RUMEN FUNCTION IN CATTLE GIVEN SUGAR CANE

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Diets based solely on sugar cane, urea and minerals support only maintenance in ruminants and provision of supplements for production appears to be essential, As part of a study of the role of these supplements, a number of measurements of rumen function were examined in bulls given sugar cane diets with or without rice polishings (a supplement which has given consistent responses in terms of production). Determinations were made of pH, ammonia, total and VFA proportions and protozoan biomass in rumen fluid on several days and for 28 hr consecutively. A new method of assessing protozoan biomass is put forward. There were no apparent differences due to the rice polishings in any of the parameters of rumen function measured over 28 hr or between days. The outstanding findings were that large holotrich protozoa populations existed in the rumen of these animals and that concentrations varied within and between days. The reasons for, and the effects of such fluctuations are discussed. It is concluded that the value of rice polishings in sugar cane diets is not mediated through an effect on rumen function but is probably due to its ability to supply essential nutrients.

Key words: Sugar cane, cattle, rumen function, protozoa, rice polishings

The humid tropics have great potential for both crop and animal production, nevertheless there has not been the development of intensive beef and milk systems as has occurred in the temperature regions. This lack of development appears to have been mainly due to a scarcity of cereal grain and to the fact that tropical crops are generally low in protein and are apparently of low nutritive value.

Recently systems of feeding sugar cane to ruminants have been developed (Bonefer et al 1975, Leng & Preston 1976, Preston 1977); these would appear to have considerable potential since dry matter yields from this crop are very high (often exceeding 40 tons/ha; Preston et al 1976) and the material is highly digestible (60-65%) (Montpellier & Preston 1977a, Ferreiro & Preston 1977).

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The technology for utilizing high levels of molasses in ruminant diets with consistently high rates of production is now well established (see Preston and Willis 1974). However, the utilization of whole sugar cane appears to be less predictable. Cattle given shredded whole sugar cane supplemented only with urea and minerals did not grow; whereas, after addition of 1 kg/d of rice polishings, production rose to 0 9 kg/d (Preston et al 1976).

A dry matter digestibility in the range of 60-65% indicates that sugar cane should be capable of supporting higher rates of production than have so far been obtained. While, on a unit area basis, calculations suggest that up to 20 cattle could be fattened/ha/year on this crop if the apparent limitations to its use could be overcome.

A puzzling feature of work reported recently using different supplements for sugar cane diets (Silvestre et al 1977), indicated that proteins of animal origin often appear to have no beneficial effects, and that vegetable protein supplements of which the most consistent has been rice polishings, are more effective (Preston et al 1976 ; Lopez et al 1976).

The objective of the present studies was to increase our understanding of digestive processes in cattle fed sugar cane, with and without supple-. meets of rice polishings, in the belief that further knowledge in this area would lead to the development of more predictable feeding systems with this crop. This initial investigation was aimed at examining rumen function since it was expected that the rumen might be the effective site of action of the rice polishings.

Material and Methods

Experimental Animals and Diets: 8 crossbred (Brown Swiss/Zebu) bulls 18 months of age and weighing between 300 and 400 kg were used in this study. They were housed in single pens in an open-sided animal house. Mean day and night temperatures were 32 and 28! at the time the experiment was carried out. No artificial light was used and day length was about 14 hr.

The animals were prepared with permanent rumen fistulas and were well accustomed to the experimental procedure. The diets consisted of freshly cut and shredded mature whole sugar cane, approximately 12-14 mth old, 18 Brix (percentage sugar in juice) and 28% dry matter. The sugar cane was supplemented with a solution of urea in molasses (817 g final molasses of 88°Brix, water 208 g and urea 283 g/litre) which was sprinkled on the surface of the sugar cane in the ratio of 50 ml/kg fresh cane. The animals had free access to a mineral mixture (50% NaCl, 45% ground rock phosphate (12% P) and 5% of a commercial trace element mixture (included Fe, Mg, Mn, Zn, Cu, Co). Four of the animals received in addition a supplement of 1 kg/d of rice polishings, the approximate composition of which was 26% starch, 13% crude protein, 13% lipids.

The daily feeding regime was as follows: all the rice polishings (according to treatment) was given at 7:30 a.m. and the sugar cane/urea molasses mixture at 8:00 a.m., the animals were fed again with the sugar cane/urea molasses at 4 p.m. The sugar cane mixture was given in excess of requirement. The animals had been established on the diet for 3 months before the experiment was initiated.

Experimental Procedures:
Fibre digestion in the rumen: The nylon bag technique was used to measure the digestibility of whole sugar cane and of fibre in the following way. Fifty g of freshly shredded whole sugar cane were placed inside the bags which were then put into the rumen of cattle which were being fed on the same diets. After 72 hr the bags were removed and washed 2 or 3 times by squeezing in water prior to drying at 100° for 24 hr. The insoluble dry matter in 50 g samples of fresh sugar cane was estimated by washing out the solubles, placing the bag in water and squeezing over a period of about 1 hr. These control bags were then dried and treated in the same way as samples from the rumen.

