THE ROLE OF RUMEN PROTOZOA IN THE METABOLISM OF RUMINANTS GIVEN TROPICAL FEEDS[1]

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The interrelationships between the rumen ciliate protozoa and the host animal are reviewed and various aspects of importance are discussed. These include the distribution of protozoa and the effect of protozoa on growth, rumen and blood metabolises and bacteria. Variations in rumen protozoa! populations are discussed in relation to diet and interrelationships between protozoa! species and the quantitative contribution of protozoa to the overall nutrition of ruminants is reviewed.

KEY WORDS: Review, ruminants, metabolism, protozoa.

The purpose of this article is to review the interrelationship between the rumen ciliate protozoa and the host animal and to emphasise those aspects which are of importance to the investigation of rumen metabolism. Readers who require detailed information on the cultivation of Entodiniomorphid protozoa should consult Coleman (1978) and on their detailed physiology and biochemistry, Coleman (1979, 1980a) where references to original papers are given.

In ruminants maintained on the diets co only fed in temperate climates e.g grass, hay and concentrates, the level of soluble sugars in the rumen tends to be low (Phillipson & McAnally 1942) and the food materials available to the protozoa principally particulate e.g starch grains, cellulose fibres and bacteria. In contrast, in animals fed on tropical feeds such as molasses (Preston & Willis 1974) or sugar cane (Valdez et al 1977) supplemented in various ways, there is likely to be a much higher level of free sugars in the rumen especially where the molasses is continuously available. As the holotrich protozoa utilize soluble sugars much more readily than Entodiniomorphid protozoa (Heard & Oxford, 1953), the former are likely to be of greater importance than the latter on these tropical feeds and this has been found in practice (Valdez et al 1977).

DISTRIBUTION OF PROTOZOA

In the author's experience sheep on farms in Great Britain all contain holotrich protozoa and a wide range of Entodiniomorphid protozoa. However, this is not true in other parts of the world e.g there were no holotrich protozoa in the sheep used in Western Australia by Purser & Moir (1959); no *Polyplastron* or *Ophryoscolex* spp. in

New Zealand cows (Clarke 1964) and no Polyplastron spp. in Indian cows (Kofoid & MacLennan 1932). There are also variations over much smaller distances. For example the author found no holotrich protozoa in cattle at the Colegio Superior Agricultura Tropical at Cardenas, Tabasco or in the animals fed on molasses at the Escuela de Medicina Veterinaria at Merida, Yucatan although they were present in animals from the slaughter house in Merida. Similarly all the experimental animals at Cardenas and Merida contained no large Entodiniomorphid protozoa although these were present in slaughter house animals. This suggests that at some time these protozoa must have been 'lost' from the experimental animals possibly due to acidosis or some other trauma. The absence of holotrich protozoa from animals fed diets rich in free sugars could have dramatic effects on rumen metabolism as an important group of protozoa responsible for the removal of these sugars are then absent. Although in Great Britain it is normally safe to assume that holotrich and Entodiniomorphid (A or B-type population - see below) protozoa will always be present, this is obviously not true elsewhere. The author urges all workers in this field to check that the protozoa! populations present in their sheep are 'normal' and, if not, to inoculate their animals with rumen contents from animals containing the missing protozoa. If this is not done different groups of workers could obtain different results although nominally doing the same experiment under the same conditions.

INFECTION OF YOUNG RUMINANTS WITH PROTOZOA

There is no resistant phase in the life cycle of any of the rumen ciliate protozoa (Strelkow et al 1933) and a young or adult animal can only be infected by direct or very close contact with an infected animal. Young animals kept isolated from normal adult animals never become infected. Ciliate protozoa are not essential for the life of a young or adult ruminant although they are normally present in all such animals on farms or in the wild (Becker et al 1929; Giesecke 1970). *Entodinium* spp. which are the smallest of the rumen ciliates are always acquired first by young ruminants (usually at the time rumination begins), followed by the holotrich and larger Entodiniomorphid protozoa (Eadie 1962a) although some adult sheep, kept under normal farm conditions at Babraham and mixing freely with infected animals, never become infected with these larger protozoa. Contrary to the commonly held belief (Hungate 1966) these large protozoa, at least, are probably acquired by young ruminants from the flock or herd at large rather than just from the mother animal (Coleman & Kemp quoted by Coleman 1980a).

