digestão ruminal.

Termos para indexação: novilhos, consumo de alimento, conteúdo ruminal, digestibilidade *in situ*, tempo de retenção ruminal.

INTRODUCTION

In a recent literature review by Gibson (1981), it was stated that, on the average, increasing the feeding frequency in ruminants caused improvements of 16.2% in daily gain and 18.7% in the efficiency of growth, the latter implying better feed utilization efficiency. Much of the improvement was obtained when the feeding frequency was increased from one or two meals per day to four

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meals. The implications of these findings, in terms of the concomitant digestive events, are varied and may require a more precise definition of the methods that are currently being used in studies on metabolism, *in vivo, in situ,* and *in vitro* digestion and other digestion aspects.

Papers dealing with the effect of feeding frequencies upon digestion parameters are scarce. Some point out that voluntary dry matter intake is not affected by increasing the number of meals from one to two a day (Coleman et al. 1984) or up to four times a day (Priego & Lora 1978). Although Priego & Lora (1978) found that increasing feeding frequency caused a trend for an increased rumen turnover rate, they also observed reduction in the total liquid pool thus resulting in no effects on the total fluid flowing out of the rumen. Goetsch & Galyean (1983) did not detect differences in terms of rumen passage of a particulate marker when they fed steers two or eight times a day. Even when more extreme frequencies were compared (hourly vs. daily feeding) no differences in rumen parameters were reported by Ulyatt et al. (1984) other than an increase in N-flow to the duodenum when daily feeding was used.

The methodology for rumen digestion studies includes different conditions of feeding. Minson (1966) established that animals in this type of study should be fed hourly based on the fact that with this method diurnal variations in the excretion rate and composition of feces and urine were eliminated (Minson & Cowper 1966). However, other feeding frequencies have been used in digestion studies: every three hours (Thiago et al. 1979), four times a day (Weakley et al. 1983) and once a day (Perez Gutierrez 1983, Soofi et al. 1983). Some have even used *ad libitum* feeding (Mehrez & Ørskov 1977) or a combination of feeding certain feeds once a day while leaving others *ad libitum* (Mapoon 1980, San Martín et al. 1983). The intention of using continuous or very frequent feeding regimes for the test animals has been to establish steady-state conditions of rumen fermentation and nutrient flow (Goetsch & Galyean 1983); however, in order to establish such schemes, there has to be an investment in machinery or labor which may not be within the reach of research stations in developing countries; in addition, very frequent feeding may not be representative of practical situations.

Given all the above considerations, two experiments were planned to compare the effects of different feeding frequencies upon voluntary intake, total rumen dry matter content, apparent rumen retention time for neutral detergent fiber, 48-hour *in situ* digestibility of pigeon pea (*Cajanus cajan*) forage and 48-hour *in situ* digestibility of brachiaria grass (*Brachiaria decumbens* cv. Basilisk).

MATERIAL AND METHODS

Experimento 1

Six feeding frequencies were tested on three 3-year old Nelore steers that had been rumen-fistulated seven months prior to the start of the experiment. The treatments were as follows:

A: Hourly feeding
B: Feeding every 3 h
C: Feeding every 6 h
D: Feeding every 12 h
E: Feeding every 3 h from 6:00 AM to 9:00 PM at which time three meals were combined and fed.
F: Feeding every 3 h from 6:00 AM to 6:00 PM at which time four meals were combined and fed.

The total feed offered per day was the same for every treatment. The amounts fed were kept constant in relation to body weight (Table 1) which meant that every animal received different amounts since they differed in weight (animals 199, 200 and 201 weighed, respectively, 323 kg, 242 kg and 358 kg). Adaptation to feeds and feeding schedule was started three weeks prior to the start of the experiment.

An unbalanced design was used with the six treatments distributed among the three fistulated steers over four 1-week periods (Table 2).

Although no effects were expected from periods (Weakley et al. 1983), the data were analyzed by using period as a covariable to adjust effects of treatments and animals. The General Linear Model procedure was used to analyze the data and, corresponding to an unbalanced design, type IV mean squares were used to specify effects (SAS Institute 1982).