Sampling Rumen Fluid: Rumen fluid was sampled through a perforated copper tube (5 cm long, 0.3 cm diameter) covered with nylon. This was attached to a flexible tube (0.5 cm diameter) which was passed through a stopper in the mouth of the rumen cannula, allowing samples to be taken without opening the rumen cannula. Rumen fluid samples were taken from the animals at intervals over 17 days. On each day samples were taken before feeding and at intervals for up to 6 hr after feeding. In one period, samples were taken at hourly intervals over 28 hours. The sample of rumen fluid (20 to 50 ml) was taken with a minimum of suction, its pH and protozoa biomass determined immediately and samples (20 ml) stored at -15° after adjusting to pH 4 with 10N H_2SO_4.

Analytical Methods:

Total VFA and proportions: Total volatile fatty acid (VFA) concentration in rumen fluid was determined by titration after steam distillation. Proportions of VFA as acetic, propionic, and butyric acids were estimated by chromatography, after extraction into ether, using a Carle 311 gas chromatography (Carle Instruments inc., Fullerton, California) with a thermal conductivity detector and hydrogen as the carrier gas. Rumen fluid (4 ml) was placed in a small stoppered tube and 0.08 ml 50% (v/v) H_2SO_4 added followed by 2 ml ether. The tubes were stoppered and shaken by inverting 30 times and placed in a deep freeze at -15°. Following freezing the ether was poured into a chilled test tube and about 50 mg anhydrous MgSO4 was added to remove any residual water. After standing for 5 min 2-5 ml were injected into a 2 m, 0.3 cm diameter stainless steel column containing LAC-IR-296 (Burrell Corp. Pittsburgh, Pa). Proportions were calculated by reference to known standards prepared in the same way (Holdeman and Moore 1972).

Ammonia in rumen fluid: This was estimated by a modification of the indoplenol method of Chaney & Marbach (1962). Rumen fluid (1 ml) was added to a 100 ml wide mouth flask with a lid that was sealed by a plastic washer; a small hole was drilled in the lid. A scintillation vial containing 0.5 ml 0.28N H_2SO_4 was placed inside and the lid tightly closed. One ml 5N NaOH was injected into the rumen fluid using a syringe and

needle and the hole sealed with a piece of waxed paper. The flasks were allowed to stand at room temperature for 8 hr and then H_2O (5 ml) was added to the acid in the scintillation vial and 1 ml was taken for analysis according to Chaney and Marbach (1962). Recoveries of ammonia from standard solutions were 80-90% and a standard curve and blanks were always analysed in the same way and at the same time as rumen fluid.

Protozoan biomass: Microscopic examination showed that large protozoa were mainly involved, which were difficult to count accurately. Large variations occurred between different counts in the same samples. Counting was also found to be too slow for the large number of samples involved. A Technique was therefore developed for rapid assessment of protozoan biomass based on a packed cell volume measured as follows: Glucose (100 mg in 1 ml) was placed in a centrifuge tube and 15 ml of rumen fluid added with mixing. The tube was incubated at 40! for 20 min. Much of the solid matter in rumen contents floated to the surface because of gas production and could be removed with a pasteur pipette. The protozoa gradually settled and after 20 min the tubes were lightly centrifuged (500 r.p.m.) and the rumen fluid was removed to leave the protozoa in 1 ml. This was then mixed, transferred to a 1 ml graduated hematocrit tube and incubated for 5 min at 40! Again l considerable separation of protozoa and residual feed materials was achieved in the tube. The tube were centrifuged at 1500 r.p.m. for 1 min and the height of protozoal biomass was immediately read off the tube as a percentage of the volume. In the majority of analyses, particularly where protozoa were in large numbers, a clear demarcation was obtained between protozoa and plant material. With small amounts of protozoan biomass (less than 0.1% of rumen fluid) there was difficulty in separation. Repeated analyses on the same sampled indicated that individual biomass was estimated with a coefficient of variation of about ± 8% at a content of about 1% biomass (volume basis) in the rumen.

Results

Preliminary studies indicated that there were considerable fluctuations in protozoa population between animals and within animals on the same day. For this reason parameters in rumen function have been monitored over a series of days and also on a continuous basis throughout a 28 hr period.

Digestibility of fibre: The digestibility coefficients obtained by the nylon bag technique were: 53.0 ± 1.3%(mean and SE of 8 estimations) for dry matter and 19.5 ± 2.2% (8 determinations) for insoluble material.

Rumen fluid pH: Generally pH values were high over the full 24 hr period, however there was a tendency towards a diurnal pattern characterized by a fall in rumen pH over a 6-8 period after feeding, with subsequent recovery to high values prior to the next feed (figure 1). This trend was also apparent between days (figure 1). There were no obvious differences in rum pH between animals with and without rice polishings.