EFFECT OF CILIATE PROTOZOA ON THE GROWTH OF RUMINANTS

There have been many studies over the past 50 years on the effect of the presence or absence of rumen ciliates on the growth of young ruminants maintained on conventional temperate diets such as hay and concentrates. Three of these (Christiansen et al 1965; Abou Akkada & El-Shazly 1964; Borhami et al 1967) showed that there could be an increase of up to 30% in rate of weight gain of faunated compared with protozoa-free animals. Nine other studies (including e.g Eadie & Gill 1971; Pounden & Hibbs 1950;and Williams & Dinusson 1973) showed that there was

no difference in the rate of growth of these two groups of animals although Pounden & Hibbs (1950) found that protozoa-free animals had rougher coats and both these authors and Eadie & Gill (1971) showed that protozoa-free animals were pot-bellied compared with faunated animals.

Recently there have been investigations on the effect of protozoa and of by-pass protein on the growth of young ruminants fed on the lowprotein/high energy diets which are used in the tropics. In the experiments on cattle (Bird & Leng 1978) defaunation had no effect on the growth rate of animals fed molasses/straw but increased both the rate of growth (43%) and the feed conversion ratio (39%) when bypass protein was also fed. Conversely in experiments on lambs (Bird et al 1978; 1979) faunated animals fed only on the basal diet of sugar/chaff lost weight whereas defaunated animals gained weight. Not until there was 10% of bypass protein (fishmeal) in the diet was the rate of weight gain higher in faunated animals. These latter results suggest that the protozoa destroy (for possible mechanism - see below) some of the limited amount of protein in this low protein diet. Why defaunation should enhance the benefits of bypass protein supplementation in cattle is not clear.

EFFECT OF RUMEN CILIATES ON RUMEN AND BLOOD METABOLITES AND BACTERIA

In faunated animals the bacterial population density is lower (Eadie & Gill 1971; Eadie & Hobson 1962; Kurihara et al 1968; 1978) and the level of ammonia higher (Christiansen et al 1965; Borhami et al 1967; Klopfenstein et al 1966; Males & Purser 1970) than in protozoa free animals. Klopfenstein et al (1966) also found higher plasma amino acid concentrations in faunated animals and produced evidence that the limiting amino acid in protozoa-free animals was lysine: no one amino acid was consistently limiting in faunated animals. Abou Akkada & El-Shazly (1965) also found higher blood haemoglobin and protein levels in faunated animals whereas Christiansen et al (1965) found no increased nitrogen retention in faunated animals.

There is no clear effect of faunation on the levels of volatile fatty acids in the rumen. In some experiments, the level of these fatty acids increased on faunation (Abou Akkada & El-Shazly 1964; Kurihara et al 1968; Borhami et al 1967) whereas in others it was unchanged (Williams & Dinusson 1973) or decreased (Males & Purser 1970). The effect of protozoa on the relative proportions of acetate, propionate and butyrate was also variable with decreased (Abou Akkada & El-Shazly 1964) or increased (Youssef & Allen 1968, Eadie & Mann 1970) acetate/propionate ratio and decreased (Youssef & Allen 1968) or increased (Bird & Leng 1978) acetate/butyrate ratio on faunation. These differences suggest that other factors such as the ration (Eadie & Mann 1970), the species of protozoa present and the age of the animal may be important. Leng (1976) states that on a high starch ration, faunation changes a high propionate fermentation to a high acetate/butyrate fermentation.

