THE INFLUENCE OF THE PRESENCE OF PROTOZOA ON RUMINANT PRODUCTION : A REVIEW

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SUMMARY

The ciliate protozoa in the rumen are quantitatively important in the digestion of the major carbohydrate, protein and lipid components of feed material ingested by the ruminant. Attempts to clarify their nutritional importance to the host animal have utilised comparative studies with faunated and ciliate-free animals. Results from these studies have not been consistent and it is suggested that some of the methods used to obtain animals free of ciliate protozoa may not represent the true effects of the ciliate-free condition on rumen function or animal production. An examination of the behaviour and metabolism of the ciliates indicates that in the absence of these organisms more protein will be available for intestinal digestion by the host animal relative to the VFA absorbed from the rumen (P:E ratio). An increase in the P:E ratio will increase the efficiency of nutrient utilisation by the animal (Leng 1982) and can be expected to increase ruminant *production under most conditions.

INTRODUCTION

The rumen is an integral component of the digestive tract of the ruminant animal. It is inhabited by a diverse population of anaerobic microbes (chiefly bacteria, fungi and protozoa) that are active in the fermentation of ingested feed. The major products of this fermentative digestion are volatile fatty acids (VFA) which are used by the host animal as a source of energy and for fattening and the digestible components of the microbial cells synthesised in the rumen (approx 60% protein). Intestinal digestion of dietary materials escaping rumen degradation provide an additional source of nutrients for host metabolism. It has been suggested by Leng (1982) that it is the availability of protein relative to VFA-energy (P:E ratio) that determines the efficiency with which metabolisable energy is utilised for productive purposes (i.e. growth reproduction) by the host animal. In contrast wool production appears to be highly correlated to the availability of protein (Reis, 1979).

Results from comparative studies with faunated and Protozoa-free animals have not been consistent and in two recent reviews it was suggested that compared with faunated animals the-ciliate-free animal will be less productive in most situations (Veira 1986; Ryle & Orskov 1987). This conclusion is challenged for while it is not possible to quantitate the contribution of the protozoa to the supply of nutrients to the host animal it is apparent that the post-rumen availability of amino acids is higher in ciliate-free animals. Therefore animals without protozoa should achieve higher levels of production whenever the availability of amino

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acids to the host animal is the primary limitation to production i.e. whenever a low P:E ratio results in an inefficient use of absorbed nutrients. This situation covers a wide range of ruminant production systems as there are very few forage based diets that provide adequate amounts of protein for optimal production. These contrasting views are discussed with attention given to the effects of the ciliate protozoa on' rumen function and nutrient availability to the host animal and to results from production studies.

THE RUMEN PROTOZOA

Protozoa are normally present in domestic ruminants. Most of the rumen protozoa are ciliates although several species of flagellates may also be present. The number of flagellates in the rumen is generally low (Eadie 1962) and their mass small (Clarke 1977) and are not considered in this discussion. The rumen ciliates are classified into two orders: Trichostomatina (family Isotrichidae, Butschi) commonly referred to as holotrichs and Entodiniomorphida (family Ophryoscolecidae, Stein) commonly referred to as entodiniomorphs. The holotrichs and entodiniomorphs are able to degrade and metabolise the principal protein, carbohydrate and lipid components of the feed material ingested by the host animal (Williams 1989). The ciliates are also responsible for the engulfment and degradation of large numbers of rumen bacteria (Coleman 1975). Estimates of the biomass of protozoa in the rumen indicate that the contribution of the ciliate protozoa to the total microbial biomass may vary from 40-80% (Harrison & McAllan 1980) and as a result their influence on rumen fun&ion must be at least comparable to that of the rumen bacteria.

DEGRADATION OF INGESTED FEED IN THE RUMEN

Microbial degradation of feed material entering the rumen begins with the rapid colonisation of feed particles by large populations of bacteria protozoa and fungi. *In vitro* studies indicate that the colonisation of plant material by the protozoa is rapid and maximal between 5 and 35 min. after exposure to the plant particles (Orpin 1985). The vast array of enzymes capable of digesting proteins and complex carbohydrates that have been isolated from the protozoa (Coleman 1983; Forsberg et al. 1984) is further evidence that protozoa actively participate in the digestion of feed material in the rumen.

