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MICROBIOLOGY OF THE RUMEN IN RELATION TO THE NUTRITION AND PHYSIOLOGY OF THE ANIMAL

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The ruminant is a symbiotic association between mammal and micro-organisms which has evolved to enable the animal to live on high fibre diets. In its natural habitat the ruminant has the advantage over a monogastric animal, but under intensive farming the rumen micro-organisms can be a liability.

The animal provides the rumen micro-organisms with the following facilities:

A means of gathering and selecting food.

Maceration and mixing of food.

A large fermentation vat.

Temperature control.

pH Control through bicarbonate and phosphate in the saliva.

Additional nutrients, e.g. urea, phosphate, in the saliva or through the ruminal wall.

Removal of inhibitory, soluble end-products.

Removal of undigested solid residues.

Removal of gas.

The anaerobic conditions are provided by the bacteria themselves.

The animal and the farmer can only control the activities of the rumen bacteria in so far as they can vary these facilities. For the rest the micro-organisms are governed only by their growth physiology and by competition and synergism between species.

In return the bacteria provide the animal with enzymes for hydrolysing cellulose and hemicellulose. This is the whole reason for the symbiosis, and consequently the cellulose- and hemicellulose-digesting bacteria are functionally the most important groups in the rumen, although they may form only a small proportion of the total microflora. On all-roughage diets the other species are largely dependent on the fibre-digesters for their energy supply and may be regarded as satellites. Thus the ruminal micro-organisms may be divided into functional groups. This constitutes the grouping together of all the different species capable of fermenting any given substrate in the diet, or one derived from it, like cellulose, starch or lactic acid. It is not a grouping in the classical bacteriological sense, and overlapping of species between groups can occur where the different species possess the enzymes to ferment more than one substrate. It does, however, provide a means whereby the amount of any given substrate in the diet and the numbers of the different species of ruminal micro-organisms fermenting it, can be compared.

Because of the importance of the fibre digesters, the Digestion and Metabolism Research (DMR) Unit has up to now put most of its effort into studying the cellulolytic bacteria of the rumen, and has been interested in the satellites only in so far as they aid or compete with the cellulolytics. Unfortunately the bacteria do not stop at hydrolysing the polysaccharides to monosaccharides, but ferment these further. Thus the animal itself normally gets a negligible amount of preformed carbohydrate from its diet, and has to synthesize its glucose requirements from propionic acid and protein. This process is wasteful of energy, but the waste is tolerable if the energy comes from cellulose or hemicellulose and could not have been obtained in any other way. Even where the energy comes from starch and sucrose, the fermentation of these substrates by the ruminal micro-organisms is an essential if wasteful process, because it has recently been shown (Ørskov, 1972) that the capacity of the ruminant animal itself to use starch and sucrose is limited.

Similarly the rumen bacteria break down dietary protein to ammonia and volatile fatty acids, which are used to resynthesize bacterial protein. On a low-protein ration this process can be turned to the advantage of the animal because the bacteria can use its waste nitrogen, which recycled to the rumen, to synthesize more protein than was in the diet. In the same way non-protein nitrogen supplements can be converted into protein. With high protein diets, however, the process is wasteful of both nitrogen and energy since the resynthesis of 100 g of protein requires approximately 16 moles of ATP or 200 Kcals. Moreover, the rate at which the animal can obtain protein is limited by the rate at which the bacteria can synthesize it, i.e. by their growth rate.

In this paper we shall attempt to examine the interactions between the animal and its micro-organisms to see how the microflora can be manipulated to give maximum productivity of the animal most economically.

