

Conference: Altering Ruminant Nitrogen Metabolism to Improve Protein Utilization

Effect of Rumen Protozoa on Nitrogen Utilization by Ruminants¹

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ABSTRACT Results obtained during the past decade indicate clearly that protozoa are actively involved in the degradation of dietary and microbial proteins in the rumen. Because of the great ability of protozoa to ingest the particulate matter suspended in the rumen, protozoa are more active in degrading insoluble than soluble proteins. This indicates that studies carried out using lysed and sonicated protozoa are not appropriate for quantifying the actual contribution of protozoa to protein degradation in the rumen. In vivo trials have confirmed that duodenal flow of both undegraded dietary protein plus bacterial protein generally is increased by defaunation. The decrease in ruminal ammonia concentration consistently observed after defaunation accounts for the lower urinary nitrogen (N) excretion found in defaunated animals, whereas the increase in fecal N excretion in the same animals probably results from a shift of plant cell wall digestion from the rumen to the large intestine. Total N excretion is not altered significantly by defaunation. A summary of literature data indicates there are contradictory effects of defaunation on ruminant performance. This implies that animal response to defaunation may depend on the specific nutrient-limiting performance on the one hand and on the modifications of digestion and metabolism resulting from defaunation on the other. Different methods are proposed to either eliminate or decrease the numbers of ruminal protozoa or to alter their makeup. However, none of these approaches has been tested under practical feeding conditions. *J. Nutr.* 126: 1335S-1346S, 1996.

INDEXING KEY WORDS:

- rumen • nitrogen • digestion • metabolism
- protozoa

Ruminants receive amino acids from two main sources: ruminally undegraded protein (RUP) originating from the diet and microbial protein synthesized within the rumen. Recent findings indicate that free

peptides originating from dietary protein degradation accumulate in the rumen (Broderick et al. 1988, Russell et al. 1991) and may be available for absorption. Endogenous proteins from digestive secretions and desquamation of cells lining the digestive tract generally have not been considered quantitatively important compared with the other sources of protein.

Ruminal protozoa may influence intestinal protein flow at a number of different levels. Protozoa can alter quantitatively ruminal degradation of dietary proteins and ruminal bacterial growth, consequently modifying the supply of both protein of feed and bacterial origin. The contribution of protozoal protein to total microbial protein supply must also be considered. For the purposes of this discussion, we will assume that protozoa have no significant impact on the flow of endogenous proteins entering the duodenum.

The ruminal protozoa

The protozoal population in the rumen is composed of flagellates and ciliates, the latter being by far the more numerous. There has been some confusion in the past in differentiating between zoospores of phycomycete fungi and flagellates. Also, Quin's and Eadie's

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ovals, which are known to be large bacteria, sometimes mistakenly have been considered to be protozoa. Ruminant ciliates are composed of two orders, both in the class *Vestibulifera*: holotrichs, belonging to the family *Isotrichidae*, the suborder *Trichostomatina* and the order *Trichostomatida*; plus the entodiniomorphs, belonging to the family *Ophryoscolecidae* and the order *Entodiniomorphida*. Because these two groups have different specificities in their feeding behavior and digestive and metabolic activities, their respective roles will be considered separately in this discussion.

Feeding behavior of protozoa in the rumen

Entodiniomorphid protozoa are particularly efficient in taking up particulate matter suspended in ruminal fluid. Anatomically, they possess a vestibulum surrounded by cilia. The cilia are used to trap particles that are subsequently driven into a vestibulum and then into cytostomes. In *Ophryoscolecidae*, a cytopharynx extends from the cytostome. Membrane and cytoplasmic movements across the cytopharyngeal microtubular ribbons are implicated in the migration of feed particles inside the digestive vacuoles of the cell. Entodiniomorphid protozoa also are able to take up soluble compounds. There is evidence that their ectoplasm is freely permeable to low-molecular-weight compounds, but a permeability barrier between the ectoplasm and the endoplasm may limit utilization of soluble compounds by protozoa (Coleman 1986).

Holotrich protozoa have considerable capacity for taking up soluble compounds, chiefly sugars, from the medium. However, they are less efficient than entodiniomorphs in the ingestion of insoluble plant particles. This is confirmed by increased holotrich numbers when animals are fed diets rich in readily soluble carbohydrates and, conversely, by decreased holotrich numbers when diets rich in digestible cellulosic materials are fed (Jouany 1989). Nevertheless, holotrichs (mainly *Isotricha*) are able to digest starch granules.