Rumen fluid ammonia concentration: Rumen ammonia concentrations rose after feeding in both groups of animals but fell at about 4 hr after feeding and then slowly declined throughout the rest of the 24 hr period. In the case of the animals without rice

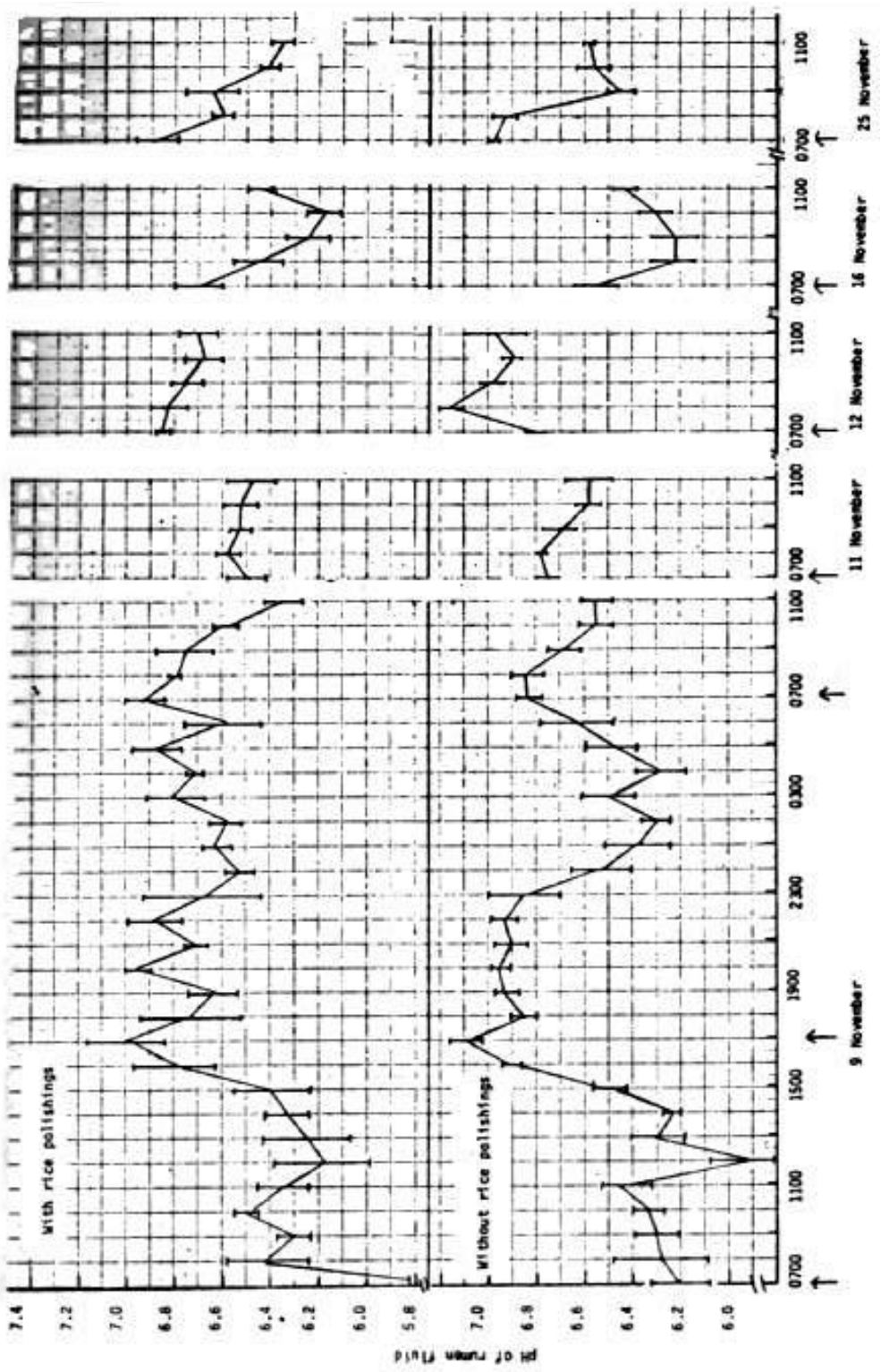

Figure 1 :
pH in rumen fluid during a continuous sampling period of 28hr on one day and over 4hr on different days (means and SE) (indicates feeding times)