It has been shown (Balch & Campling, 1962, Campling, 1970) that the voluntary intake of animals fed long roughages is related to the capacity of the reticulo-rumen, and to the rate of disappearance of digesta from it. The first depends on the animal, the latter partly on mastication by the animal, but mainly on the activities of the cellulose- and hemicellulose-digesting bacteria in the rumen. Purser & Moir (1966) showed that the propensity of sheep to eat was related to the volume of their rumens. We have also selected animals which will be good eaters of poor quality roughage for our microbiological studies, on the

*Abbreviated in text to DMR Unit

Table 1

Influence of cellulotytic bacteria on consumption and digestion of teff hay diets

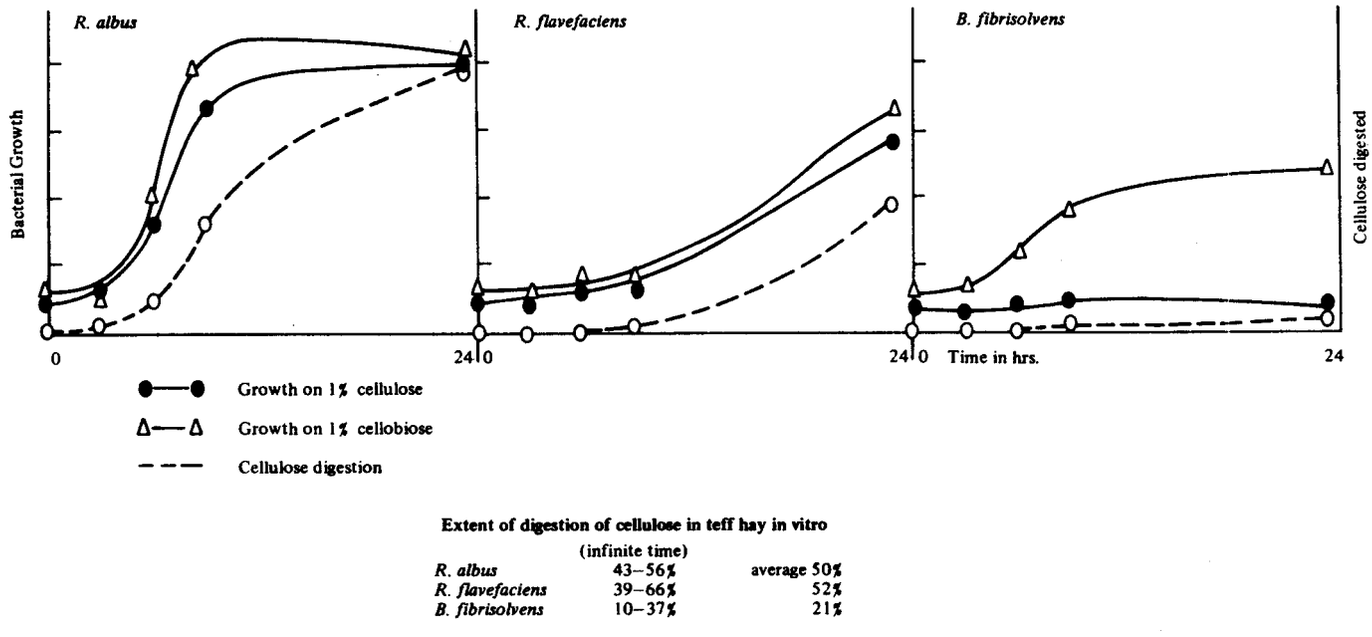
Sheep fed 1200 g teff hay (3,7% C.P.) sprayed with supplement

Supplement	No. of cellulolytic bacteria (millions/ml)	Types of cellulolytic bacteria		Hay consumption (g/day)	Cellulose digested		Hemicellulose digested		Wt. loss over 12 weeks %
					%	g/day	%	g/day	
None	41	Butyrivibrios	31%	440	57	78	45	75	19
		Ruminococci	67%						
3% Urea	39	Butyrivibrios	9%	901	72	188	64	219	8
		Ruminococci	87%						
3% Urea + 1,4% BCFA *	98	Butyrivibrios	3%	1015	72	219	67	276	4
		Ruminococci	94%						

* BCFA = Mixture of *iso*butyric, *iso*valeric, 2-methyl butyric and *n*-valeric acids

basis of their having a body conformation which will allow a large rumen capacity, i.e. animals with a large barrel-like ribcage. The extent to which we have succeeded has convinced us that this is a factor to which breeders should pay some attention, particularly in the case of wool sheep, since the present trend in wool prices is such that they can only be run economically on the veld.