It is clear that uptake of substrates and formation of digestive vacuoles inside the cell are preliminary stages of the digestive process of ciliates. This strategy is complementary to that of bacteria, which degrade insoluble compounds using extracellular enzymes after adhesion to feed particles, and that of fungi, which degrade plant structures from deep within the particles during their vegetative stage of growth by means of branched rhizoids.

Effect of protozoa on the degradation of dietary proteins

Considering the differences in feeding strategies between protozoa and other ruminal microbes, it can be concluded that determining enzyme activities in autolyzed or sonicated cells is not appropriate for quantifying

the contribution of ciliates to protein degradation in the in vivo rumen. Thus, only results obtained using live protozoa, under conditions similar to those within the rumen, will be presented here.

In testing the effect of different proteins on the growth of entodiniomorphid protozoa cultivated in vitro, it was found that these ciliates do not metabolize soluble proteins (Michalowski 1989) and do not grow unless insoluble proteins are supplied (Muszynski et al. 1985). In vitro studies with both A-type protozoa (Ushida and Jouany 1985) and B-type protozoa (Jouany et al. 1992) indicated that the production of ammonia from fish meal, soybean meal, lupins and peanut meal was significantly greater in ruminal contents obtained from faunated sheep than defaunated sheep (Table 1). Conversely, the mixed population of protozoa had no effect on ammonia production when casein was used as substrate. These results may be explained by the high capacity of entodiniomorphid ciliates, which represented 99% of the tested population, for ingesting and digesting insoluble particles and by their low activity toward highly soluble proteins such as casein.

Because water is involved in the interaction between proteins and extracellular proteolytic enzymes, the solubility of proteins is a major characteristic determining their sensitivity to bacterial degradation (Nugent and Mangan 1981). Feeding strategies of protozoa vary. Ingested insoluble proteins are degraded within the cell of entodiniomorphid protozoa by active proteases present at high concentration; these enzymes are not diluted by being secreted into ruminal fluid.

Holotrichs show multiple forms of protease (Lockwood et al. 1988, Lockwood et al. 1989), but the actual contribution of holotrichs to protein degradation has never been studied in individual genera (Williams and Coleman 1992). For this reason, we tested the effect of the inoculating *Isotricha* spp. into defaunated rumens of cattle fed three different protein sources and compared these results with those obtained using an inoculum with mixed B-type protozoa (Jouany et al. 1992). It was interesting to note that addition of *Isotricha* spp. alone into defaunated rumens decreased ammonia production 25, 33 and 15% for fish meal, soybean meal and casein, respectively, the smallest effect being obtained with the highly soluble protein casein. Onodera and Kandatsu (1970) also found that isolated holotrichs rapidly degraded casein, whereas isolated entodiniomorphids had no effect. This suggests that addition of the genus *Isotricha* into the defaunated rumen will lower bacterial deamination activity and that the contribution of *Isotricha* themselves to ammonia formation will increase with solubility of protein sources. In an in vivo study, Matsumoto et al. (1989) also observed that ruminal ammonia concentration decreased after the holotrich *Dasytricha ruminantium* was inoculated into defaunated goats.

When single genera of Ophryoscolecids, such as *Eudiplodinium*, or a mixture of *Epидinium* plus *Entodi-*

TABLE 1

Influence of the ruminal fauna on degradation of different proteins during 6-h in vitro incubations

	Defaunated	<i>Isotricha</i>	<i>Eudiplodinium</i>	<i>Epidinium</i> + <i>Entodinium</i>	Total mixed fauna (Type B)
No. ciliates, 10 ³ /mL	0	7.5	41.0	44 + 174	336.6
Extent of degradation to ammonia N, %					
Fish meal ¹	22.7bc	17.2d	26.1ab	20.2cd	26.5a
Fish meal ²	17.0b	—	—	—	27.9a
Soybean meal ¹	41.8b	28.1d	44.0b	37.2c	51.1a
Soybean meal ²	37.2b	—	—	—	47.6a
Casein ¹	83.2a	70.5bc	74.1b	67.0c	82.0a
Lupin ²	60.7b	—	—	—	70.9a
Peanut meal ²	60.2b	—	—	—	71.7a

¹ Data from Jouany et al. (1992).² Data from Ushida and Jouany (1985).a,b,c,d Means within rows having different superscript letters are different ($P < 0.05$).