In each period the following activities were carried out: weighing the feed refused every day; sampling of feeds offered and refused; placing of four nylon bags in the rumen; withdrawing the bags after 48 hours of incubation; manual emptying of the rumen, homogenization, sampling and reposition of contents; weighing the animals and change to the following treatment. Net feed intake was calculated on the basis of the feed offered and feed refused during three consecutive days in the second half of each period.

TABLE 1. Feeds offered and their contributions of crude protein (CP), neutral detergent fiber (NDF) and total digestible nutrients (TDN) per 100 kg liveweight per day.

Ingredient	As-fed kg	DM kg	CP g	NDF kg	TDN[1] kg
Brachiaria decumbens, hay (IFN 2-13-214)	1.42	1.22	105	0.94	0.52
Pigeon pea (*Cajanus cajan*), fresh (IFN 2-03-715)	1.05	0.62	60	0.38	0.39
Sorghum (*Sorghum vulgare*), ground (IFN 4-13-558)	0.43	0.36	30	0.07	0.29
Total	2.90	2.20	195	1.39	1.20
National Research Council (1976) requirements[2]	-	-	169	-	1.15

[1] Calculated from Florida. University (1974).

[2] Estimated for steers weighing 350 kg and gaining 350 g/day.

TABLE 2. Scheme followed for the allocation of treatments to animals in experiment 1.

Period	Animal	Treatment A	B	C	D	E	F
1	199	X					
	200	X					
	201		X				
2	199		X				
	200			X			
	201			X			
3	199				X		
	200				X		
	201					X	
4	199					X	
	200						X
	201						X

In situ digestibility of the pigeon pea forage and brachiaria grass was determined by using the nylon bag technique of Mehrez & Ørskov (1976). The bags measured 16 cm x 8 cm, had 2500 pores/cm^2 which measured 40 μ in diameter. The seams were double sewn with polyester thread and needle holes were sealed with waterproof glue. Three grams of the forage, dried at 65°C and ground to 1 mm particle size, were weighed into the bags. The bags were then closed and tied with nylon fishing line. Each animal, in each period, received four bags, two for each type of forage. These bags were tied to a bolt weighing 500 g and, after soaking in water, were placed in the ventral sac of the rumen. After 48 h, the bags were removed and washed under running water gently squeezing until the rinsing water was colorless, a procedure that took approximately 5 min per bag. They were then dried at 65°C for 24 h. The proportion of DM which had disappeared was calculated from the amount incubated and the residue left in the bag.

Rumen contents were obtained at exactly midpoint between two consecutive feedings, and after the nylon bags were withdrawn. After removal of the cannula top, the solid material was taken out by hand; halfway through, the removal of the material included prior mixing of the solid material with the rumen liquor so as to minimize the use of cups to extract the remaining liquid. Extreme care was exercised in checking the rumen folds for occult material. After complete emptying, the contents were weighed and mixed in a concrete mixer during 10 min; between 4 and 5 kg were taken for dry matter, crude protein and neutral detergent fiber (NDF) analyses. The contents were then returned to the rumen. The complete rumen emptying and repositioning procedure took about 30 min and the animals seemed to suffer no discomfort.

The apparent retention time (ART) for NDF was calculated according to the formula developed by Minson (1966),

$$ART\ (h) = \frac{\text{Weight of fraction in the rumen}}{\text{Intake of fraction per hour}}$$

Thus, the feeds offered, the refused feeds and rumen contents were analyzed for DM, NDF and crude protein although ART was calculated only for NDF.