The importance of the bacteria in controlling intake is best shown by the results of the work of Van Gylswyk (1970) which is summarized in Table 1. He fed a group of sheep first poor teff hay (3,7% crude protein), then the same hay sprayed with urea, and finally the hay sprayed with urea and branched chain volatile fatty acids (BCFA). With the unsupplemented hay the growth of the cellulolytic bacteria in the rumen was limited by the shortage of nitrogen. When this limitation was removed by adding urea, the greater proliferation of the fibre-digesting bacteria enabled the animals to increase their daily intake by 40%. This bacterial proliferation did not, however, show up as an increase in the numbers of cellulolytic bacteria in the rumen. Simply counting bacteria in the rumen is not a good measure of their multiplication, because the bacteria pass con-



Extent of digestion of cellulose in teff hay in vitro

	(infinite time)	
R. albus	43–56%	average 50%
R. flavefaciens	39–66%	52%
B. fibrisolvens	10–37%	21%

Fig. 1. – *Rate of cellulose digestion by different species of cellulolytic bacteria in* vitro

tinuously out of the rumen with the digesta, and the faster the cellylolytic bacteria grow and digest cellulose, the faster the passage of digesta. To determine rate of growth of bacterial in the rumen you must thus multiply the numbers present by their rate of flow out of the rumen. Unfortunately in these experiments the flow of digesta was not measured, but we now do this in all our microbiological studies. With adequate nitrogen, the next factor limiting the multiplication of the cellulolytic bacteria was the availability of BCFA, which are essential for the growth of some species. When these were given together with urea, the numbers of cellulolytic bacteria more than doubled. In this case the sheep were eating virtually all the hay offered, so the flow of digesta was limited by the intake, with the result that the increased growth rate caused their numbers to rise in the rumen. These BCFA are normally derived from protein in the diet, and these results explain why you get a falling off in production if you replace more than a certain percentage of protein by urea. With protein the BCFA do not become limiting; with high levels of urea, they may.

More important than the increased growth of cellulolytic bacteria was the change in predominant types. Supplementation favoured the growth of *Ruminococcus albus* and *R. flavefaciens* to a greater extent than that of *Butyrivibrio fibrisolvens*. As the numbers of ruminococci increased, so did the amount of cellulose digested per day. Studies of the cellulolytic activity of the different species in pure culture showed the reason for this. As can be seen in Figure 1 the ruminococci grow rapidly on cellulose and degrade it equally rapidly. By contrast *B. fibrisolvens* grows much more slowly on cellulose than it does on simpler sugars, and even with long incubation periods cannot break down cellulose to the same extent as the ruminococci (Van Gylswyk & Labuschagne, 1971; Koch & Kistner, 1969). It is thus a much less effective cellulolytic organism than the ruminococci.

Since the type of cellulose-digesting bacteria in the rumen can have such a profound effect of fibre digestibility and animal performance, one may digress a moment to consider the time required for a new population pattern to establish itself after a change of diet. Gouws & Kistner (1965) studied this for the change – over from poor teff hay to lucerne hay and vice versa. The predominant cellulolytic bacteria in the rumen of sheep fed lucerne are ruminococci, and these overgrew the butyrivibrios, which were present in large numbers when teff was fed, within a week. However, when the dietary change was made in the