nium were inoculated into defaunated rumens, no significant effect on degradation and fermentation of fish meal and soybean meal was found (Jouany et al. 1992). However, a decrease in ammonia production was observed when casein was used as substrate (Table 1). Comparing the results obtained with the mixed fauna and the isolated genera of Ophryoscolecids, two hypotheses are proposed. Because protozoal numbers were much higher in animals containing mixed fauna, we may assume that there is a threshold for entodiniomorphid ciliates which must be reached before protein degradation will be effective. Also, there may be synergic effects of different genera on protein degradation by mixed fauna that do not occur in the rumens of animals inoculated with only individual genera of entodiniomorphids. For example, Onodera and Yakiyama (1990) suggested that holotrichs may produce some casein-coagulating substances that render casein sensitive to entodiniomorphs. The decrease in casein degradation after inoculation with the simplified ciliate populations in defaunated rumens could be due to a negative effect of Ophryoscolecids on the bacterial biomass even though their own activity against casein is very low.

Ushida and Jouany (1985) showed in situ that the potentially degradable fraction of insoluble protein in soybean meal and its rate of degradation were both increased 11% when mixed A-type protozoa were inoculated into defaunated sheep. Ruminal protein degradability, calculated using the model of Ørskov and McDonald (1979) with passage rate = 0.06/h, was 13% higher in faunated animals.

It can be concluded from these results that mixed A-type or B-type protozoal populations both stimulate degradation of low soluble, particulate dietary proteins in the rumen but are relatively inactive toward soluble proteins (Fig. 1). Similar results were reported previously by Nugent and Mangan (1981) using azocasein and by Forsberg et al. (1984) using several protein substrates (azocasein, casein, soy protein, hemoglobin and gelatin). Conversely, if present in the rumen as a single

genus of protozoa, *Isotricha* spp. may have a protective effect against degradation independent of protein solubility. However, this original result has not been confirmed as yet by further in vitro or in vivo experiments.

Ingestion and digestion of bacteria by protozoa

All ciliates ingest bacteria as their principal source of protein amino acids. According to Coleman (1986), the uptake of bacteria from the ruminal liquid phase may be either selective or nonselective, depending on the protozoa and the bacteria. Small entodinia have been shown to be by far the major contributor to bacterial protein turnover in the rumen (Wallace and McPherson 1987). Bacteria are necessary for the growth of ciliates in culture (Coleman 1962, Onodera and Hen-

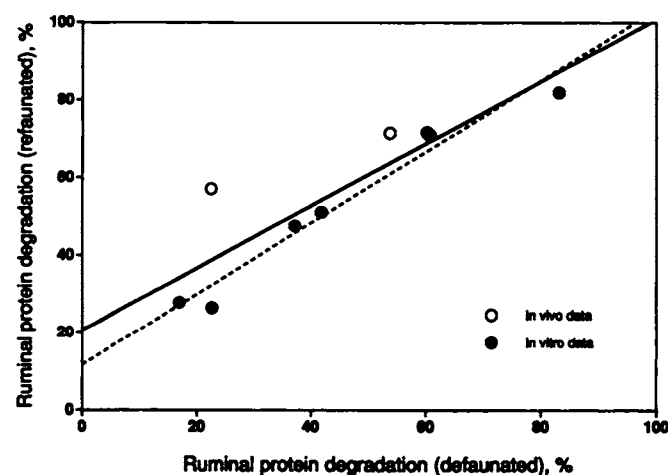


FIGURE 1 Effect of protozoa on protein degradation in vitro in ruminal fluid from sheep or in vivo in the sheep rumen. In vitro data are from Ushida and Jouany (1985) and Jouany et al. (1992); in vivo data are from Ushida et al. (1986). Linear regression using both in vitro and in vivo data (—): $Y = 20.7 + 0.802 X$ ($r^2 = 0.794$); linear regression using in vitro data only (- - -): $Y = 11.7 + 0.917 X$ ($r^2 = 0.924$).

derson 1980). Coleman (1975) reported that a single protozoon can take up 10^2 – 10^4 bacteria/h. Applying a value of 10^9 bacteria/mL to the rumen, these estimates indicate that predation could almost renew the entire bacteria biomass every hour in a rumen harboring a high concentration of protozoa (10^5 – 10^6 /mL). This explains the increase in bacterial biomass after the elimination of ciliates from the rumen (Eadie and Hobson 1962, Teather et al. 1984). The qualitative composition of the ruminal flora also is altered by the presence of ciliates. Itabashi and Katada (1976) and Ushida et al. (1987) observed that the numbers of small gram-negative rods, selenomonas-like bacteria and gram-negative cocci were higher in defaunated animals, whereas gram-positive strains were unchanged. The decrease of amyolytic bacteria after defaunation (Kurihara et al. 1978) probably results from extensive uptake of starch granules and the associated bacteria by protozoa in faunated animals.