EFFECT OF DIET ON PROTOZOAL POPULATION DENSITIES

When large amounts of starch are fed to a ruminant without a period of adaptation during which increasing amounts are gradually fed, the pH of the rumen drops rapidly due to the production of lactic acid, and all the protozoa are killed (Ryan 1964, Eadie et al 1967). In less extreme cases where the pH drop is less severe, the Entodinium spp. and Isotricha spp. are the last protozoa to disappear (Eadie 1962a) and of these the small entodinia are the most resistant (Abou Akkada et al 1959). The number of protozoa also decreases rapidly during starvation of the animal (no protozoa visible after 4 days starvation - Warner 1962) and this is why the rumen contents of animals taken from commercial slaughter houses often contain no protozoa.

Under the normal feeding regimes used in temperate climates, the number of Entodiniomorphid protozoa (principally *Entodinium* spp.) increases as the proportion of concentrate in the diet increases (Abe et al 1973) until the maximum population density occurs when concentrates are fed to 80% of appetite (Eadie et al 1970, Christiansen et al 1964). However, the physical form of the ration is also important and Christiansen et al (1964) found that with pelleted or reground pelleted rations all the protozoa disappeared and suggested that this could be due to the increased rate of passage of the digesta.

In a basal ration of wheaten chaff, alfalfa and starch, linseed or linseed oil decreased the protozoa! population while the addition of gluten or urea increased the population of all protozoa! types (Purser & Moir 1966). Reichl (1961) also found a positive correlation between the protozoa! population density and the content of nitrogen-free compounds in the diet and a negative correlation with the protein content.

The population density of the holotrich protozoa does not always follow that of the Entodiniomorphid protozoa (Eadie et al 1970) and often increases when the total ciliate population is decreasing. On a typical temperate diet, a decrease in feed intake and water consumption tends to increase the number of holotrich protozoa (Dehority & Purser 1970). The number of holotrich protozoa is high in animals fed on diets rich in free sugar such as beet (Oxford 1955), molasses (Boodoo et al 1978) or sugar cane (Valdez et al 1977) but little is known about the effect on the protozoa of gradually increasing amounts of e.g sugar cane in the diet. Appreciable numbers of entodinia (approx. 105/ml) are also present in animals fed on sugar cane (Valdez et al 1977) and molasses (Coleman unpublished observations) diets. Under these conditions the entodinia do not become full of storage material as would occur if starch were fed, suggesting that glucose can be utilized to provide energy but not for the rapid synthesis of polysaccharide although 14C-glucose is incorporated into-this material (Coleman 1969b: Coleman & Hall 1974: Coleman & Laurie 1976).

Addition of certain chemicals to the diet increases the numbers of rumen ciliates, Aureomycin and tyloein increase the number of protozoa but not bacteria (Klopfenstein et al 1964; Purser et al 19653. Diethylstilboestrol in the ration may increase (Christiansen et al 1964; Ibrahim et al 1970) or have no effect (Dearth et al 1974) on the protozoa! population but has no effect when implanted in the animal (Slyter et al 1970). Where diethylstilboestrol is stimulatory it appears to have a selective effect on the *Isotricha* spp. and the larger Entoniniomorphid protozoa, the

relative abundance of the *Entodinium* spp. decreasing slightly (Ibrahim et al 1970). In contrast methionine hydroxy analogue in the diet specifically increased the numbers of entodinia, the other species tending to decrease in numbers (de Vuyst et al 1975) while Patton et al (1970) found a ten fold increase in protozoa! numbers on adding this compound to hay, silage and grain rations. On purified dietsβ-sitosterol is also stimulatory (Hino & Kametaka 1974). Many antimicrobial compounds decrease the rumen ciliate population density but these will not be considered in detail except to note that monensin decreases the numbers of protozoa and increases the numbers of bacteria(Jouany & Senaud 1978).