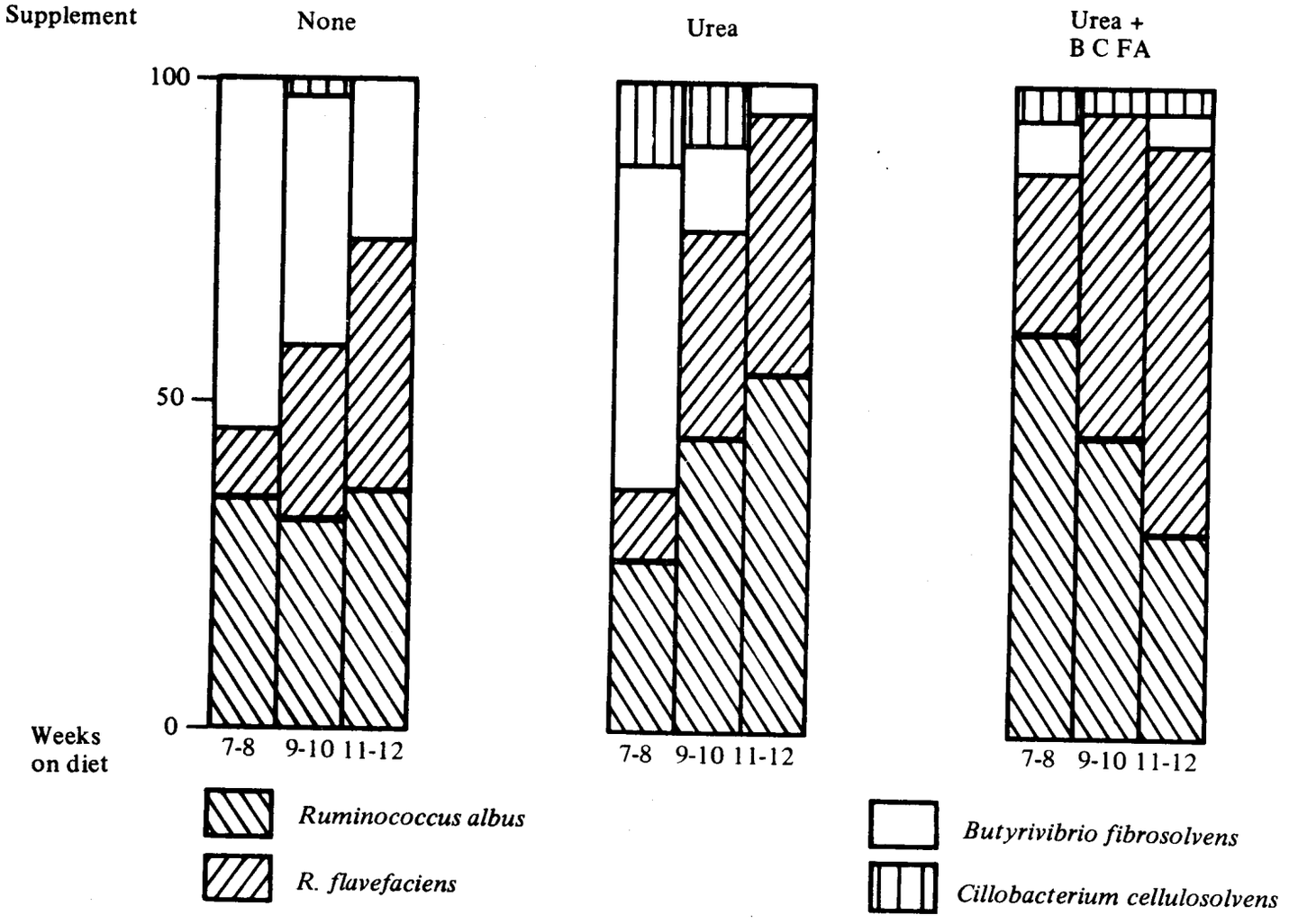


Fig. 2. – *Relative proportions of cellulolytic bacteria in rumen of sheep fed poor teff hay and supplements*