Protozoal biomass accounts for 20–70% of the total microbial biomass in the rumen (Coleman 1979). It is now accepted that only 20–40% of protozoa leave the rumen (Collombier et al. 1984, Harrison et al. 1979, Weller and Pilgrim 1974) and that protozoa remaining in the rumen die and lyse. Ankrah et al. (1990a) estimated that ~50% of the decrease in Ophryoscolecidae numbers after feeding may have resulted from dilution due to intake of feed and water and ~50% from passage out of the rumen. However, these authors did not favor either explanation for the decline in Ophryoscolecidae numbers.

As a consequence of bacterial predation and subsequent protozoal lysis, N recycled within the rumen—corresponding to the difference between total incorporation of N into microbial cells (total protein synthesis) and the net outflow of microbial N (net protein synthesis)—is much greater in faunated than in defaunated animals.

Bacterial proteins are degraded within protozoal cells to small peptides then free amino acids, which are in turn incorporated into protozoal proteins without further interconversion (Coleman 1972). Part of the peptides originating from bacterial protein degradation also are excreted by protozoa (Denholm and Ling 1984), as are free amino acids (Coleman 1975) and their *N*-acetyl and *N*-formyl derivatives (Coleman 1967). Decarboxylation and deamination of amino acids can occur within protozoal cells. Release of small molecular weight nitrogenous products may account for as much as 50% of the bacterial protein ingested by protozoa (Coleman 1975). As a consequence, the concentration of free amino acids and peptides is higher in faunated than in defaunated rumens (Hsu et al. 1991a, Itabashi and Kandatsu 1975, Ivan et al. 1991b). Therefore, microbial uptake of peptides and free amino acids and total microbial protein synthesis are greater in faunated than in defaunated animals.

Effect of protozoa on the duodenal N flow in ruminants

Most workers have observed that passage of ruminal undergraded proteins RUP originating from the diet increases or remains unchanged when animals are defaunated. The increase may range as high as 216% (Table 2). Although this is not unexpected, based on the studies discussed previously, the positive relationship between insolubility of dietary protein and increased RUP flow after defaunation is not always apparent in the summarized data. Lack of sensitivity of the methods used to partition duodenal flow between N of dietary and microbial origins may partly explain the difficulty of detecting effects of defaunation on RUP. Absence of an apparent effect of defaunation on RUP also may be due to an increase in retention time of insoluble proteins associated with defaunation. Removal of protozoa decreases the rate of degradation of plant cell walls (Jouany 1991) and will be associated with an increase in ruminal volume. Retention time of plant particles likely would increase in defaunated animals because fractional outflow rate for ruminal contents generally is inversely related to ruminal volume (Demeyer 1989, Faichney and Griffiths 1978, Orpin and Letcher 1983/84, Ushida et al. 1986). Thus, defaunation probably would not alter RUP flow on low quality roughage diets. However, this has never been clearly established, possibly because of variable effects of defaunation on digesta kinetics (Demeyer 1989, Jouany et al. 1988). Meyer et al. (1986) did not detect an increase in RUP flow in defaunated animals.

A decrease in bacterial biomass after inoculation of protozoa into defaunated rumens also is observed at the duodenum, probably reflecting increased bacterial predation of bacteria and increased competition for dietary nutrients from protozoa. Bacterial protein flow to the duodenum may nearly double after defaunation (Table 2). When protein yield is expressed in efficiency terms as amount of microbial N formed per unit of organic matter fermented, the improvement of microbial protein synthesis due to defaunation ranges from 40 to 125% (Table 3). Such a large response may be explained both by an increase in net synthesis and a decrease in the amount of ruminally fermented organic matter when protozoa are eliminated from the rumen. There is no explanation for the decrease in duodenal flow of bacterial N reported by Punia et al. (1987) after defaunation.

As a consequence of increases in both dietary and microbial proteins entering the duodenum of ruminants, the intestinal supply of nonammonia N is always significantly improved by defaunation (Table 2). The effect of defaunation on duodenal supply of nonammonia N is greater when animals are fed diets more favorable to protozoal growth (Ushida et al. 1991).