Carbohydrate digestion

There is apparently very little competition between the two groups of protozoa for energy substrates as the holotrichs use soluble sugars and the entodiniomorphs utilise starch and more complex carbohydrates (Hungate 1966). The holotrichs are attracted to sources of soluble carbohydrates by chemotaxis (Orpin & Letcher 1978) and attach to damaged ends of plant material entering the rumen (Bauchop 1989). The holotrichs rapidly assimilate soluble sugars which may be converted to storage polysaccharide reserves within the cell (Williams & Harefoot 1976). All the entodiniomorphs with the exception of the smallest. *Entodinium spp.* can ingest starch (Williams 1989)

and it has been shown that the highest starch degrading activity is found in the protozoa1 population (Williams & Strachan 1984). Results from microscopic, ***in vitro*** and ***in vivo*** studies indicate that protozoa have a significant role in the degradation of plant cell wall polysaccharides. Enzymes capable of degrading cellulose, hemicellulose and pectin have been isolated from the entodiniomorphid protozoa (Coleman 1983; Orpin 1983/84) and it has been demonstrated that protozoa may account for 5-90% of the cellulolytic activity in the rumen (Coleman 1985).

Although it is apparent that protozoa have a significant role in the digestion of fibre in the rumen, it is not possible to measure their contribution directly. From a survey of the early ***in vitro*** and nylon bag ***(in sacco)*** studies, Demeyer (1981) calculated that the protozoa were responsible for approximately 34% of the total microbial digestion of fibre. Of course in the absence of the protozoa the digestion of fibre in the rumen will not be depressed by this amount because of increases in the size of the bacterial population (Bryant & Small 1960; Eadie & Hobson 1962) and fungal population (Soetanto 1986; Romulo et al. 1986) and changes in the enzyme activity of the bacterial population (Jouany & Senaud 1979; Kurihara et al. 1978). In a more recent review Veira (1986) noted that the apparent digestibility of organic matter in the rumen of ciliate-free sheep was on average only 85% of that measured in faunated sheep given concentrate and roughage diets. However this situation may not be true for animals receiving high roughage diets. For example defaunation had no effect on the digestibility of Timothy grass (***Phleum pratense)*** in sheep given high roughage diets (Orpin & Letcher, 1983/84) and the digestibility of cereal straw was increased by defaunation (Romulo et al. 1986). In this latter study a number of sporangia and zoospores were increased in the absence of protozoa indicating that fungal growth may have been greater in these animals.

Rumen environment

In addition to their direct contribution to the enzymic degradation of carbohydrate in the rumen, the presence of the ciliates has been shown to influence other components of the rumen ecosystem which may effect the rate and extent of carbohydrate digestion.

(i) Digesta outflow from the rumen Defaunation is often associated with changes in rumen volume and digesta outflow but the results from defaunation studies are not consistent (Orpin & Letcher 1983/84; Kayouli et al. 1984). An increase in the time plant material remains in the rumen should increase the extent of microbial digestion. There is no proven explanation for the existence of a relationship between digesta kinetics and the presence of ciliate protozoa.

(ii) pH of rumen fluid Compared with faunated animals the pH of rumen fluid in defaunated animals is often lower (Whitelaw et al. 1972; Veira et al. 1983) and the difference is exacerbated when the diet is rich in starch. This effect may be due to an increase in the amylolytic activity of the bacteria in the absence of the ciliates (Kurihara et al. 1978)

or may be due to differences between the bacteria and protozoa with respect to VFA production rates. Some protozoa1 species also metabolise lactic acid (Newbold et al. 1986). Cellulolytic activity is inhibited at low pH (Stewart 1977) and by lactic acid accumulation (Fay & Ovejero 1986).

(iii) Ammonia concentration in rumen fluid Ammonia concentrations in the rumen are consistently higher in faunated than in ciliate-free animals (Abou Akkada & El Shazly 1964; Christiansen et al. 1965). The lower ammonia concentration in the defaunated rumen may be the result of: lower production rate (i.e. lower degradation of dietary and bacterial protein), an increased rate of utilisation, an increased. rate of absorption across the rumen wall or an increase in rumen volume. Low ammonia concentration in the rumen of ciliate-free animals may limit carbohydrate digestion (Veira 1986; Ryle & Orskov, 1987). However, varying levels of urea supplementation had no effect on the productivity of ciliate-free lambs (Bird & Leng 1984).