opposite direction, it took about 4 weeks for the butyrivibrios to re-establish themselves in two of the three sheep studied, while in the third animal they had not come up even after 5 weeks. In view of these results we now allow 6 weeks for our sheep to adapt to a new diet before starting to examine the microbiology of the rumen. By contrast, digestion trials are commonly carried out after a 10 day preliminary feeding period (Stielau, 1960), and recently a study was published (Ishaque, Thomas & Rook, 1971) where measurements of carbohydrate and nitrogen metabolism in the rumen were made after sheep were allowed "not less than 6 days" to adapt to a change from a diet of long hay to one containing 80% concentrates plus 20% ground hay. Not surprisingly the authors found large differences between the parameters in different sheep and in the same sheep at different times, and we would like to stress the need to allow sufficient time, i.e. 6 weeks if possible, for the ruminal flora to adapt to a change in diet before attempting to take any observations. In making this review, we found that the amount of data which had to be disregarded on this account, from what were otherwise well-planned and critical experiments, most distressing. Even with a 6 week preliminary feeding period, Van Gylswyk (1968) found that the proportions of the different cellulolytic species varied with time as can be seen in Fig. 2. This was particularly marked when teff hay plus urea was fed, when the percentage of ruminococci increased from 37% to 95% between the 7th and 12th weeks. This may explain why some workers (for example Schaadt, Johnson & McClure, 1966; Ludwick, Fontenot & Tucker, 1971) have found long term adaptation of sheep to urea supplements. This is not adaptation to urea as such, because the micro-organisms are always able to hydrolyse endogenous urea which is recycled to the rumen. It is a gradual change in the ruminal flora to species which can make optimal use of the additional NH_3 provided. This phenomenon is not confined to NPN supplements. We found a similar trend when teff hay was supplemented with protein in the form of egg albumin.

To get maximal production on high roughage rations one must thus encourage the growth of the right cellulose-digesting bacteria. One can go so far as to say: **if you feed the cellulose-digesting bacteria correctly, they will feed the sheep.** In order to do this consciously one needs information not only on the absolute nutritional requirements of the various species, but also on the influence of the concentration of nutrients on their growth rates. A certain amount is known about the absolute nutrient requirements of the different cellulolytic rumen bacteria (Hungate, 1966), but there is little information about the effect of levels of nutrients on growth rates. This, however, is critical because an organism can only increase its numbers in the rumen if it can grow faster, in the nutrients available there, than the rate at which it is washed out of the rumen by the flow of digesta. For this reason Dr. Kistner and his co-workers in the DMR Unit have a major project on the study of the growth kinetics of cellulolytic rumen bacteria in continuous culture with either nitrogen, BCFA or carbohydrate as limiting substrates.

However, even with optimum conditions for the growth of cellulose-digesting bacteria in the rumen the amount of long roughage an animal can consume daily is limited, on the one hand by the inherent lowness of the enzymatic digestion of cellulose which in its native state is not very accessible to attack, and on the other hand by the effort the animal has to make to masticate it. Freer, Campling & Balch (1962), for example, found that their cows were spending up to 16 hours per day eating and ruminating when fed hay *ad lib.* Both these limitations may be avoided by grinding the ration. This permits the animal to eat more food, but normally results in lower digestibility of fibre because the food particles pass out of the rumen before the cellulolytic bacteria have had time to complete their job. However, this decrease is not as great as it might be because the grinding increases the surface available for bacterial attack and can expose cellulose and hemicellulose which would otherwise have been inaccessible to the enzymes because of encrustation with lignin. Thus the net result of grinding is a net increase in the intake of digestible matter. The markedly less time spent in eating and rumination saves energy, so the increase in productive energy is considerable (Weston & Hogan, 1967). Nevertheless, even the ground material still remains fibre which is more slowly fermented than readily available carbohydrates.