Experiment 2

One limitation in developing or perfecting methodologies involving the use of fistulated animals is the availability of them. One way to circumvent this problem is through the use of designs such as the one used in experiment 1 that makes the most use of available animals. However, in that particular experiment only three degrees of freedom resulted for the experimental error. Mehrez & Ørskov (1977) recommended the use of one bag, two different days and three sheep in order to obtain acceptable precision in DM disappearance studies (standard error of the mean = 1.85%), although they found a high variability among sheep. In experiment 1, applying Mehrez & Ørskov (1977) variance values, the standard error of the mean would be 2.52%. Also Weakley et al. (1983) found no differences among cows on *in situ* digestion studies; however, to increase the precision of the test between different feeding frequencies, experiment 2 was planned as a switchback design (Cochran & Cox 1957) with two treatments, hourly feeding and feeding every 6 h, and six fistulated animals randomly distributed among the two sequences of treatments. Three animals were the same as those used in experiment 1 but now with 4 years of age and averaging 465 kg liveweight, while the three others averaged 2 years in age and 245 kg in weight having been fistulated two months before the start of experiment 2. The feeds and amounts offered are shown in Table 3.

All experimental procedures were the same as those already described for experiment 1 except that the *in situ* digestibility of brachiaria grass was not included. The statistical analyses consisted of t-tests (Cochran & Cox 1957) of the calculated ratios of the treatment values per animal according to the following formulae:

$$\text{Ratio (6/1)} = \frac{\text{2 x observed value when feeding every 6 h (middle period)}}{\text{Sum of observed value when feeding every hour (first and last periods)}}$$

$$\text{Ratio (6/1)} = \frac{\text{Sum of observed values when feeding every 6 h (first and last periods)}}{\text{2 x observed value when feeding every hour (middle period)}}$$

RESULTS AND DISCUSSION

Experiment 1

The average values for net feed intake, 48-h *in situ* dry matter digestibility (ISDMD) of pigeon pea forage, ISDMD of brachiaria grass, rumen contents and apparent retention time of NDF are shown in Table 4. The corresponding analyses of variance are presented in Tables 5 and 6.

Daily feed consumption was not affected significantly by treatments and averaged 1.93 kg DM/100 kg LW. This was 12% less than the amount offered, the refused portion constituted largely by pigeon pea forage (68%). Observing the mean values for treatments it is clear that in all cases there was a situation of unrestricted consumption. It was important to establish this in order to proceed with an analysis of variance for this criterion. As the experimental error was not different from the variation among days of intake measurement, and due to the small number of degrees of freedom for the experimental error, both experimental and sampling errors were pooled to test period, animal and treatment effects.

With respect to the ISDMD data, the values obtained give an indication that both forages had an acceptable nutritional quality; the legume was composed of leaves and fine stems (diameter ≤ 6 mm) and the brachiaria had been fertilized and cut 40 days after the previous cutting. For these parameters, no differences due to feeding frequencies were noted although in treatment F the ISDMD of pigeon pea was 10% lower than the values observed in the other treatments. Animal effect was found to be important ($P < 0.05$); it would seem, then, that methodologies for *in situ* evaluation of specific feeds should not be based on one animal only. The results of Mehrez & Ørskov (1977) support this observation.

Insofar as the measurements of rumen contents are concerned, the values do not appear to correlate with intake and statistically are not different when feeding frequencies are compared. The apparent retention time of NDF averaged 23.8 h, much shorter than the retention time for brome grass

TABLE 3. Average quantities of feeds offered per 100 kg liveweight per day in experiment 2.

Ingredient	As-fed kg	DM kg	CP g	NDF kg	TDN[1] kg
Sugarcane (*Saccharum officinarum*) tops, fresh (IFN 2-17-517)	6.42	1.43	53	0.85	0.76
Pigeon pea (*Cajanus cajan*), fresh (IFN 2-03-715)	0.84	0.40	46	0.23	0.25
Ground ear corn (*Zea mays*) (IFN 4-02-849)	0.42	0.35	39	0.06	0.26
Total	7.68	2.18	138	1.14	1.27

[1] Calculated from Florida. University (1974).

The ration was calculated to meet maintenance requirements (National Research Council 1976).

TABLE 4. Average values for the five parameters used to evaluate the six diferent feeding frequencies studied in experiment 1.