Protein digestion

The bacteria, protozoa and fungi found in the rumen all have proteolytic activity. There are contrasting views on the relative importance of these groups, Blackburn & Hobson (1960) concluded that more than half the proteolytic activity was present in the protozoa1 population while Brock et al. (1982) and Wallace (1985) suggest that the bacteria are responsible for most of the proteolytic activity in the rumen. It is apparent that the characteristics of the dietary protein are important. Bacteria appear to have a major role in the degradation of soluble proteins (Nugent & Mangan 1981) while the entodiniomorphs only utilise insoluble particulate protein sources including bacteria and chloroplasts (Coleman 1975). The holotrichs are able to use both soluble and particulate sources of protein (Abou Akkada & Howard 1962).

Only a proportion of the peptides and amino acids formed by the digestive activities of the protozoa are used for protozoa1 protein synthesis. The remaining amino acids and peptides are either excreted directly into the rumen liquor or catabolysed within the cell giving rise to ammonia which is also secreted into the medium (Warner 1956). Protein degradability is generally higher in the rumen of faunated animals and the difference between faunated and ciliate-free animals is exacerbated with low-solubility proteins (Ushida & Jouany 1985).

END PRODUCTS OF RUMEN FERMENTATION

(i) Volatile fatty acids The fermentation products of the rumen protozoa cultured in vitro are H_2, CO_2, formate, acetate, propionate, butyrate and lactate (Heald & Oxford 1953; Coleman 1978). The proportions of the major metabolites produced *in vitro* are influenced by the nature and concentration of the substrate and by environmental conditions (Williams 1986). Therefore it is not surprising to find that defaunation of the rumen is not associated with consistent changes in VFA proportions (Jouany et al. 1981).

(ii) Methane. The production of methane during fermentation represents a loss of energy. Methanogenic bacteria have been observed to be attached to protozoa (Vogels et al. 1980) and the protozoa have been reported to be the major methane - producing fraction in the rumen (Krumholz et al. 1983). Compared with faunated steers the production of methane was lower in the rumen of ciliate-free steers fed a high barley ration (Whitelaw et al. 1984), which is consistant with an increased microbial cell yield and lower VFA production in the ciliate-free animals.

(iii) Microbial protein microbial protein synthesised in the rumen is a major source of protein for the ruminant. The quantity of microbial protein that is available to the host animal is a function of; the efficiency of microbial cell synthesis (weight of dry cells (g) produced per mole of ATP generated from substrate fermentation, termed YATP) the amount of organic matter degraded in the rumen and the proportion of microbial cells which leave the rumen. Microorganisms use ATP for two purposes : to provide energy for cell growth and to provide energy for cell maintenance (Me) (Stouthamer & Bettenhaussen 1973), and the Me for a particular microorganism is positively correlated to gene'ration interval (Isaacson et al. 1975). The ciliate protozoa have a longer generation interval relative to the bacteria (Warner 1965) and therefore a smaller proportion of the available ATP will be used for growth in protozoa1 cells. Unfortunately for the host animal only a small proportion of the protozoa1 cells in the rumen flow to the lower digestive tract (Weller & Pilgrim, 1974). This retention of protozoa in the rumen results utilmately in the lysis of large numbers of protozoa (Leng et al. 1981; Leng 1982; Ffoulkes & Leng 1988) and compared with ciliate-free animals a higher recycling of N within the rumen (Cottle 1980). In addition the predatory activity of the protozoa (Coleman 1975) will significantly reduce the availability of bacterial cells for intestinal digestion.

