EFFECT OF SAMPLING BY FISTULA OR AT SLAUGHTER ON ESTIMATION OF RUMEN PROTOZOA[1]

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Six Zebu bulls fitted with rumen cannulas and with a mean live weight of 150 kg were used to determine the effects on estimates of rumen protozoa population when samples were taken through the rumen cannula at intervals after feeding or directly from the mixed rumen contents after slaughter. Comparisons were also made of covering the tube with gauze when rumen fluid was taken by suction. All of the animals received chopped whole sugar cane supplemented with urea and ammonium sulphate: three of them received 1 kg daily of a 20% crude protein concentrate while the others had 5 kg/d of fresh cassava forage. The estimates of rumen protozoa concentration (according to the biomass technique) were lower when gauze was used to cover the sampling tube (.54 vs.71% P <.002). The estimates were higher when samples were taken from the rumen at slaughter after mixing (.65%) than without mixing (.43%) (P <.0005). There were no significant differences in the protozoa population (sampled by suction tube) between the live and dead animal when mixing was not practised. The samples taken over the period 0 to 4 hr after feeding showed a similar tendency to what has been reported previously, namely a maximum concentration, at 1 hr after feeding followed by a decline. However the absolute value, corresponding to mixed contents in the slaughtered animal 4.5 hr after feeding, was as high or higher than the peak value reported at 1 hr taken for sampling via the cannula. The results show that samples of rumen fluid taken through the cannula will lead to underestimates of the true protozoa population, due to the settling out of the protozoa at the bottom of the rumen after feeding "because of their greater density through storage of starch from ingested sugars), and because rumen contractions are less intense at this time, resulting in less efficient mixing.

Key words: Cattle, sugar cane, rumen protozoa, sampling technique

Diets based on sugar cane and urea appear to be uniquely different from all other rations normally fed to ruminants in that they sus tain very high populations of holotrich protozoa in the rumen (Leng and Preston 1976; Valdez et al 1976; Minor et al 1976a,b) exact function of these organisms within the overall nutrition of cattle fed on sugar cane is still not understood but it has been postulated that because they have not been detected in the omasum other than in a few cases and then at very reduced concentrations (Minor et al 1976a,b) that they die in the rumen and are then fermented by bacteria.

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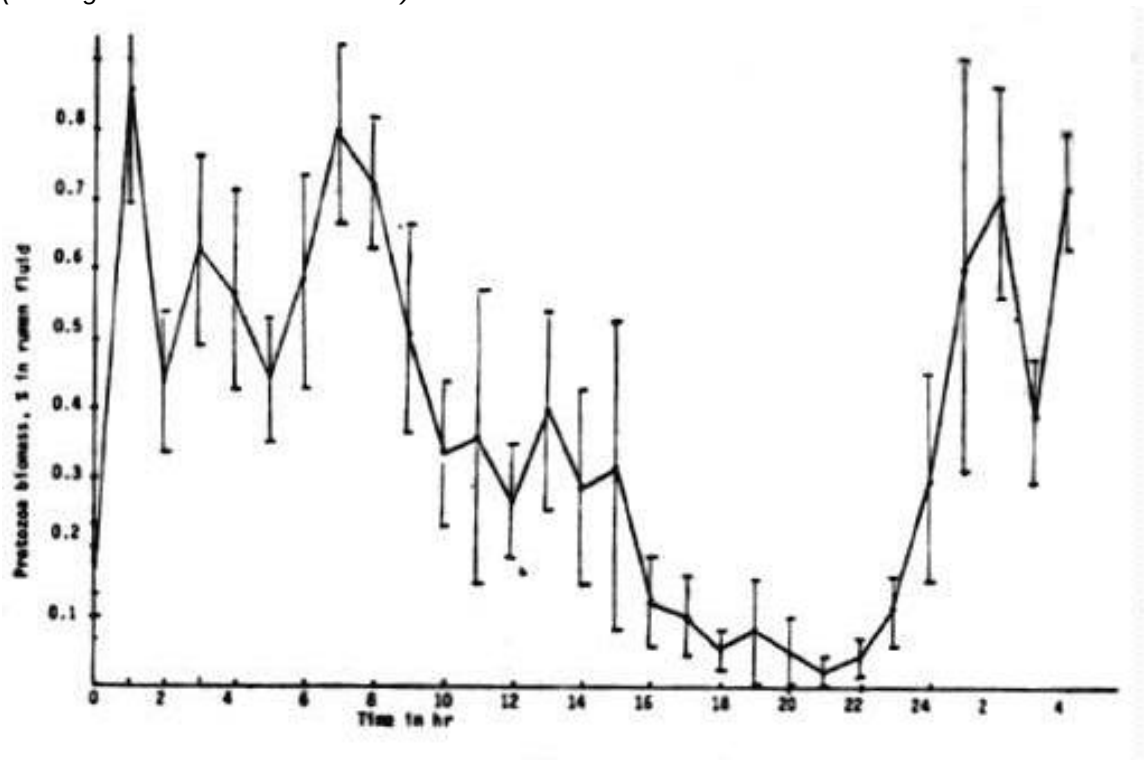
Figure 1:
Diurnal variation in protozoa biomass (mean of 4 animals) on a diet of sugar cane and urea (feeding was at time zero and 7hr)



It has been estimated that up to 100 g/d of protein might be lost to the animal in this way (see Leng and Preston 1976). Other interesting observations relate to an apparent diurnal variation in the protozoa population (figure 1). These data based on samples taken via a rumen cannula, indicate that the protozoa population is low before feeding, rising to a peak about 1 hr after feeding, falling subsequently to reach minimum values again before the next morning feed (Valdez et al 1976). Marked variations between animals have also been observed.