Thus where really high production is required, as in the case of milking cows, and the finishing off of slaughter animals, it becomes necessary to feed a more rapidly fermented source of energy, such as starch, present in grain, or sugars in molasses. The feeding of these with adequate nitrogen speeds up the production of volatile fatty acids and of microbial protein by encouraging the faster-growing satellite bacteria. When all-roughage diets are fed, these bacteria grow on the constituent units of plant fibre released by the cellulose and hemi-cellulose digesters which, in general, they actually out-number by a factor of 10 or more. Because of this, quite large amounts of starch can be added to roughage-based diets without upsetting the balance of the ruminal flora providing the additions are made gradually. This is to allow sufficient time for the different functional groups to adjust to the higher growth rate on the increased concentrations of substrate added directly to the diet, and thus no longer limited by the rate of fibre digestion.

Nothing like the detailed study of the ruminal micro-organisms which has been done in the DMR Unit for all-roughage diets has yet been carried out on diets containing concentrates. However, where microbial data are lacking, changes in the relative proportions of the ruminal fatty acids can be used as an indication of changes in the composition of the ruminal flora. On an all-roughage diet the volatile fatty acids are produced in the approximate molar proportions of 70 for acetic: 20 for propionic: and 10 for butyric acid; and these proportions can be maintained when concentrates are fed as will be seen later.

The introduction of starch or soluble sugars increases the rate at which these acids are produced so that a severe strain is imposed on the buffering capacity of the rumen;the more so because the reduced hay intake provides

Table 2

Functional ruminal flora and ruminal levels of lactic and volatile fatty acids produced from starch-containing rations

Dietary proportions (%) of		Ruminal flora (No./ml or g of Ruminal Contents)				Acids (m-equiv./1 of ruminal fluid)					References * *
Hay	Starch*	Protozoa	Bacteria			pH	lact.	acet.	prop.	but.	
		Ciliate	Cellulolytic	Amylolytic	Lactic utilizers						
100	0	10^5	10^7	10^7	10^8	6,5	<0,1	79	20	11	(2) (6) (7)
50	35	10^6	–	–	–	>6,0	<0,1	66	17	15	(5)
		10^6	10^9	10^9	–			71	18	20	(6)
27	60	–	–	–	–	6,3	1,5	68	22	8	(9)
0	80	0	10^7	10^9	10^9	5,5	<1	80	80	18	(4)
(to appetite)				*Bacteriodes*	*Veillonella*						
0	80	10^6	10^7	10^9	10^9	6,3	≤1	59	8	28	(1) (4) (5) (8)
(restricted)		*Entodinium*			*P. elsdenii*						
		Epidinium									
0	90	0	0	$>10^9$	0	≤4,0	333	0	0	0	(3)
(uncontrolled)				*Lactobacillus*							
				S. bovis							

*Starch calculated from % concentrates fed.

** 1. Abou Akkada & Howard (1960) 6. Giesecke *et al.* (1966)
2. Balch & Rowland (1957) 7. Gilchrist (1965)
3. Dirksen (1970) 8. Gutierrez & Davis (1962)
4. Eadie *et al.* (1967) 9. Phillipson (1952)
5. Eadie *et al.* (1970)

less stimulus for the secretion of saliva which is the main source of buffer salts. Thus there is a tendency for the pH of the rumen to fall below that of 6,5 to 6,8 associated with the all-roughage diet. Unlike the activity of the amylases which is not very sensitive to low pH, the activity of some of the enzymes involved in volatile fatty acid production slows down as the pH falls. Under these conditions there appears to be a shift in the rate limiting step from the hydrolysis of the starch to the formation of volatile fatty acids from the soluble sugars produced by the hydrolysis. This results in an accumulation of lactic acid, because the ruminal hydrogen, which is normally utilized in the production of volatile fatty acids, is passed on to pyruvic acid, a precursor of lactic acid (Walker, 1968).