Results from both ***in vitro*** and ***in vivo*** studies indicate that the efficiency of microbial synthesis is increased in the absence of the protozoa. The synthesis of microbial N in rumen fluid incubated ***in vitro*** (collected from faunated and defaunated goats) was 15% higher in the protozoa-free incubation (Takahashi & Kametaka 1976) and Demeyer & Van Nevel (1979) reported a 33% increase in the net synthesis of microbial protein in a protozoa-free incubation (rumen fluid collected from faunated and defaunated sheep). Comparative studies with faunated and defaunated sheep have demonstrated that on average the post-rumen supply of microbial protein is 20% higher in the defaunated animals (for review see Bird & Leng 1985).

NUTRIENT AVAILABILITY FROM THE CILIATE-FREE RUMEN

The size and composition of the ciliate population in the rumen is variable and controlled by a complex set of factors (Hungate 1966). Accordingly the contribution of the ciliates to rumen function is not a constant and not easily predicted. It is obvious that there are both costs and benefits for the host animal associated with the presence of a ciliate

population in the rumen. With the exception of high roughage diets, the digestion of carbohydrate in the rumen is often reduced when the ciliates are removed. Therefore the amount of energy (VFA) available to the animal will also be reduced. However in the ciliate-free animal this loss will be partially compensated for by an increased digestion of carbohydrate in the hind gut (see Veira 1986), and an increase in the outflow of starch (when present in the diet) from the rumen (Veira et al. 1983). In contrast more microbial protein and dietary protein will be available to the host animal when protozoa are removed from the rumen. Therefore while it is not possible to predict the quantities of nutrients available from the rumen, with or without protozoa, the elimination of protozoa will result in an increase in the total protein available relative to VFA absorbed from the rumen (P:E ratio). An increase in the P:E ratio should increase the efficiency of nutrient utilisation by the animal (Leng 1982) and can therefore be expected to increase ruminant production under most conditions.

CILIATE-FREE ANIMALS AND PRODUCTION

Background

Interpretation of results from the first comparative study of faunated and defaunated animals was that rumen protozoa were not essential to the host (Becker & Everett 1930). In this study the growth rates of faunated and protozoa-free lambs were 134 g/d and 151 g/d respectively, which represented a 12% improvement in growth rate of the ciliate-free lambs . (difference statistically non-significant). Further comparative studies with calves were conducted as a result of an observation that under dairy farm conditions protozoa and some characteristic rumen flora (normally‘ seen in mature animals) were not established in calves until they were several weeks old (Pounden & Hibbs 1948b). In these studies calves were isolated from their dams at 3 days of age (calves were therefore ciliate-free) and reared in isolation from other ruminants. Rumen inoculations (freshly obtained cuds from mature cows) were given to some of the calves and successfully established populations of protozoa and some of the characteristic rumen bacteria in these animals. Growth rates of the inoculated and non-inoculated calves over a 6 week period were the same (Pounden & Hibbs 1948a). Attention was again focussed on the role of protozoa in-ruminant nutrition when it was demonstrated that the protozoa could metabolise carbohydrate to produce VFA (Heald & Oxford 1953; Guitierrez 1955). As a result of the studies of Pounden and Hibbs (1948a) isolation of newborn animals was used as the method for obtaining protozoa-free animals in contrast to the chemical drenching method ($CuSO_4$) used by Becker and Everett (1930). Experiments with lambs (Abou Akkada et al. 1964; Eadie & Gill 1970), buffalo calves (Borhami et al. 1967), ***Bos indicus*** calves (El Sayed Osman et al. 1970) and ***Bos taurus*** calves (Itabashi & Matsukawa 1979) demonstrated that inoculation of the isolated animal with rumen fluid increased liveweight gain (see Table 1). These positive responses were attributed to the establishment of protozoa in the inoculated animals and have had a considerable impact on the perceived role of protozoa in the nutrition of the ruminant. However,

as was noted by Pounden and Hibbs (1948a) the isolated animal would not only be lacking ciliate protozoa but also some of the microflora normally found in the rumen, some of which would have been introduced with the rumen fluid inoculation. Therefore the growth responses obtained in these studies cannot be attributed solely to the establishment of the protozoa. The only conclusion that can be drawn from these studies is that the inoculated animals had faster growth rates than the non-inoculated animals in the early months following inoculation. That this benefit can be maintained or that it can be attributed to the presence of protozoa is not substantiated by these studies.