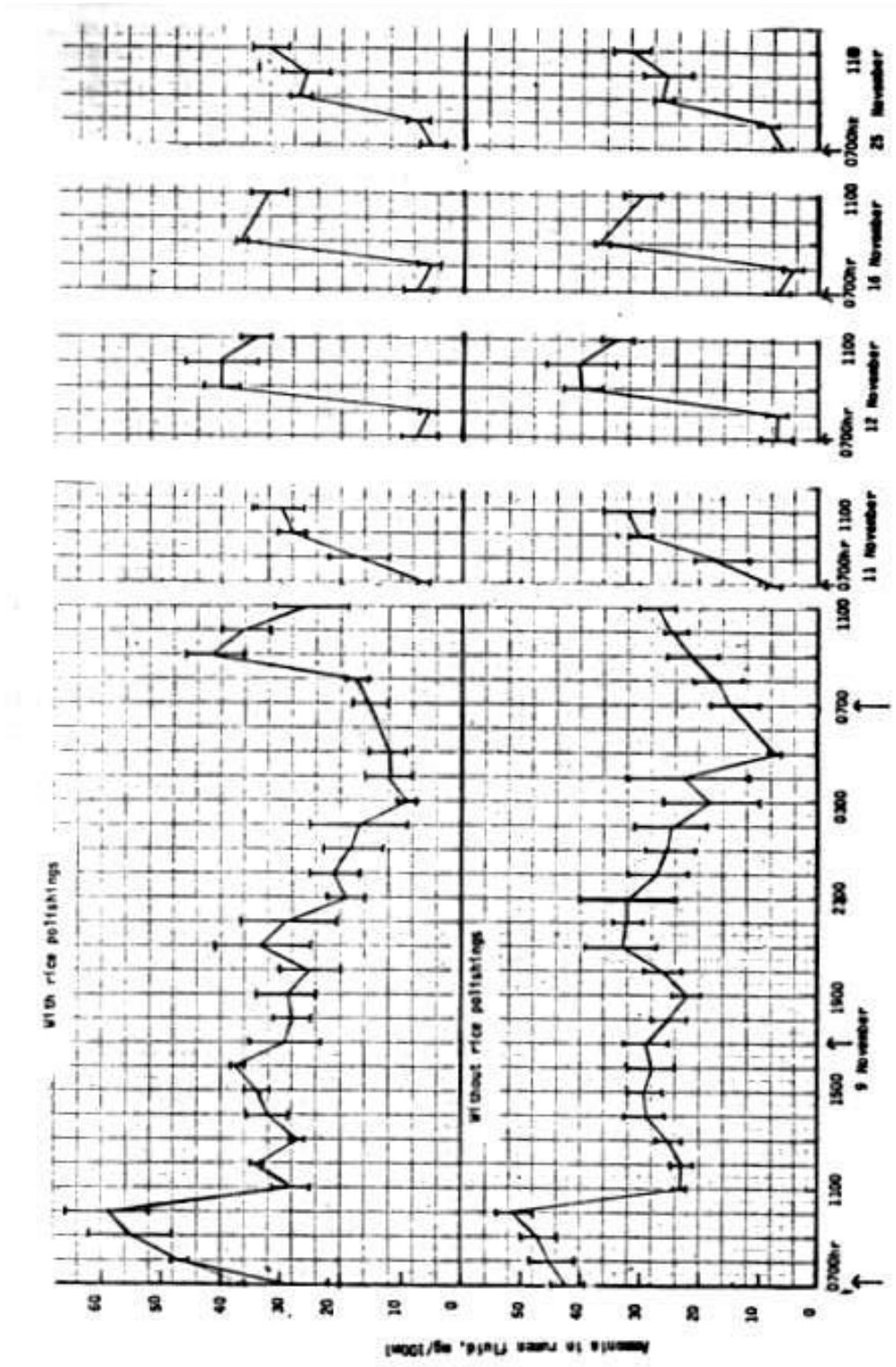

Figure 2 :
Ammonia in rumen fluid (mean values and SE)(indicates feeding times)