DIURNAL VARIATION IN PROTOZOAL POPULATION DENSITY

Sampling rumen contents. The validity of the results of all experiments on the numbers of protozoa in the rumen, but especially those on diurnal variation, where the changes in population density are relatively small, depend upon the accuracy of the sampling method. In ruminants fed 'dry' temperate climate materials such as hay, grass and concentrates, the rumen contents are not homogeneous and have a crust of solid matter overlaying liquid. Protozoa may be sequestered in this crust but the author considers it unlikely that, in animals on these diets in England, protozoa sink to the bottom of the liquid layer because it is necessary to centrifuge rumen contents at over 300g for 5 min to sediment the small entodinia which comprise over 70% of the population. This view is supported by the observations of Purser & Moir (1959) and Boyne et al (1957) who found no difference in the ciliate population densities between the top and bottom of the rumen. In contrast in animals fed on sugar cane where the predominant ciliates were holotrichs, Minor et al (1977) showed that the protozoa tended to sink to the bottom of the rumen and that counts on samples taken via the rumen cannula were 25% lower than those obtained after killing the animal and mixing the rumen contents. This phenomenon has been ascribed to a combination of heavy starch-filled holotrichs naturally tending to sink rapidly especially in animals fed a partly liquid diet (molasses) and the finding, that, in the absence of substrate, holotrich protozoa tend to 'flock' together and sink (Valdez et al 1977).

Regardless of the problems involved in sampling it must be remembered that Boyne et al (1957) showed that there was a three fold day-to-day variation in the numbers of ciliates in one animal kept on a constant ration under constant conditions.

Protozoal populations in vivo. In animals fed once daily on conventional temperate rations, the population density of all Entodiniomorphid protozoa decreases after feeding (for 4-16h) and then increases slowly or rapidly (depending on the species) until the pre-feeding level is reached (Warner 1962, 1966a; Purser & Moir 1959; Senaud et al 1973; Michalowski 1977). The number of dividing protozoa usually increases directly after feeding and Warner (1966a) was able to explain the observed changes in the population densities of Entodintomorphid protozoa on the basis of the division rate (calculated from the number of dividing protozoa) and the dilution rate of the rumen contents.

In contrast Warner (1966a) and Michalowski (1977) both found that the numbers of holotrichs decreased steadily for 12-20h after feeding and that no division forms were present during this period. This decline in numbers was followed by a rapid increase (over 4-6h) in the number of holotrichs (10% observed to be dividing) until the original population density was reached (Warner 1966a; Purser 1961; Michalowski & Muszynski 1978). However Clarke (1965 - using cows fed on red clover) and Valdez et al (1979 - using cattle fed on sugar cane) found an increase (3 fold) in the number of both Dasytricha ruminantium and Isotricha spp. in the first 2h after feeding and this was followed by a decline in numbers to the original level or below over the next 2-10h. Clarke (1965) explained the decrease in the number of holotrichs as being due to their bursting because of excessive synthesis of intracellular starch while Valdez et al (1977) believed the almost complete disappearance of holotrichs observed in their experiments was due to settling of these protozoa to the bottom of the rumen. The author does not favour the first explanation because it now seems probable (Van Hoven & Prins 1977; Prins & Van Hoven 1977) that this bursting phenomenon is an artifact induced by artificial conditions in vitro. The second explanation should be easily amenable to investigation by use of a sheep where it is relatively easy to obtain samples from all parts of the rumen. The reason why the holotrich protozoa show these different behaviour patterns in the different experiments is not clear although the difference between them may be illusory. It appears to the author that the increase in the number of holotrichs always begins before feeding and continues during feeding, which will be from 2h in animals fed hay and concentrates to 10h in animals fed sugar cane ad lib, and always begins to decline when feeding ceases.