As shown by Veira et al. (1984), Ivan et al. (1991b) and Hsu et al. (1991b), total amino acid flow to the

TABLE 2
Effect of defaunation on duodenal nitrogen (N) flow¹

Reference	Nonammonia N		Feed N		Microbial N		Method ²	Protozoal N
	F	D	F	D	F	D		
	<i>g/d</i>							<i>% of microbial N</i>
Lindsay and Hogan (1972)	18	21	6	7	12	14	DAPA	—
	—	—	11	13	18	19	DAPA	—
Weller and Pilgrim (1974)	—	—	—	—	—	—		20
Ulyatt et al. (1975)	—	—	—	—	—	—		3.7–6.9
Jouany (1978)	—	—	—	—	—	—		10–55
Harrison et al. (1979)	—	—	—	—	—	—		23–28
Collombier (1981)	23	28*	9	10	14	18*	DAPA	—
Steinhour et al. (1982)	—	—	—	—	—	—		32–51
	—	—	—	—	—	—		37.5–58.1
Borhami et al. (1983)	—	—	—	—	—	—		10.6–44.7
Jouany and Thivend (1983)	25	25	9	9	16	16	DAPA	15
Michalowski and Harmeyer (1983)	—	—	—	—	—	—		20–30
Veira et al. (1983)	16	17	—	—	—	—	—	—
Cockburn and Williams (1984)	—	—	—	—	—	—		10–46
John and Ulyatt (1984)	—	—	—	—	—	—		11.0–19.0
Ushida et al. (1984)	23	33	11	15*	12	18*	DAPA	21
	—	—	8	15*	15	18	Purines	—
Veira et al. (1984)	—	—	—	—	—	—		16.0–30.0
Punia et al. (1984a)	—	—	—	—	—	—		26–29
Punia et al. (1984b)	—	—	—	—	—	—		21
Rowe et al. (1985)	19	22*	7	7	12	15	¹⁵ N	—
Kayouli et al. (1986)	23	30*	6	14	16	16	DAPA	—
	—	—	9	9	10	17	DAPA	—
Meyer et al. (1986)	19	22	7	5	8	16*	DAPA	36
	—	—	—	—	12	17*	³⁵ S	—
Ushida et al. (1986)	24	30	9	11*	15	19	DAPA	7
	—	—	8	12*	16	18	—	—
Punia et al. (1987)	50.6	57.0	7.7	23.3	42.9	33.7	DAPA	23.7
	51.0	52.4	7.3	23.1	43.7	29.3	DAPA	26.8
Itabashi et al. (1989)	40.2	42.2	—	—	—	—		—
Ushida et al. (1990)	13	16*	8	8	6 ^a	8*		22
	13	19*	7	9	7 ^a	9*		32
Broudiscou et al. (1994)	16.4	23.9	7	10	9.4	13.9*		—
Punia and Leibholz (1994)	—	—	—	—	—	—		26.1–29.4

¹ F = Faunated animals; D = defaunated animals; DAPA = diaminopimelic acid.

² Marker method used to determine proportion of microbial N in duodenal N flow.

* Significant effect of defaunation ($P < 0.05$).

duodenum is significantly increased by defaunation. These authors observed that duodenal flow of both essential and nonessential amino acids increased to the same extent after defaunation. However, the increase was greatest for alanine and least for lysine. This reflects the large increase in the proportion of bacterial protein in the duodenal flow after defaunation, because bacteria have higher alanine and lower lysine contents than protozoa (Cockburn and Williams 1984, Czerkowski 1976). It also indicates that although the outflow of protozoa from the rumen may be small, their influence on microbial protein synthesis can significantly alter amino acid distribution in the duodenal digesta of faunated animals.