Another relevant finding has been that protozoa concentrations determined on slaughtered animals where thorough mixing of rumen contents and efficient sampling could be ensured, were very much higher than the average reported in samples of rumen fluid taken by rumen cannula (Minor et al 1976a,b). An explanation of these differences was put forward by Priego and Leng (1976) on the basis of their observations that the time when rumen protozoa apparently reach their maximum concentration was also the period of most intense rumen contractions. They proposed that the apparent lower values of rumen protozoa observed during periods when animals were resting or ruminating arose because these organisms were settling to the bottom of the rumen or migrating to the walls of- this organ and thus not becoming available at the point were rumen sampling was carried out, which generally was at, or about, the centre of the rumen contents.

Two methods have been used to estimate protozoa populations. The one used most frequently, and considered to have the most biological significance is based upon precipitating the protozoa from rumen fluid by adding solutions of glucose and subsequently determining biomass by direct reading in a haematocrit tube (Leng et al 1976). The conventional method of counting the protozoa under the microscope was found to give very variable results in view of the difficulties of achieving repeatability due to the large size of the organisms.

Another difference between methods of sampling by fistula and in the slaughtered animal was that for sampling through the rumen cannula a hand suction pump was used, and either a nylon stocking or two layers of gauze were used to cover the orifice of the tube that was suspended in the rumen. In contrast, when samples were taken of rumen fluid from slaughtered animals, generally the rumen fluid was expressed by hand

The objective of the experiment reported here was to obtain further information on the differences between samples taken by cannula and at slaughter, and to examine the effect of filtration through gauze.

Materials and Methods

Treatment: Two treatments were studied. The first was a comparison of samples taken through the rumen cannula by suction compared with those obtained directly from total rumen contents after slaughter. The other treatment was the effect Oc straining the rumen contents through gauze.

Animal and Diets: 6 Zebu steers with rumen cannulas were used. These were approximately 2 years old and weighed about 150 kg. The basal ration was chopped whole sugar cane supplemented with an aqueous solution of urea and ammonia sulphate (180 g urea 50 g ammonium sulphate and 770 g water/litre) at the rate of 50 ml/kg of fresh sugar cane. Two of the animals also received 1 kg/d of a protein concentrate and 2 had 5 kg/d of chopped cassava forage. Minerals were given to all the animals Two animals (one on cassava and one on concentrates) received the sugar cane/urea after it had been prefermented for 24 hr while the other two received it fresh immediately after chopping and mixing.

Procedure: After the animals had been receiving the experimental diets for 20 days, samples of rumen fluid were taken before and 1, 2, 3 and 4 hr after feeding. Immediately after the 4 hr sample, the animal was slaughtered and samples taken directly, after thorough mixing of the rumen contents. A hand suction pump with a 10 mm diameter copper tube was used to with-draw the samples. One sample was taken with two layers of gauze secured over the end of this tube, while another sample was taken with the tube completely open. A third sample of rumen fluid was taken from the slaughtered animals, using simple hand pressure to obtain the rumen fluid.

Protozoa biomass was determined on all samples by the packed cell volume method (Leng et al 1976).

Results and Discussion

Values for protozoa biomass in the 6 animals that were slaughtered are set out in table 1. Methods for taking the samples were analysed by the "t" test for paired comparisons and are presented in table 2. The effect of time of sampling on values for biomass in rumen fluid taken via the cannula are compared in figure 2 with the final absolute value obtained after slaughter and thorough mixing of the rumen contents. Protozoa biomass could not be detected in two of the animals. One of these was receiving the concentrate supplement but was in extremely emaciated condition while the other received the supplement of cassava forage.