In animals fed a proportion of their ration every 3or 12h the rhythm of change in protozoa! population density was the same within these periods as over the 24h period (Warner 1966b, Michalowski & Muszynski 1978) although the mean number of protozoa was 30% higher in animals fed twice rather than once a day. In animals on pasture the pattern was that of animals fed once a day as left to themselves animals tend to feed at one time of day (Warner 1966c)

INTERRELATIONSHIPS BETWEEN PROTOZOA

In sheep in Great Britain there are three types of protozoa! population: type O is characterised by the presence of *Entodinium* spp. and holotrich protozoa only, and types A and B which are like type O with the additional presence of *Polyplastron multivesiculatum* and *Diploplastron* affine (type A) or *Epidinium* spp., *Eudiplodinium maggii* and *Eremoplastron bovis* (type B) (Eadie 1962b). These populations are quite stable in individuals in flocks of sheep and in a flock of 5 year old ewes at Babraham in 1975, 16 had type B populations, 10 type A and 2 type 0. Although the populations are quite stable in normal farm animals they can be very easily upset in animals with a rumen cannula by the careless use of a contaminated sampling tube. The introduction of type A or type B protozoa into a rumen containing no large Entodiniomorphid protozoa (i,e type O) will rapidly convert the population into the corresponding type, The introduction of *Polyplastron multivesciculatum* (in type A) into a rumen containing type B protozoa results in the death of the B-ciliates and their replacement (in 7-11 days) by the type A protozoa. During this changeover the *Polyplastron*

multivesiculatum increases in size and engulfs firstly all the epidinia and then the *Eudiplodinium maggii* until all have disappeared (Eadie 1962b, 1967). However in calves the E.maggii also increases in size and it is possible for this and P. multivesiculatum to exist together. The holotrich protozoa and the smaller*Entodinium* spp. are not involved in the changeover. In vitro P.multivesiculatum only grows well in the presence of epidinia which are engulfed at the rate of 2/day: the maximum rate of engulfment is 10 epidinia /*P.multivesiculatum*/day (Coleman et al 1972). Certain other protozoa such as *Eudiplodinium maggii* and *Diplodinium monacanthum* are engulfed and will support limited growth of *P.multivesiculatum* but *Entodinium* spp. will not support growth and are not engulfed. As epidinia unlike entodinia have bacteria attached to their surface, it is possible that *P.multivesiculatum* recognises epidinia but not entodinia as bacteria and engulfs the former but not the latter.

   Entodinium bursa and *Entodinium caudatum. E.caudatum* which is a common member of the rumen ciliate population is characterised by the presence of two short and one long caudal spine. However on inoculation into medium in vitro (Coleman 1960) or into a protozoa-free rumen, this protozoon grows well but gradually loses its caudal spines. This change has been permanent during 20 years cultivation in vitro. *Entodinium* bursa, which also occurs commonly in the rumen, will only grow in vitro in the presence of the spineless form of *E.caudatum* which is engulfed at a rate of over 2/h (Coleman et al 1977). On growth of an excess of *E.caudatum* in the presence of *E. bursa*, the former gradually develops caudal spines over the course of 2-3 weeks. As *E.caudatu*m is engulfed posterior end first, the presence of the caudal spines is a good defence mechanism and such spined protozoa are engulfed much less readily. *E.caudatum* also possesses a second defence mechanism against engulfment in that it increases in size in the presence of *E.bursa* until it is too large to be taken up (Coleman et al 1977).

   Entodinium bursa and the spined form of *E.caudatum* will grow together as the only ciliate protozoa in the rumen of a sheep (Coleman 1980b). Under these conditions there is a cyclic (4-6 days) rise and fall (2-10 fold difference between maximum and minimum) in the population density
of *E.caudatum* associated with smaller and irregular fluctuations in the numbers of *E.bursa*. The changes in the population of *E.caudatum* are not associated with changes in spinatation but with small changes in size the length of the protozoon decreasing slightly when the numbers are increasing and increasing when the numbers are decreasing. It is not known if the changes in the population density of *E.caudatum* are due to engulfment by *E.bursa* or to changes in the rate at which the protozoa pass from the reticulum into the omasum. If this latter explanation is correct then measurements of rate of passage of protozoa made over just one day could give a false estimate of the true importance of protozoa to the nutrition of the host. Whatever the cause, the changes in the population densities observed may explain in part the daily fluctuations in protozoa! numbers in the rumen observed by Boyne et al (1957).