Microbial protein quality and digestibility in the small intestine

Unless the protein is digestible, an increase in bacterial nonammonia N flow to the small intestine will not supply more amino acids to the host animal. Using ³⁵S- and ¹⁵N-labeled mixed microbes, intestinal digestibility of microbial protein was estimated to be 85% (Salter and Smith 1977, Siddons et al. 1985). Storm et al. (1983) found that 81% of bacterial N disappeared in the intestine of sheep fed by intragastric infusion. Gulati et al. (1989) reported an apparent intestinal digestibility of ¹⁴C-labeled amino acids in fungal proteins is >90%. Although not measured in ruminants,

TABLE 3

Effect of defaunation on ruminal microbial protein yield

Reference	Microbial protein yield	
	Faunated	Defaunated
	<i>g N incorporated/kg fermented organic matter</i>	
Meyer et al. (1986)	27.4	42.7*
Kayouli et al. (1986)	34.9	49.6
	18.2	40.7
Ushida et al. (1986)	26.9	60.6*
	37.3	59.2*
Ushida et al. (1989)	18.0	26.0*
Ushida et al. (1990)	17.5	26.3*
	17.8	32.5*
Broudiscou et al. (1994)	18.5	34.5*

* Significant effect due to defaunation ($P < 0.05$).

McNaught et al. (1954) showed that the true digestibility of protozoal protein was greater than that of bacterial protein in rats. Thus, increasing the outflow of protozoa from the rumen may be effective to both limit intraruminal microbial protein turnover and to improve the protein status of productive ruminants. However, the concentration of plasma free amino acids is higher in defaunated animals (Itabashi et al. 1983, Itabashi et al. 1990, Itabashi and Matsukawa, 1979), which probably indicates that more amino acids are available for absorption when protozoa have been eliminated from the rumen.

Losses of N in the feces and urine

Data summarized from the literature indicate that fecal N losses increase after defaunation (Table 4). The lower ruminal digestion of cell wall carbohydrates that is observed generally after defaunation is compensated for by greater digestion in the large intestine (Ushida et al. 1991). As a consequence, there is more microbial protein formed in the large intestine and greater outflow in the feces. Conversely, urinary N always decreases with defaunation. This probably results from both decreased ammonia concentration in ruminal fluid and increased capture for microbial protein synthesis of blood of urea N recycled to the large intestine. Thus, differences in total N losses between faunated and defaunated animals largely disappear, and the net effect of defaunation is a shift of N excretion from the urine to the feces (Table 4).

Effect of defaunation on animal performance

Reported effects of defaunation on animal performance are contradictory (Table 5). Live-weight gains in defaunation studies must be considered with care, because chemical defaunation often results in short-term decreases in feed intake and body weight (Demeyer et al. 1982). Furthermore, as noted by Chalmers et al. (1968), defaunated animals often are pot-bellied, indicating an increase in the weight and volume of digesta in the rumen and large intestine; this may account for some of the reported differences in live-

TABLE 4

Effect of defaunation on N excretion and ruminal ammonia concentration¹

Reference	Species	Fecal N		Urinary N		Total N		Ruminal NH ₃ -N	
		F	D	F	D	F	D	F	D
		<i>g/d</i>						<i>mg/L</i>	
Lindsay and Hogan (1972)	Sheep	5.4	5.9	—	—	—	—	271	220
		7.2	8.2	—	—	—	—	208	193
Ikwuegbu and Sutton (1982)	Sheep	3.0	3.6	7.2	6.1	10.2	9.7	167	71
Rowe et al. (1985)	Sheep	3.6	5.3	—	—	—	—	104	104
		8.3	9.2	10.7	9.3	19.0	18.5	—	—
		10.7	11.8	12.8	11.5	23.5	23.3	—	—
Ushida et al. (1986)	Sheep	8.0*	8.6	—	—	—	—	191*	98
		7.4*	8.0	—	—	—	—	140*	54
Punia et al. (1987)	Cattle	26.8	29.6	44.2	39.4	71.0	69.0	190*	171
		26.6	27.3	15.6	12.3	42.2	39.6	23*	9
Kruezer and Kirchgessner (1988)	Sheep	5.5	5.0	11.2	10.2	16.7	15.2	—	—
		5.5	6.2	10.1	8.1	15.6	14.3	—	—
		6.0	6.3	8.5	8.0	14.5	14.3	—	—
		7.7	7.7	10.8	11.4	18.5	19.1	—	—
		7.4	7.6	10.5	10.7	17.9	18.3	—	—
Hsu et al. (1991c)	Sheep	6.6	7.3	—	—	—	—	235	154

¹ F = Faunated animals; D = defaunated animals.

* Significant effect of defaunation ($P < 0.05$).