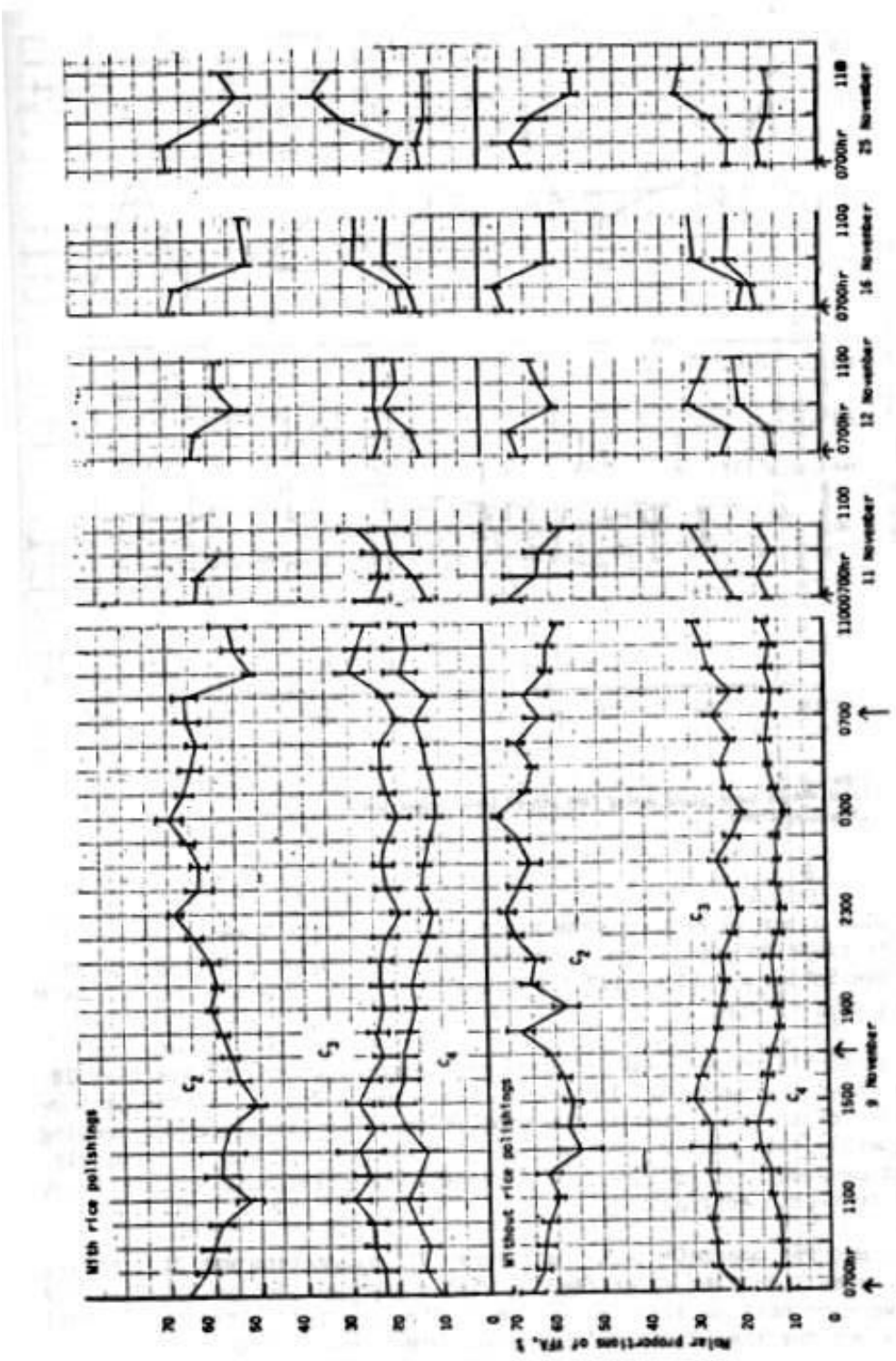

Figure 3:
Molar proportions of VFA in rumen fluid (mean values and SE) (Indicates feeding times)

polishings, ammonia levels did not reach the same value after 24 hr as at the start of the study. However, ammonia concentrations in general were always very high relative to many feeds (e.g. see Satter & Roffler 1977) (figure 2). This trend was also consistent between days (figure 2).

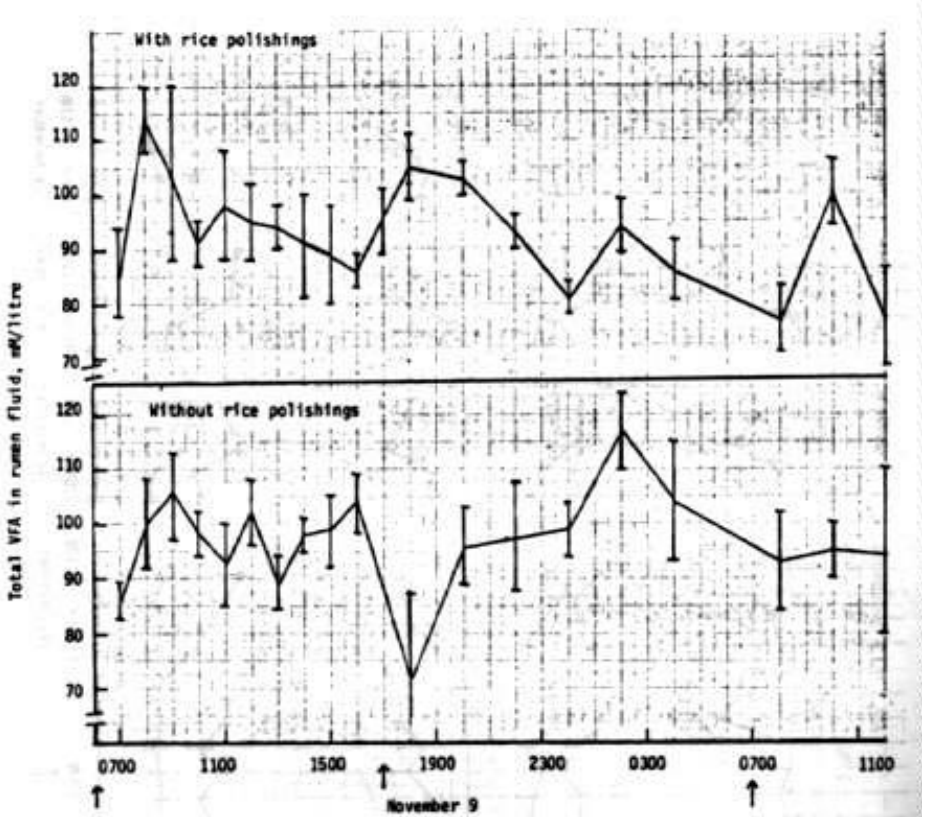

Figure 4:
Total VFA in rumen fluid during 28hr period (mean values and SE)(↑ indicates feeding times)

Rumen fluid VFA proportions: The mean molar proportions of VFA over 2 hr are shown in figure 3. In general there was a decline in the proportion of acetate and a rise in the proportion of propionate after feeding; subsequently this pattern was reversed. This trend was particularly evident between days (figure 3) for acetate and propionate while butyrate tended to remain constant.