Parameter	Treatment						Observations/ animal/ treatment
	A	B	C	D	E	F	
Net feed intake, kg DM/100 kg LW/day	1.86	1.94	2.03	1.88	1.97	1.93	3
48-h DM digestibility in situ of pigeon pea forage, %	65.1	65.2	64.5	63.7	63.5	56.5	2
48-h DM in situ digestibility of brachiaria grass, %	66.1	67.4	65.3	65.9	64.0	66.7	2
Rumen contents, kg DM/100 kg LW	1.75	1.80	1.74	1.96	1.68	1.62	1
NDF apparent retention time, h	24.2	24.0	21.3	28.0	23.9	21.2	1

TABLE 5. Analyses of variance for feed intake, and in situ DM digestibility (ISDMD) of pigeon pea and brachiaria grass, experiment 1.

Source of variation	Feed intake		ISDMD			
			Pigeon pea		Brachiaria	
	d.f.	M.S.	d.f.	M.S.	d.f	M.S.
Period[1]	1	0.03062 ns[2]	1	46.54 ns	1	8.434 ns
Animal	2	0.00254 ns	2	81.89 *	2	12.839 *
Treatment	5	0.03028 ns	5	29.61 ns	5	6.677 ns
Experimental error	3	0.01717 }[3]	3	34.65 }[3]	3	1.917 }[3]
Sampling error	24	0.01145	12	10.90	11[4]	2.010
Overall mean	1.93 kg DM/100 kg LW/day		63.1%		66.0%	
Model C.V.	5.69		6.27		2.14	

* Significant ($P < 0.05$).

[1] Covariable

[2] ns = nonsignificant

[3] Pooled error as the F test for experimental error proved to be nonsignificant.

[4] One nylon bag in treatment E was accidentally punctured causing the reduction of 1 degree of freedom for sampling error.

TABLE 6. Analyses of variance for rumen contents and NDF apparent retention time (NDF-ART), experiment 1.

Source of variation	Rumen contents		NDF - ART	
	d.f.	M.S.	d.f.	M.S.
Period	1	0.03308 ns[1]	1	19.559 ns
Animal	2	0.00506 ns	2	3.678 ns
Treatment	5	0.03089 ns	5	16.497 ns
Experimental error	3	0.03634	3	30.611
Overall mean	1.76 kg DM/100 kg LW		23.77 h	
Model C.V.	10.84		23.28	

ns = nonsignificant.

(of similar characteristics as the brachiaria used in this study) reported by Ndlovu & Buchanan-Smith (1985), although these workers used sheep as test animals which consistently show lower retention time values than cattle (Rees & Little 1980).

Experiment 2

The individual values, overall mean and standard error are shown in Table 7 while Table 8 contains the corresponding ratios (6/1) and the results of the t-tests of the null hypothesis that the ratio (6/1) is not different from 1.0.

From Table 8, it is evident that hourly feeding does not differ from feeding every 6 h in the estimation of digestion parameters. This finding corroborates the results obtained in experiment 1.

The level of feed consumption in experiment 2 was somewhat inferior to that observed in

TABLE 7. Net feed intake (kg DM/100 kg LW/day), rumen contents (kg DM/100 kg LW), NDF apparent retention time (h) and 48-h in situ DM digestibility (ISDMD) of pigeon pea forage (%) observed in experiment 2.

Animal	Live Weight kg	Treatment sequence[1]	Parameter: Feed intake	Rumen contents	NDF-ART	ISDMD
199	474	1	1.69	1.38	29.9	58.9
		6	1.50	1.42	36.2	54.4
		1	1.52	1.41	35.6	53.1
200	458	6	1.88	1.39	26.4	57.1
		1	1.77	1.41	29.7	53.3
		6	1.55	1.35	34.5	52.4
201	462	1	1.71	1.20	25.5	59.2
		6	1.67	1.46	33.4	51.4
		1	1.52	1.33	32.9	55.3
202	230	6	2.00	2.03	36.7	51.4
		1	1.96	1.80	32.6	52.3
		6	1.83	2.06	41.7	53.9
203	262	1	1.87	2.21	45.4	51.4
		6	1.60	2.08	47.8	54.0
		1	1.79	2.39	48.3	51.9
204	244	6	2.01	2.38	45.3	50.3
		1	1.93	2.39	44.0	51.8
		6	1.89	2.78	52.1	53.2
Overall mean			1.76	1.79	37.6	53.4
Standard error of mean (n = 12)			0.048	0.141	2.18	0.56

[1] Refers to the treatment applied in every period (1 = feeding every hour, 6 = feeding every 6 h).