TABLE 5
Effect of defaunation on animal performance¹

Reference	Species	Performance		Feed intake	
		F	D	F	D
Milk yield and feed intake, kg/d					
Yang and Varga (1989)	Cows	27.7	24.6	19.4	18.1
		23.6	20.8	—	—
Wool growth, g/d					
Fenn and Leng (1990)	Sheep	65	81	—	—
		76	84	—	—
Bird and Leng (1984)	Lamb	8	11	—	—
Weight gain, g/wk and milk intake, L/d					
Eadie and Gill (1971)	Lamb	221	231	1.7	1.7
		274	227	0.27	0.27
		141	144	0.9	0.84
		87	-61	1.2	1.2
		71	75	0.30	0.30
Weight gain and intake feed, g/d					
Abou Akkada and El Shazly (1964)	Lamb	120	96	—	—
		96	111	—	—
		91	106	—	—
Christiansen et al. (1965)	Lamb	280	210	—	—
		Borhami et al. (1967)	Calf	380	330
400	310			169	175
360	240			163	150
410	320			159	174
Williams and Dinusson (1973)	Calf	equal	equal	—	—
		Boodoo et al. (1978)	Calf	122	244
78	290			—	—
Bird and Leng (1978)	Calf	451	490	3760	3650
		530	757*	4150	4230
Bird et al. (1979)	Lamb	0	35	390	455
		70	130	645	690
		140	160	660	685
		180	150	745	735
		equal	equal	—	—
Itabashi and Matsukawa (1979)	Calf	66	46*	495	447
		Ramaprasad and Ragahavan (1981)	Lamb	181	213*
102	140*			878	964
239	192			1766	1895
135	208*			1127	1530*
equal	equal			—	—
Archer et al. (1984)	Sheep	122	135	865	890
		8 ^b	11	—	—
Bird and Leng (1984)	Lamb	122	135	865	890
		8 ^b	11	—	—
		122	132	870	930
		88	125*	1015	1085
Van Nevel et al. (1985)	Lamb	109	99	1166	1189
		100	120*	1085	1114
		217	204	—	—
Ivan et al. (1992)	Lamb	58	72	825	840
		Bird et al. (1994)	Sheep	41	14
-20	-1			535	710
-69	-62			455	510

¹ F = Faunated animals; D = defaunated animals.

* Significant effect of defaunation ($P < 0.05$).

weight gain between defaunated and faunated animals. Also, the specific effects of the defaunating treatment may be different from the sensu stricto effects of protozoal elimination. For instance, Kreuzer (1986) reported that the effects on growing ruminants depended on the method used for defaunation. Use of chemicals to kill

protozoa may influence other aspects of the ruminal ecosystem as shown by Eadie and Shand (1981).

Greater flow of protein to the duodenum consequent to defaunation explains why it improves growth of young animals, which have high protein requirements, when they are fed low protein diets [Jouany et al. 1988,

Ushida et al. 1991, Veira 1986). For the same reason, birthweight of lambs born to defaunated ewes grazing pasture was significantly greater than lambs from faunated ewes in two of three experiments (Bird and Leng 1985 cited by Bird 1989). It should be noted that Ankrah et al. (1990b) observed no effect of defaunation on lamb growth in a study where protein utilization also was unaffected.

The effect of defaunation on wool growth is more consistently positive (Table 5). Such a result may be explained by a greater supply in defaunated animals of sulfur amino acids, which are first limiting for wool production (Ivan 1989).

Only the report of Yang and Varga (1989) is available on the effect of defaunation on milk production in dairy cows. Yield of milk, total solids, ash and lactose was reduced in defaunated cows. This may be a direct result of greater feed intake in faunated animals. However, elimination of protozoa decreased milk lactose content but significantly increased milk protein content. Milk fat from faunated cows contained a greater proportion of short-chain fatty acids than that of defaunated cows; this may result from greater formation of ruminal butyrate in the former. The lower proportion of unsaturated fatty acids in milk from faunated cows likely is a direct effect of protozoa, mainly entodiniomorphid ciliates, on ruminal biohydrogenation of lipids.

Increased fecal energy losses are more than compensated for by decreased methane and urinary losses, resulting in improved metabolizability of dietary energy in defaunated animals (Kreuzer et al. 1986, Whitelaw et al. 1984). Nolan (1989) proposed that the fauna-free state promotes an increased protein:energy ratio in absorbed nutrients, which improves animal performance when essential amino acids are the primary limiting nutrients. However, Bird (1989) observed that live-weight gain was greater when ciliate-free animals were fed either high or low quality forage diets; the same effect also was noted in grazing animals. The most likely cause of negative responses to defaunation may be decreased ruminal digestibility of plant cell walls and the consequent lower availability of digestible energy for animal production. A depression in live-weight gain generally has been observed in defaunated animals fed diets rich in cereal starch. This result may be explained by ingestion by protozoa of large amounts of starch and the associated bacteria, which stabilizes conditions in the rumen of faunated animals and decreases the negative effects on ruminal cellulolysis, such as depressed pH, which may occur with feeding of high starch diets.