Rumen fluid VFA concentration: Peak rumen VFA production was apparently reached some 1 to 2 hr after feeding with a gradual decline subsequently. There were no obvious differences due to the rice polishings supplement. Mean values for the 28hr sampling period are given in figure 4.

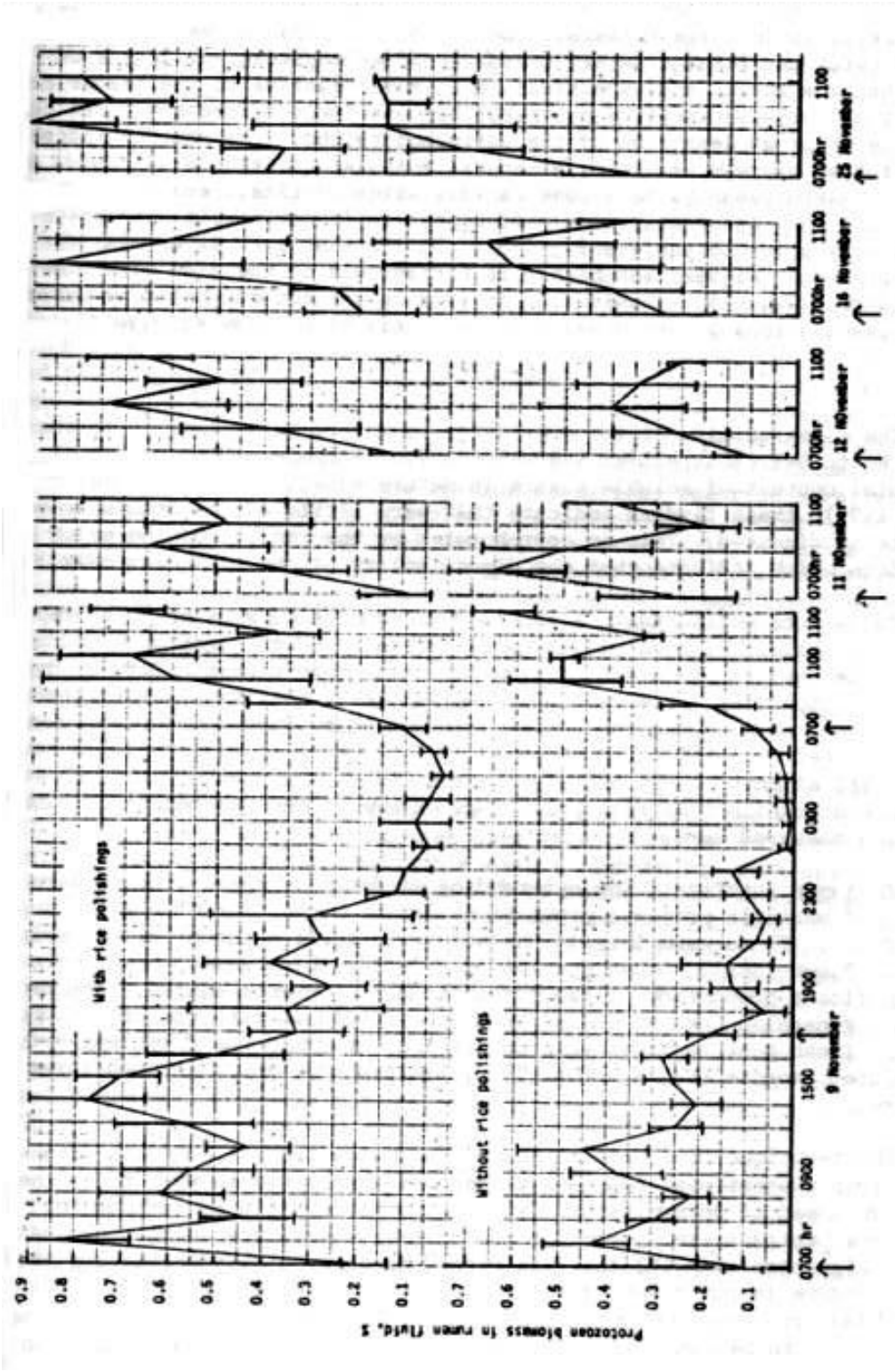

Figure 5:
Protozoan biomass in rumen fluid (mean values and SE) (feeding times)

Protozoan biomass in rumen fluid: Protozoa were present in the cattle on these diets in large numbers. The predominant species were *Isotricha intestinalis* and *Dasytricha* sp; *Entodinea* sp were present at levels of less than 1 x 105/ml and were small in biomass relative to the holotrichs. The method for assessment of protozoan biomass included the entodinea but was made up of at least 95% of the large holotrichs. The diurnal changes in protozoan biomass were similar in all animals on both diets (figure 5). Protozoan biomass rose rapidly after feeding, remained high for 6-8 hr and then apparently slowly decreased to low or negligible levels immediately prior to the morning feed. During the 28 hr experiment the maximum protozoan biomass was higher in the animals on the diet with rice polishings (figure 5). However, this finding was not supported by subsequent examinations of protozoan biomass on different days (figure 5).