IMPORTANCE OF THE PROTOZOA TO THE NUTRITION OF THE HOST

The contribution of rumen ciliates to the amino acid requirements of the host depends on (a) the proportion of the rumen nitrogen present in the protozoa and (b) the comparative rate of passage from the rumen of protozoa!, bacterial and non-microbial nitrogen.

Contribution of protozoa to total rumen nitrogen. As shown above the number of protozoa in the rumen depends on the ration on which the host is fed but the following estimates have been made of the contribution of protozoa to the total microbial population: (a) in a ewe fed on lucerne hay, 20% of the nitrogen (Weller et al 1958); (b) in a whether fed on air-dried alfalfa pellets, 44-53% of the weight (Hungate et al 1971); (c) in a cow fed alfalfa hay and barley without and with diethylstilboestrol 45% and 60% respectively of the protein-amino acids (Ibrahim & Ingalls 1972); (d) in a cow fed a semi-purified diet of wheat straw glucose and starch without and with diethylstilboestrol, 50% and 73% respectively of the protein amino acids (Ibrahim & Ingalls 1972); (e) in a sheep fed a semi-purified diet of starch, solka floc (cellulose) and urea 45% of the α-amino nitrogen (Harrison et al 1979); (f) in wethers fed on hay and concentrates, 40-53% of total rumen nitrogen (Michalowski 1979). No results are available for animals on restricted high grain rations but calculations from the results of Hungate et al (1971) and Eadie et al (1970) produce an estimate of 80%. Unfortunately it is less easy to make similar calculations for animals on molasses diets but from the results of Hungate et al (1971) and Boodoo et al (1978) an approximate estimate of 55% is obtained.

Rate of passage of protozoa from the rumen. In the earlier work it was assumed that all fractions left the rumen at the same rate (Ibrahim & Ingalls 1972) and calculations of the significance of the protozoa to the nutrition of the host were made on that basis. However, Hungate et al (1971) showed that the microbial fraction in the rumen turned over at a rate of 1.25 per day compared with a rate of 2.27 per day for a soluble marker (polyethylene glycol-PEG). Calculation suggested that the protozoa turned over even more slowly at a rate of 0.69 per day. This work was extended by Weller and Pilgrim (1974) who made direct measurements of the passage of protozoa by removal of samples twice a day from the reticuloomasal orifice of sheep fed on hay and showed that protozoa left the rumen at only 20% of the rate of a soluble marker and that the amount of protozoa! nitrogen leaving the rumen represented only 2X of the dietary nitrogen. The population density of protozoa in omasal fluid is only 8-10% of that in rumen fluid (Bird et al 1978). More recently Harrison et al (1979) using sheep on a semi-purified diet, have shown, by assuming that protozoa! nitrogen is the difference between total microbial and bacterial nitrogen, that the protozoa left the rumen at only 41% of that predicted from the rate of rumen outflow. Although this is a low value, it does mean that 19-24% (depending on the method of calculation) of the total microbial amino nitrogen in the duodenum was protozoa! and is a significant proportion of the total nitrogen available to the host. The results of Weller and Pilgrim (1974) and those of Harrison et al (1979) therefore agree in showing that protozoa are selectively retained in the rumen but disagree on the extent of this retention.

Possible mechanisms of protozoal retention. Two mechanisms for the retention of protozoa have been put forward. The first is the attachment of the protozoa to large plant particles which form part of the crust in the rumen. There is evidence that *Epidinium* spp. (Bauchop & Clarke, 1976), *Isotricha* spp. (Orpin & Letcher 1978) and other protozoa including, but to a much smaller extent, *Entodinium* spp. (Orpin 1979a,b) attach themselves to plant particles. With the holotrichs, at least, this was the result of chemotaxis to surfaces liberating certain soluble sugars. However this chemotaxis is probably only important during the first 2h after eating with Isotricha spp. (Orpin & Letcher 1978) and from 4-10h with *Epidinium ecaudatum caudatum* (Amos & Akin 1978) suggesting that in animals fed once a day sequestration of the protozoa on food particles would only be important in the first 12h after feeding. Unfortunately there is no evidence on when during the day, the protozoa are selectively retained. Although it has always been assumed that the decrease in protozoa! numbers after feeding is &e to the increased dilution rate that occurs at this time (Michalowski & Muszybski 1978,Warner 1966a) it is possible that some of the protozoa could become sequestered in the crust.