Of all forms of starchy feeds, flaked maize is the most rapidly hydrolysed and is most often associated with the presence of lactic acid in the rumen. Lactic acid itself can be metabolized in the rumen if the pH does not fall too low. Large quantities of it in 70 lb (31,8 kg) of silage eaten daily by a cow were shown by Balch & Rowland (1957) to be completely fermented to acetic, propionic and butyric acids in molar proportions typical of an all-roughage diet. But it is accumulation of lactic acid in the rumen from 2,5 to 5 hours after feeding concentrates containing either starch or sugars which is an indication of a deranged carbohydrate fermentation and a harbinger of trouble.

This is clearly shown in Table 2. It can be seen that the pH (6,5) of the ruminal contents of animals on a 100% hay diet fed *ad libitum* is close to neutral despite high concentrations of acetic, propionic and butyric acids, but only negligible amounts of lactic acid. At this pH there are large numbers of ciliate protozoa (10^5) (Giesecke, Lawlor & Walser-Kärst, 1966), cellulolytic (10^7), amylolytic (10^7) and lactic acid-utilizing (10^8) rumen bacteria per millilitre of ruminal contents (Gilchrist, 1965). Giesecke *et al.* (1966) and Eadie, Hylgaard-Jensen, Mann, Reid & Whitelaw (1970) found that reducing the hay in the diet to 50% and adding 35% starch in the form of 43% mixed grains or rolled pelleted barley brought about a rise in the levels of all the functional groups examined with little or no change in the pH or the concentrations of acetic, propionic and lactic acid. A rise in the butyric acid concentration probably reflected the 10-fold rise in the level of starch-digesting entodiniomorph protozoa which persist at pH values below 6,5 and two genera of which (*Entodinium* and *Epidinium*) are known to produce butyric acid as a major product of starch digestion (Abou Akkada & Howard, 1960; Gutierrez & Davis, 1962). The 100-fold rise in the levels of cellulolytic as well as amylolytic bacteria despite the reduction of hay in the diet was probably due to the fact that the cellulolytic species were growing on the maltose and glucose produced from the starch, as some of them, particularly butyrivibrios as distinct from ruminococci mentioned earlier in connection with all-roughage diets, can do. The situation regarding pH and pattern of fermentation acids, at least, was

Table 3

Animal performance and ruminal bacteria of sheep fed poor teff hay ad lib. *and dosed with 10 g urea and 100 g sucrose as molasses*

Weeks on diet	Hay eaten g/day	Live wt. (lb)	Rumen pH	Counts (millions/ml of ruminal fluid)				
				Cellulose	Starch	Glucose	Xylose	Lactate
8	560	83	6,8	3	35	10	80	200
10	680	83,5	6,7	3	120	110	130	15
12	210	79,5	6,5	0,06	–	–	–	–
13	250	77,5	5,3	0,5	3000	6000	2500	5

found by Phillipson (1952) to remain unchanged even when the diet fed *ad libitum* contained only 27% of hay and 66% of starch as flaked maize. These would, however, appear to be critical proportions of these two dietary constituents, because 73 mequiv./l of lactic acid was found in the ruminal contents of young sheep when the hay was reduced to 15% and the flaked maize increased.

This does not mean that diets containing only concentrates can not be successfully fed to ruminants especially beef cattle. Eadie, Hobson & Mann (1967) showed that young steers thrive exceedingly well on a diet of cubed barley grain fed to appetite thrice daily. From the table it can be seen, that despite the strictest adherance to this regimen, which the authors claim to be essential to prevent pH control getting out of hand, the pH of the ruminal ingesta of these animals fell to 5,5. This was due to a surge in propionic acid production, resulting in a 4-fold increase in the concentration of this acid above that found in hay-fed animals. This low pH is inimical to the ciliate protozoa which disappeared and to the cellulolytic bacteria, the levels of which fell 100-fold. On the other hand, the levels of the less acid-sensitive lactic acid-utilizing bacteria and the amylolytic bacteria remained high (10^9/g), and particularly those of two genera *Bacteriodes* and *Veillonella* known to be highly active propionic acid producers (Kay & Hobson, 1963). From the data of Storry & Rook (1966) and Storry & Sutton (1969) it would appear that a rise in the rumen concentration of propionic acid above a critical level of about 30 mequiv./l is sufficient to trigger off the low milk-fat syndrome (Davis & Brown, 1970; Trenkle, 1970). Thus an all-concentrate diet fed in this way is not suitable for milking cows, since it is capable of producing up to 30 mequiv./l of ruminal propionic acid at least. It is, however, eminently suited to bringing ruminants into prime condition for slaughter.