Discussion

The digestibility of the dry matter of mature sugar cane is in the range 60 to 64% (Montpellier & Preston 1977a; Ferreiro 8 Preston 1977), Sir the total content of soluble sugars in mature cane is over 50% (See Ferreiro et al 1977), these finding indicate that very little of the insoluble components is digested. This is corroborated by the results of the nylon bag technique which indicate that the digestibility of the fibre was only 19%.

Effects of rice polishings: A considerable amount of work has been reported on the effect of rice polishings in diets based on sugar cane and urea. The consistent results are as follow: no effect on digestibility of dry matter (Montpellier & Preston 1977b); significant positive linear effects (for up to 1 kg/d of rice polishings) on voluntary feed intake, daily weight gain and feed conversion (Lopez et al 1976; Preston et al 1976). In contrast with these effects on the animal, the results of the present study showed: no systematic effect of rice polishings on any of the parameters of rumen function measured here.

Overall rumen function: The outstanding findings in these studies were the large and variable protozoa populations in the rumen of tattle given sugar cane diets. These were largely the holotrich species (about 80% Isotricha and 20% Dasytricha). Other species of protozoa which were present were a. insignificant proportion of the total biomass. On most cattle diets protozoa are generally present in the rumen but rarely in such concentrations. Only on fresh pasture grass (Clarke 1965) or on grain based rations fed in restricted amounts (Eadie & Mann 1970) are protozoa found in such large numbers.

The technique developed to measure protozoa biomass has many advantages over conventional counting procedures, but its main virtue is the relative speed of determination. Conventional counting procedures for the large protozoa are inherently inaccurate, however, at high protozoa biomass (greater than 1%) this new method appears to be accurate to ± 8%. The procedure is put forward for comparative purpose rather than to indicate total protozoa present in the rumen at any one time. This latter measure appears only to be possible in slaughtered animals where rumen fluid can be thoroughly mixed (Minor et al 1977b).

The pattern of change of protozoa biomass over a 24 hr period indicates that the protozoa probably settle in the rumen on becoming full of starch as they do in test tubes. In studies of protozoan metabolism (Valdez, Preston & Leng 1976 unpublished observations) it was found that protozoa incubated in boiled rumen fluid without substrate rapidly clumped on the sides of the incubating flasks or attached themselves to any fibrous material in the flask; whereas with additions of glucose, sucrose or glucose and sucrose, no clumping occurred even though the protozoa settled in the flasks if they were not shaken. In the flasks without substrate, clumps were observed to form within 30 seconds of cessation of shaking and there was a definite migration to a clump. Microscopic examination of these clumps showed that there was no apparent adhesion of the organisms which were moving freely, suggesting that the word clumping is a misnomer and flocking may better describe this phenomenon. The in vitro results tend to suggest that in the presence of substrate these organisms are active. In vivo studies suggest that subsequently they become heavy and tend to sink in the rumen and as soon as the substrate is exhausted begin to migrate into groups. In the feeding situations reported here, where the energy source is already in solution, the protozoa may confer an advantage to the host animal, in that they can rapidly store soluble sugars which presumably are then fermented at a rate to meet their requirements for maintenance and reproduction, allowing the host animal a more continuous supply of VFA. The protozoa probably settle in the rumen once they have stored sufficient starch. On this same feeding regime it was reported that the frequency of rumen contractions, and their strength increased after feeding (Priego & Leng 1976) which would tend to redistribute the protozoa through rumen contents at a time when substrate was readily available.

The migration and flocking and also the settling in the rumen would tend to reduce the outflow of protozoa from the rumen (see Weller & Pilgrim 1974). In fact, subsequent studies have shown that the concentration of protozoa in the omasum is negligible even though in the same animal concentrations in the rumen often exceeded 5% of rumen volume as biomass (Minor et al 1977a). The increase in the apparent protozoan biomass in the rumen shortly after feeding is probably due mainly to a redistribution of protozoa. Warner (1965) has observed that the holotrich protozoa tend to be dividing just before feeding, and the differences in magnitude of the protozoan biomass between days in the present study suggest that protozoa can double their populations in 24 hr and also that there are some deaths of protozoa. If protozoa die then the soluble parts of their cells presumably become substrates for the remaining microbial community (see Leng 1976).

In the first experiment there was an apparent significant reduction in protozoan biomass in cattle fed with rice polishings, however when the protozoan biomass was examined with time the between day variation was large and also there appeared to be cyclical variation in the protozoan biomass. Overall, there appeared to be no differences between the groups of animals, supplemented with or without rice polishings, the difference in the first 24 h sampling period being fortuitous.

A point that should be emphasized is that in making comparisons of protozoal biomass between days, only the maximum measurements of packed cell volume of protozoa should be used. This is still a minimum value for the protozoan biomass since other studies from these laboratories have shown that point samples taken in this way consistently underestimate the true population density when these are measured in mixed rumen fluid from slaughtered animals (Minor et al 1977b). The values reported here are therefore to be considered as relative values only.