The second possible mechanism for protozoal retention is the settling to the bottom of the rumen of starch-filled holotrich protozoa (Valdez et al 1977). This phenomenon is probably only important in animals fed on diets rich in soluble sugars and containing a large number of holotrich protozoa. Although starved and therefore less dense holotrichs might be expected to swim away from the bottom of the rumen the observation of Valdez et al (1977) that holotrich 'flock' together in the absence of fermentable substrate suggests that this would not occur. The reduced ruminal contractions in animals 12-24h after feeding (Priego & Leng 1975) would also encourage protozoa to settle to the bottom of the rumen.

The evidence that protozoa are selectively retained in the rumen has given rise to speculation as to what happens to these protozoa especially at very low dilution rates. It is often assumed (e.g Harrison et al 1979) that minimum generation times quoted for protozoa! growth in vitro are the same as those found in vivo and that ciliate protozoa must always grow at their maximum rate. As conditions for growth in vitro are at best a good approximation to conditions in vivo, there is no reason why protozoa should not grow faster in vivo than in vitro and there is evidence that this occurs (e.g minimum mean generation time of *Entodinium caudatum* in 5 1/2h in vivo (Warner 1962) and 37h in vitro (Coleman 1969a)). Rumen bacteria can be maintained in a conventional continuous flow apparatus at very variable growth rates (Hobson 1965) and the protozoa can presumably behave in the same way. It is therefore not necessary to invoke death and lysis of the protozoa to explain the maintenance of protozoa when rumen outflow is very slow (Harrison et al 1979; Leng 1976). The growth rate of ciliates can also vary greatly with the supply of food (Warner 1962) and if this is only supplied for a short time each day, the protozoa will probably divide once or twice rapidly and rest for the remainder of the time till the host is next fed.

As rumen ciliates will only grow in continuous culture in vitro when the fermentation vessel has a large dead space (Weller & Pilgrim 1974; Czerkawski & Breckenridge 1977, 1978) in which the protozoa become sequestered it is probable that the same situation may occur in vivo. Provided that the population density is large compared with that in the free space (>10 times was found in in vitro experiments

(Czerkawski & Breckenridge 1978) the protozoa can grow in the dead space at a comparatively slow rate and if necessarily intermittently. Protozoa would enter the free space from the dead space (e.g by ruminal contractions in vivo). Once in the free space, the protozoa could remain there, pass into the omasum or swim back into the dead space and an equilibrium would be set up. As in vivo, the dead space is the crust, the investigator only sees the protozoa in the free space when sampling the rumen. It is therefore obvious that we still have much to learn about the behaviour and growth of protozoa in the rumen.

Although the protozoa may be less important in the nutrition of the animal than was previously thought, they are digested more rapidly than the bacteria by rats (McNaught et al 1954; Bergen et al 1968) presumably because they are completely destroyed by the acid conditions in the stomach (Smith 1969). As the abomasum is also acid, the protozoa may also be digested more rapidly than bacteria by the ruminant.

ROLE OF THE PROTOZOA IN RUMEN METABOLISM

Regardless as to whether rumen protozoa are important to the nutrition of the host they play an important role in rumen metabolism.