On the other hand, if this same diet is fed at a restricted level of intake the situation is quite different. When Eadie *et al.* (1970) restricted the level of intake of the cubed barley diet by heifers thrice daily to 80% of appetence, the ruminal concentration of volatile fatty acids was only about half of that for the steers similarly fed but to 100% of appetence, and the ruminal pH could be maintained at 6,3 instead of falling to 5,5. This permitted the reappearance of high levels (10^6/g) of the cliate protozoa *Entodinium* and *Epidinium* which produce butyric acid, as well as high levels (10^9/g) of *Peptostreptococcus elsdenii* known to produce butyric acid by a reaction involving one molecule each of acetic and propionic acids and the loss of one molecule of carbon dioxide. This resulted in a completely new pattern of ruminal volatile fatty acids, with exceptionally low concentrations of propionic and high concentrations of butyric, while those of acetic changed the least. Since many workers (Rook & Balch, 1961; Bergman, Reid, Murray, Brockway & Whitelaw, 1965) found butyric acid to be as effective a precursor of milk-fat as acetic the diet managed in this way could be profitably fed to milking-cows.

Uncontrolled intake of starch concentrates under conditions of inadequate nitrogen intake leads eventually in acute cases to a marked fall in rumen pH to 4,0 or lower due to large accumulations of lactic acid, and the disappearance of almost all the ruminal flora except for the amylolytic bacteria like *Lactobacillus spp* and *Streptococcus bovis*. The animal refuses to eat, particularly hay. The rumen acidiosis brings about a cessation of ruminal movements and a metabolic acidiosis of the animal itself which dies if not appropriately treated (Dirksen, 1969). We (Gilchrist, 1965) observed the different stages of this process in a case of chronic acidosis brought on over 13 weeks in a sheep given an inadequate nitrogen diet consisting of poor teff hay (4,6% C.P.) *ad libitum* and a daily dose of 10 g of urea with 100 g of sucrose in the form of molasses *per fistulam*. Table 3 shows that from the eighth to the thirteenth week there was a 100-fold increase in the levels of the fast-growing amylolytic and other potential lactic acid-producing bacteria, which was accompanied by a steady fall in the pH of the ruminal contents form 6,8 to 5,3. These fast-growers would have first chance at what dietary nitrogen there was available, leaving the slower-growing cellulolytic and lactic acid-utilizing bacteria in a nitrogen-limited condition. This and the fact that these two functional groups also tend to be acid-sensitive, would slow down their growth rate even more, so that their levels fell 50– and 40-fold respectively, during that period when the levels of the fast-growing starch and sugar fermenters increased. These changes in the ruminal flora were accompanied by a fall in hay consumption which roughly paralleled that of the cellulose digesters, and a loss of live weight by the animal amounting to 6,5 lb (3 kg) in 5 weeks.

In conclusion, we would like to make a strong plea that you always bear in mind that a ruminant has a rumen full of micro-organisms. These have the first go at any food that you feed to the animal. Therefore when you make up your diet and plan your dietary regimen, you must think of what the micro-organisms will do with the food you give them, and what they will pass on to the animal. For only if you can get the bacteria to co-operate with you, will you get the maximum of animal production per unit of feed. If you ignore the ruminal micro-organisms and get them up against you, they will waste your money which you put into the feed, and at worst, kill your animals.

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