There were highly significant differences between methods for taking the samples of rumen fluid. When the rumen contents were obtained by suction, biomass was significantly lower ($P<.002$) with gauze over the tube than without it. Values were also higher when total rumen contents were mixed by hand before sampling as compared with inserting the sample tube into the center of the rumen and taking out the sample by suction ($P <.005$). There also appeared to be differences between samples taken directly from the rumen after slaughter as compared with those taken via the rumen cannula immediately prior to slaughter (in both cases by suction and without mixing), however the data were highly variable and the difference was not significant

Table 1:
Mean values for protozoa biomass (as % packed cell volume in rumen fluid) in individual animals 1

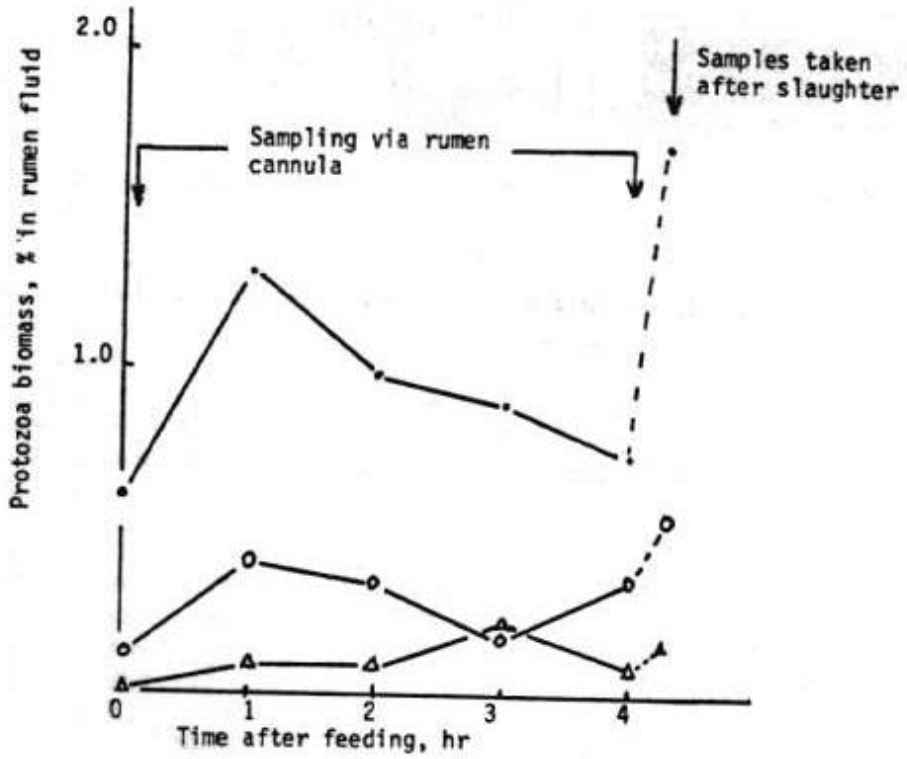
	By suction through the rumen cannula					After slaughter	
	Time of sampling, hr					By suction	by hand
	0	1	2	3	4		
Concentrate supplement							
Prefermented cane	.59	1.30	.99	.91	.74	1.46	1.71
Fresh cane	.12	.41	.34	.19	.36	.27	.54
Cassava forage supplement							
Prefermented cane	-	.09	.09	.21	-.09	-	.16
Fresh cane	-	-	.03	-	-	-	.18

In two animals given fresh sugar cane (one on concentrate and one on cassava), no protozoa were detected.

Table 2:
Effect ("t" test) of different methods of sampling rumen contents on estimates of protozoal biomass (% of rumen fluid)

	Mean values	Difference	Probability
Effect of straining through gauze			
With	.54	.17±.032	.002
Without	.71		
Method of sampling[1]			
By suction without mixing	.43	.21±.027	.0005
By hand after mixing	.65		
Effect of slaughter			
By cannula in the live animal	.46	.21±.20	.32
Directly from the rumen after slaughter	.76		

Figure 2:
Protozoa biomass in rumen fluid taken via the cannula in the live animal, and directly from the rumen in the slaughtered animal (individual data from 3 animals)



The data for the 3 animals which showed highest protozoa concentrations (figure 2), show very clearly that the apparent rise and fall in protozoa biomass after feeding is not a true effect. For in the case of each of the animals, biomass taken after slaughter (4.5 hr after feeding) was the same (one animal) or considerably higher (two animals) than the peak value recorded for the cannula sample 1 hr after feeding. These data show conclusively that the accuracy of sampling rumen contents for holotrich protozoa will be a function of the degree of mixing of the contents, and in the live animal this will vary according to the intensity of rumen contractions. These findings thus support the hypothesis put forward by Priego and Leng (1976) that after feeding the protozoa settle at the bottom of the rumen, or migrate to the rumen wall, thus resulting in apparently lower values for protozoa biomass when samples are withdrawn from within the mass of contents, as happens with the use of a suction tube through the rumen cannula.

Although, it was not the objective of this experiment to make comparisons between diets, it is interesting to record that the protozoa population was very much less in those animals receiving sugar cane supplemented with cassava forage than in those receiving a concentrate supplement (.62 vs.03; SE dif ±.14; P <.001). This aspect will be discussed in greater detail in a subsequent paper.

Conclusions

Reliable estimates of the holotrich protozoa population in cattle fed on sugar cane can only be obtained when the rumen contents are mixed thoroughly prior to sampling. Such a procedure is feasible in slaughtered animals, however, in the live animal it would require some means of mixing the rumen contents, perhaps by recycling them through a pump.

Determinations of protozoa numbers or biomass from samples taken via a rumen cannula, will always under-estimate the true population.

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