Removal of carbohydrates from bacterial attack. In animals on temperate diets of hay and concentrates the ciliate protozoa engulf the starch grains in the ration and therefore render them unavailable for bacterial attack. The Entodiniomorphid protozoa ferment the starch comparatively slowly to acetic and butyric acids which are absorbed through the rumen wall. In the absence of protozoa starch is fermented by the bacteria to butyric acid and then to lactic acid (Dirksen 1970) which is only slowly absorbed through the rumen wall with a resultant drop in rumen pH (Williams & McKenzie 1965). As protozoa also engulf bacteria (Coleman 1964) the ciliates remove not only the starch but also the enzymes that attack the starch. When an animal is transferred from a high roughage to a high grain ration, it is essential that the changeover be carried out gradually in order to give the entodinia in the rumen time to increase in numbers and be able to engulf the increased amount of starch. If the process is done too rapidly the excess starch is fermented to lactic acid which kills the protozoa and produces an acidosis with a rumen pH below 5. On recovery the rumen pH rises and conditions again become suitable for ciliate protozoa. Reinfection is most likely to occur with the small entodinia and it is possible to obtain, unknowingly, animals with an abnormal fauna.

Although there is no evidence, it is probable that on the sugar rich tropical diets, the holotrich protozoa, which rapidly take up soluble sugars and convert them to starch, may perform a similar function. This starch would be fermented to acetic, butyric and little lactic acid, (Van Hoven & Prins 1977; Prins & Van Hoven 1977). On the basis of the protozoa! population densities given by Boodoo et al (1978) and assuming that they were all Isotricha spp. which synthesised amylopectin at the rate given by Prins & Van Hoven (1977), then 5.8g of glucose would be converted to starch by each litre of rumen fluid each h. If the calves (100kg) used by Boodoo et al (1977) had a rumen volume of 5 litres then 29g glucose would be fixed each hour from an hourly intake of molasses (70g dry wt) and sugar cane (47g dry wt = 28g sugar),

Although many assumptions are made in these calculations it is apparent that an appreciable proportion (30%) of the sugar consumed by the animal could be converted into amylopectin by the protozoa.

Digestion of bacteria by protozoa. All rumen ciliates engulf bacteria and some also kill and digest them with release of low molecular weight compounds (principally amino acids) into the surrounding fluid (Coleman 1967, 1975; Coleman & Laurie 1974, 1977; Coleman & Sandford 1979b). The extent of the engulfment and digestion depends on both the protozoa! and bacterial species and the number of protozoa and bacteria present (Coleman & Sandford 1979a). Rates of engulfment of mixed rumen bacteria (at 109 bacteria/ml) vary from 130 to 21200 bacteria/protozoon/h depending on the protozoa! species with rates of digestion varying from 0to 36000 bacteria/protozoon/h. Average values calculated for the rates of digestion under conditions holding in the rumen are (in bacteria/protozoon/h): 1500 (Coleman 1975), 345 (Coleman & Sanford 1979a), 1080 (with *Streptococus bovis* only - Jarvis 1968), 1200 (calculated from Cottle et al 1978). Values of 2.4 to 45g bacteria digested per sheep rumen/day have been obtained for animals containing high protozoa! populations (106/ml). As a large proportion of the digestion products are degraded to ammonia and volatile fatty acids, this represents an important loss of amino acids to the host. The relevance of these results to conditions in vivo can be seen by examining the results of Bird et al (1978) who investigated the effect of faunation on the growth of lambs fed a high energy - low protein diet supplemented with urea. One animal on this diet consumed only 11g protein/ day which if incorporated directly, after breakdown to amino acids, would give rise to a maximum of 22g bacteria. Although some bacterial protein is obviously synthesised from ammonia derived from the urea, the loss of bacterial protein due to the activities of the protozoa is of the same order as that present in the diet. This could account for the loss of weight of faunated compared with defaunated animals under these conditions. In contrast animals given supplementary protein consumed 59g protein/day and as faunated animals then grew faster than the defaunated, this suggested that the digestion of bacteria was not depriving the animal of protein and that the increase digestibility of the protozoa compared with bacteria was providing valuable extra nutrient.

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