Table 1 Liveweight (LW) gains of faunated (+F) and ciliate-free (-F) ruminants isolated from their dams at birth

Ref.	Diet[o]	Age(d)	Animals Type	LW gain (g/d) F+	F-
1.	Milk, concentrate berseem	51-219	lambs	106[a]	91[b]
2.	a) milk	10-120	buffalo calves	380	330
	b) milk, concentrate, roughage	10-120	"	400[a]	31[b]
	c) milk, concentrate roughage	10-120	"	360[a]	240[b]
	d) milk, concentrate	10-120	"	410	320
3.	Milk, concentrate	10- 90	Zebu calves	310[a]	240[b]
4.	a) milk, concentrate grass	49-147	lambs	250	229
	b) concentrate, grass	252-413	"	71	83
5.	a) milk, concentrate, grass	0-140	calves	750	670
	concentrate, grass	140-252	"	490	540
	b) milk, concentrate hay	0-140	"	720	680
	concentrate, hay	140-252	"		

[+]Faunated lambs were inoculated with rumen fluid prior to the commencement of the experimental period.
[o]In all the studies, the diet was changed during the experimental period with milk gradually being replaced with concentrate and roughage.
Values with different superscripts differ significantly ($P<0.05$).
References: 1. Abou Akkada *et al.* (1964); 2. Borhami *et al.* (1967); 3. El Sayed Osman *et al.* (1970); 4. Eadie & Gill (1971); 5. Itabashi & Matsukawa (1979).

Bodyweight gain

With the exception of the studies in which the ciliate-free animals were obtained by isolation of newborn animals, most comparative studies with faunated and defaunated animals have demonstrated that ciliate-free animals grow at a faster rate than their faunated controls (see Bird 1989 for review). Bodyweight gain responses to defaunation have been obtained with lambs (Bird et al. 1979; Bird & Leng 1984a; Bird et al. 1991; Demeyer et al. 1982; Kreuzer & Kirchgessner 1987; Habib & Leng 1991), steers (Bird & Leng 1978) and with buffalo heifers (Bird et al. 1989). In addition to these pen-feeding studies positive growth responses have also been obtained

under grazing conditions with ciliate-free; lambs (Bird & Leng 1991), hoggets (Bird & Leng 1984b) and calves (Perdok & Leng 1984) (see Table 2). In contrast to these results, the growth rates of lambs given diets rich in starch were depressed in the ciliate-free animals (Christiansen et al. 1965; Demeyer et al. 1982), indicating that protozoa may have an important role in rumen function when diets contain high levels of starch.

Table 2. Growth rates of faunated (+F) and defaunated (-F) ruminants grazing pasture

Ref.	Production System		animals	Liveweight gain (g/d) +F	-F
1	Native pasture	1983	lambs	84	99
		1984	hoggets	89	109
2	Green oats		hoggets	78	104
3	Native Pasture		calves	210	340

References: 1. Bird & Leng, 1991; 2. Bird & Leng, 1984b; 3. Perdok & Leng, 1984.

The weight gain responses of the ciliate-free animals are associated with a significant improvement in feed conversion efficiency (Table 3). Therefore compared with faunated animals the use of metabolisable energy for growth must be more efficient in ciliate-free animals. This finding supports the concept that it is often the availability of total protein Table 1 (amino acids) relative to oxidisable substrates (mainly VFA) that is the primary limitation to the efficient use of absorbed nutrients (Leng 1982). The higher feed intakes of the ciliate-free animals which occurred in some studies (Table 3) may be due to an increased rate of fibre digestion in the rumen (Romulo et al. 1986), more amino acids available for intestinal digestion (Egan 1965) or in hot climates an increase in the efficiency of utilisation of absorbed nutrients and a reduction in heat stress (i.e. less heat production) (Leng 1990).

Wool production

Wool growth is highly correlated to the intestinal absorption of sulphur animal acids (Reis & Schinckel 1961). Therefore the increased availability of protein from the ciliate-free rumen could be expected to increase wool production. With the exception of the studies of Cottle (1986a,b) comparative studies with faunated and defaunated sheep have demonstrated that wool production was significantly higher in ciliate-free animals over a wide range of dietary conditions and production levels (see Table 4). These production responses were achieved without a change in N intake (pen-feeding studies) indicating that there is a more efficient conversion of dietary N into wool growth in the ciliate-free animal. The absence of a wool growth response in the studies of Cottle (1986a,b) may have been due to the high level of oats fed to the sheep in these studies.