The overall results suggest that there are distinct phases in substrate utilization. Shortly after feeding, the protozoa are apparently actively storing starch from soluble sugars and presumably bacteria are fermenting soluble sugars quite rapidly. The decrease in acetate and the increase in propionate shortly after feeding is therefore probably related to bacterial fermentation of soluble sugars. The large holotrich protozoa are thought to produce acetic, butyric and lactic acids (Hungate 1966), and therefore the second phase in which acetate rises to original levels before feeding is probably largely due to fermentation of stored starch by protozoa with secondary fermentation of lactic acid by bacteria to a mixture of VFA. With depletion of sugar in solution and of stored starch in protozoa, cellulose digestion which is minimal, increases in importance as indicated by higher acetate proportions with time. This concept implies that a succession of microbial communities are developing and becoming of increasing importance. Possibly all three processes can occur simultaneously provided the availability of rumen ammonia is high. Ammonia levels were extremely high throughout the 24 h period examined and were in excess of that required for instance for maximum starch utilization (see 0rskov 1976).

The main points brought out by this study are that there appear to be no differences in protozoan biomass or any other parameter in the rumen of cattle given sugar cane diets with or without rice polishings. The high values of protozoan biomass appear to be maintained by high and constant pH values. Since little change occurs in rumen fermentation, this suggests that the effect of rice polishings on animal performance is possibly mediated directly through provision of essential nutrients for absorption.

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References

Clarke R T J 1965 NZ J Agric Res 8:1
Donefer E, James L A & Laurie C K 1975 Use of sugar cane-derived feedstock for livestock Proc III Wld Conf Anim Prod Melbourne,Australia
Eadie J H & Mann S 0 1970 Development of the rumen microbial population: high starch diets and instability in: -Physiology of Digestion and Metabolism in the Ruminant (ed A T Phillipson), p 406, Oriel Press: Newcastle-upon Tyne
Ferreiro H M and Preston R R 1977 Digestibility and voluntary intake of derinded sugar cane stalk with and without addition of cane tops Trop Anim Prod 2: 90-99
Ferreiro H M, Sutherland T M & Preston T R 1977 Brix and dry matter content as indices of urea requirements in diets based on sugar cane Trop Anim Prod 2: 213-218
Noldeman L V & Moore W E C 1972 Anaerobic laboratory manual, V P I anaerobic Laboratory Blackburg, Virginia)
Hungate R E 1966 The rumen and its microbes Academic Press: New York
Leng R A 1976 In "Prom plant to animal protein" (ed Sutherland T M, McWilliam J M &
Leng R A) Rev Rural Sc II p 84 Univ New England Press: Armidale Australia
Leng R A & Preston T R 1976 Sugar cane for cattle production: Present constraints, Perspectives and Research Priorities. Trop Anim Prod 1:1-22
Lopez J M, Preston T R, Sutherland T M & Wilson A 1976 Rice polishings as a supplement in sugar cane diets: effect of level of rice polishings in wet and dry season conditions - Trop Anim Prod 1: 164-171
Minor S, MacLeod N A, Preston T R & Leng R A 1977b Effect of sampling by fistula or at slaughter on estimation of rumen protozoa Trop Anim Prod 2: 62-67
Minor S, MacLeod N A & Preston T R & Leng R A 1977a Digestive tract parameters in bulls fed sugar cane, urea and different protein supplements Trop Anim Prod 2: 163-174
Montpellier F A & Preston T R 1977a Digestibility of tops, rind, derinded stalk and the entire plant of sugar cane Trop Anim Prod 2: 13-17
Montpellier F A & Preston T R 1977b Digestibility and voluntary intake on sugar cane diets: effect of chopping the cane stalk in particles of different sizes Trop Anim Prod 2: 40-43
Orskov E R 1976 In from plant to animal protein Rev Rural Sc II p 123 (ed Sutherland T M McWilliam J M & Leng R A) Univ New England Press: Armidale
Preston T R & Willis M B, 1974 Intensive Beef Production 2nd edition Pergamon: Oxford
Preston T R, Carcano C, Alvarez F J & Gutierrez D G 1976 Rice polishings as a supplementing a sugar cane diet: effect of level of rice polishings and of processing the sugar cane by derinding or chopping Trop Anim Prod 1: 150-161
Preston T R 1977 Nutritive value of sugar cane for ruminants Trop Anim Prod 2: 125-142
Priego A & Leng R A 1976 Ruminal Contractions in Cattle Fed Sugar Cane Trop Anim Prod
Satter L D & Roffler R E 1977 Protein requirement and non protein nitrogen utilization Trop Anim Prod 2: 238-259
Silvestre R, MacLeod N A Preston T R 1977 Supplementation of sugar cane/urea for growing cattle effect of maize grain and different levels and sources of protein Trop Anim Prod
Yeller R A & Pilgrim A F 1974 Br J Nutr 32: 341
Warner A C I 1965 In Physiology of Digestion in the Ruminant Butterworths: London

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