Potential practical methods to achieve defaunation

It was shown in early studies (Beckert 1929) that it is possible to eliminate protozoa from the ruminal ecosystem. Several methods are available for research

purposes. Some are based on use of chemicals that are toxic to protozoa: copper sulfate (Beckert et al. 1929, Christiansen et al. 1965); dioctyl sodium sulfosuccinate (Abou Akkada et al. 1968, Orpin 1977); alcohol ethoxylate or alkanates (Bird et al. 1979, Bird and Leng 1978, Bird and Leng 1984); calcium peroxide (Demeyer 1982); coconut oil (Newbold and Chamberlain 1988, Sutton et al. 1983); linseed oil (Broudiscou and Lassalas 1991, Ikwuegbu and Sutton 1982); and soya oil hydrolysate (Broudiscou et al. 1990). Another method involves isolating young animals within a few hours of birth before protozoa become established (Abou Akkada and El Shazly 1964, Borhami et al. 1967, Chalmers et al. 1968, Eadie and Gill 1971, Williams and Dinusson 1973). Defaunation also can be achieved by emptying the rumen, carefully washing the ruminal mucosa and treating the digesta by heating or freezing before reintroducing it into the rumen. Heating ruminal contents to 50°C for 15 min kills holotrich ciliates (Eadie and Oxford 1957), whereas freezing then thawing the digesta eliminates all protozoa (Jouany and Senaud 1979). Gossypol, a naphthalene derivative found in cottonseed, has been shown in vitro to kill many of the protozoa but to maintain holotrichs alive (Ismartoyo et al. 1994).

Recent studies have been carried out on defaunation of animals in practical settings. Lu and Jorgensen (1987) found that increasing the level of saponin infused in the rumen decreased protozoal numbers. Based on this report, several scientists tried feeding saponin-rich plants to ruminants (Navas-Camacho et al. 1984). Navas-Camacho et al. (1993) noted that addition of 300 g/d of sun-dried leaves of *Enterolobium ciclocarpum* to the diet of sheep decreased entodiniomorphs and holotrichs numbers by 35 and 90%, respectively, without affecting bacterial concentration. The fungal population, estimated from sporangia numbers, increased by 45% in treated animals. Although dry matter digestibility and N retention were lowered, wool growth rate was increased by 47% and daily live-weight gain by 44%. The authors explained these findings as due to increased amino acid absorption in the intestine resulting from increased flow of dietary and bacterial protein in response to defaunation with the supplementation of leaves from *E. ciclocarpum*.

Feeding large amounts of starch in high concentrate diets usually depresses pH and increases total volatile fatty acids (VFA) concentration and molar proportion of propionic acid in the rumen. As a consequence, ad libitum intake of high starch diets may result in elimination of protozoa from the microbial ecosystem (Eadie et al. 1970). However, defaunation under these conditions is only apparent because protozoa reappear soon after animals are fed a diet with reduced concentrate content.

Once defaunated, animals normally must be maintained isolated from any contact with other ruminants to remain protozoa-free. This constraint is difficult to comply with under field conditions. Thus, a successful

defaunation treatment for use in feeding practice would be one that could be applied continuously throughout the desired period.

In conclusion, it is clear that defaunation may yield improved N utilization by ruminants by increasing amino acid supply to the intestines and by reducing urinary N excretion; reduced urinary N losses would have favorable effects on the environment. Qualitative control of the composition of the protozoal population is an alternative approach to improving N utilization. The nutritional consequences of modifying the ruminal protozoal population should consider not only the effects on N utilization by ruminants but also the many interrelated factors, such as cellulolysis, VFA production, methanogenesis, kinetics of digesta passage, starch degradation, ruminal pH and redox potential, ruminal osmolality, fatty acid hydrogenation, choline supply from protozoal phosphatidyl-choline and sensitivity to pesticides and plant or yeast toxins. The resultant effects of defaunation involve many interactions when studies are carried out at the whole animal level, and N metabolism represents only one part of these integrated processes. The effect of defaunation on N utilization will predominate when productive ruminants are fed protein-limiting diets.

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