TABLE 8. Calculated ratios (6/1) and statistical tests for various parameters of digestion studied in experiment 2.

Animal	Parameter			
	Feed intake	Rumen contents	NDF-ART	ISDMD
199	0.93	1.04	1.11	0.97
200	0.97	0.97	1.03	0.98
201	1.03	1.15	1.14	0.90
202	0.98	1.14	1.20	1.01
203	0.87	0.90	1.02	1.05
204	1.01	1.08	1.11	1.00
Mean	1.01	1.05	1.10	0.98
Sx	0.086	0.089	0.062	0.046
Calculated t (5 d.f.)	0.097	0.522	1.612	-0.328
Significance	ns	ns	ns	ns

experiment 1, possibly due to the use of the sugarcane tops which evidently are of lesser quality than the brachiaria grass used in experiment 1. Also, it is apparent that the ISDMD of the pigeon pea forage was lower than that observed in the previous experiment, a fact that cannot be explained by differences in quality as both experiments were conducted in the same season, in two consecutive years, and the chemical characteristics were similar.

A closer look at the data in Table 7 leads to a suggestion of an animal age influence on relative feed intake, rumen contents and NDF apparent retention time. This, of course, does not interfere with the statistical analysis as each animal was, in fact, its own control due to the characteristics of the design. Even though it was not part of the objective of this study, the two groups of animals, 4-year old (animals 199, 200 and 201) and 2-year old (animals 202, 203 and 204) were compared using s_d = 0.095, 0.282, 4.368 and 1.262 for feed intake, rumen contents, NDF-ART and ISDMD, respectively (n = 6 for each age group). The t-test indicated that, with the exception of the ISDMD, both age groups differed significantly ($P \leq 0.05$) the young animals showing higher values for the three parameters. Hence, age of the animal is a factor that should be taken into consideration in digestion studies. A sequel to the present article will specifically address the subject of age of the animal as a factor influencing rumen digestion.

Attention is given to the fact that, when feed intake is calculated on the basis of metabolic weight, the two age groups do not differ (76 g vs. 74 g per $kg^{0.75}$ for old and young animals, respectively). However, it is felt that the use of this expression is not warranted as the feed used was coarse and of low quality; therefore, consumption must not have been regulated by metabolic factors. The relatively low levels of intake provide further support for this contention.

Both experiments reported herein provide evidence to the fact that feeding frequency is not an important factor in the methodology for rumen digestion studies, at least insofar as feed intake, rumen contents, NDF retention time and *in situ* digestibility are concerned. Data from other researchers point to nonsignificant effects of feeding frequency on feed intake (Priego & Lora 1978), rumen particulate passage rate (Priego & Lora 1978, Goetsch & Galyean 1983) or reticulo-rumen pool size or digestibility (Ulyatt et al. 1984). Thus, the review by Gibson (1981) demonstrating improved feed efficiency at high frequencies of feeding may be a consequence not of changes in digestion kinetics but, rather, of improved nutrient utilization at the tissue level as suggested by the data of Ulyatt et al. (1984). In connection with this idea, it is known that variations in feeding frequency alter the patterns of rumen fermentation (Sutton 1979, Goetsch & Galyean 1983).

At least with respect to the parameters studied, the conclusion that feeding frequency is not an important factor must be taken with the underlying condition that the animals used in both experiments were fed without quantitative restrictions . Different results may be obtained under restricted feeding or at a higher level of feeding which can certainly be obtained with rations of better quality than the ones used in this study.

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