Table 3 Feed intake, bodyweight change and feed conversion efficiency (FCE) of faunated (+F) and defaunated (-F) ruminants given straw based diets

Ref	Diet	Total DM intake (kg/d)		Growth rate (g/d)		FCE DMI/gain	
		+F	-F	+F	-F	+F	-F
	Lambs						
87	Wheatstraw (WS)+urea (14g/d)	0.45	0.51	-69	-62	-	-
	WS+urea (14g/d)+lucerne (150g/d)	0.53	0.71	-20	-1	-	-
76	WS+urea (10g/d)+cottonseed meal (CSM) (80g/d)	0.60	0.65	9	26	67.3	25.0
	WS+urea (10g/d)+maize (M) (160g/d)	0.64	0.68	22	45	29.3	15.2
	WS+urea (10g/d)+CSM (80g/d)+M (160g/d)	0.67	0.73	43	58	15.6	12.4
	Buffalo heifers						
78	Ensiled WS+burseem (3kg/d) (basal diet B)	3.13	2.57	220	263	14.2	9.8
	B+ground nut cake (GNC) (250g/d)	3.16	3.08	277	370	11.4	8.3
	B+GNC (500g/d)	3.50	3.24	360	434	9.7	7.5
	B+GNC (750g/d)	3.48	3.14	400	477	8.7	6.6

* Wheatstraw was offered *ad lib.*

References: 1. Bird & Leng (1991); 2. Habib & Leng (1991); 3. Bird *et al.* (1990)

Other positive production responses that have been reported to be associated with the ciliate-free condition include increased milk production from dairy cows (Moate 1989) and increased birth weights of lambs (Bird & Leng 1991). In the study of Moate (1989) the daily production of both milk fat and milk protein were increased following defaunation treatment.

Table 4 Wool growth (g/d) of faunated (+F) and defaunated (-F) sheep given a variety of diets

Ref	Diet	Clean wool growth (g/d) +F	-F
1	Wheatstraw (WS)+urea (14g/d)	1.6	2.3
	WS+urea (14g/d)+lucerne (150g/d)	2.2	3.2
2	WS+urea (10g/d)+cottonseed meal (CSM) (80g/d)	3.7	4.7
	WS+urea (10g/d)+maize (M) (160g/d)	4.1	4.9
	WS+CSM (80g/d)+M (160g/d)	5.6	6.2
3	Oaten chaff+sugar+ fishmeal (4%)+urea (3%)	8.0	10.8
	Oaten chaff+sugar+ fishmeal (4%)+urea (5%)	7.9	11.0
4	Green oats (grazing)	10.0	11.9
5	WS (10%)+oats (89%)	6.6	6.5
	WS (10%)+oats (80%)+ crushed lupins (10%)	6.8	7.6
6	WS (10%)+oats (80%)+ whole lupins (10%)	8.2	7.9

References: 1. Bird et al. (1991); 2. Habib & Leng (1991); 3. Bird & Leng (1984a); 4. Bird & Leng (1984b); 5. Cottle (1986a); 6. Cottle (1986b).

CONCLUSION

The ciliate protozoa in the rumen are quantitatively important in the digestion of the major carbohydrate, protein and lipid components of feed material ingested by the ruminant. Removal of the ciliates may result in a reduction in the digestion of carbohydrate and protein in the rumen. However with the exception of high starch diets their presence is apparently not essential to the host animal and in many situations their presence represents a constraint to production. Ciliate protozoa actively degrade dietary and bacterial protein in the rumen and only a small proportion of protozoa1 cells are available for intestinal digestion. In many ruminant production systems this behaviour of the rumen ciliates results in a critical loss of protein to the host animal. Consequently the ciliate-free animal can be expected to achieve higher levels of production whenever the availability of amino acids to the host animal is the primary

limitation to production, i.e. whenever a relatively low P:E ratio results in an inefficient use of absorbed nutrients.

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