Metabolic changes in the rumen following protozoal inoculation of fauna-free sheep fed a corn silage diet supplemented with casein or soybean meal

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Original article

Metabolic changes in the rumen following protozoal inoculation of fauna-free sheep fed a corn silage diet supplemented with casein or soybean meal*

M Ivan**, LL Charmley, L Neill, M Hidiroglou

Animal Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A OC6

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Summary – Fauna-free wethers were fed bi-hourly a corn silage diet containing casein or soybean meal as a protein supplement. The wethers were inoculated *via* rumen cannula with a mixed population of ruminal ciliate protozoa. Ruminal fluid was sampled daily for 4 d before and for 13 d (and on d 28) after inoculation. Protozoal populations reached peak numbers on d 8 and decreased to new levels after d 9 for wethers on both supplements. Protozoa decreased ($P < 0.01$) the concentrations of total volatile fatty acids, increased ($P < 0.01$) the pH and decreased ($P < 0.01$)he concentrations of total and non-ammonia nitrogen in ruminal fluid. The concentrations of ammonia nitrogen increased with increasing numbers of protozoa for wethers on both supplements, but the concentrations decreased after d 7 to approximately pre-inoculation levels for the casein-supplemented diet. The increasing numbers of protozoa were associated with the increased concentrations of total and free α-amino nitrogen ($P < 0.01$) and sulfide ($P < 0.05$) and with the decreased concentrations of soluble Cu ($P < 0.05$) in the ruminal fluid in soybean meal-supplemented wethers but not in those receiving casein. It was concluded that dietary proteins with differing physical characteristics are metabolized to a different extent by ruminal ciliate protozoa, which in turn can affect the metabolism of other dietary nutrients such as nitrogen and sulfur and contribute to copper–sulfur interaction.

protozoa / casein / soybean meal / ruminal metabolism / sheep

Résumé – Modifications métaboliques dans le rumen après inoculation de protozoaires chez des moutons exempts de faune et nourris à l'ensilage de maïs supplémenté par de la caséine ou de la farine de soja. *Des moutons exempts de faune ont été nourris toutes les 2 h avec de l'ensilage de maïs contenant, soit de la caséine, soit de la farine de soja comme supplément protéique. Les moutons ont reçu, au moyen d'une canule dans le rumen, un mélange de protozoaires de rumen. Le liquide ruminal a été prélevé chaque jour pendant 4 j avant l'inoculation et 13 j (ainsi qu'au jour 28)*

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** Correspondence and reprints

après l'inoculation. La population de protozoaires a atteint sa valeur maximale au jour 8 et a décru les jours suivants chez tous les moutons. La présence de protozoaires a entraîné une diminution ($P < 0.01$) de la concentration en acides gras volatils totaux, une augmentation ($P < 0.01$) du pH et une diminution ($P < 0.01$) des concentrations de l'azote non ammoniacal dans le liquide ruminal. Les concentrations d'azote ammoniacal ont augmenté en même temps que le nombre de protozoaires chez tous les moutons, mais les concentrations ont diminué après le jour 7 à un taux approximativement égal à celui observé avant l'inoculation chez les moutons qui ont reçu de la caséine. L'augmentation du nombre de protozoaires a été associée à l'augmentation des concentrations de l'α-amino azote libre et total ($P < 0.01$) et du sulfure ($P < 0.05$) et à une diminution du cuivre soluble ($P < 0.05$) dans le liquide ruminal des moutons supplémentés en farine de soja mais pas dans celui de ceux qui ont reçu de la caséine. On peut conclure que les protéines alimentaires qui présentent des caractéristiques physiques différentes sont métabolisées de façon différente par les protozoaires ciliés du rumen, ce qui peut affecter le métabolisme des autres substances nutritives comme l'azote et le soufre, et contribuer à une interaction entre le cuivre et le soufre.

protozoaires / caséine / farine de soja / métabolisme ruminal / mouton

INTRODUCTION

Recent *in vitro* studies (Hino and Russell, 1987; Michalowski, 1989) suggest that the source of dietary protein is an important factor affecting its susceptibility to fermentation by ciliate protozoa in the rumen. Therefore, the many conflicting reports regarding the effects of protozoa on the metabolic parameters of ruminal fermentation (Veira, 1986) are probably a result of differences in physical characteristics such as solubility (Michalowski, 1989) or particle size (Coleman and Sandford, 1979) of the dietary proteins fed. Recent findings show that ruminal ciliate protozoa decrease the bioavailability and liver uptake of copper (Cu) in sheep receiving soybean meal (SBM) (Ivan *et al*, 1986; Ivan, 1988, 1989) but not in sheep receiving casein (CA) (Ivan, 1989) as a protein supplement. Differences in metabolism of the 2 supplements by the protozoa and hence in the production of sulfide (which decreases the biovailability of dietary Cu (Suttle, 1974)) by the ruminal bacteria was suspected as being a major factor in the interaction between ruminal protozoa, dietary protein and dietary Cu metabolism. This hypothesis was tested in the present experiment by illustrating metabolic changes after the ruminal inoculation with a mixed population of ciliate protozoa. The protozoal population was expected to be stable after 8 d following the inoculation (Veira *et al*, 1984).

MATERIALS AND METHODS

Eight wethers (Canadian Arcott) containing surgically implanted rumen and duodenal cannulas were used. The surgery had been performed at least 2 months before the measurements were started. The wethers, weighing 40–49 kg, originated from a fauna-free flock; they were thus fauna-free from birth. Fourteen d before the initiation of measurements, the wethers were allocated to 2 groups of 4 and

placed in plastic metabolism cages (Ivan and Hidiroglou, 1980) in a temperature-controlled room. One group received a CA-supplemented corn silage diet (CA diet) and the other an SBM-supplemented corn silage diet (SBM diet). Nine hundred g dry matter (DM)/d was delivered to each wether in 12 equal portions, at 2-h intervals, by automatic feeder (Buckley *et al*, 1985). The intake of each wether was maintained constant for the duration of the experiment. The composition of the diets is summarized in table I. The wethers had continuous access to drinking water.

At the beginning of the experiment all 8 wethers were fauna-free. After receiving experimental diets for 14 d, sampling of feed and ruminal fluid was carried out from all wethers for 17 d. Each wether was inoculated with a mixed population of ciliate protozoa after the first 4 d of sampling. Ruminal fluid was also sampled on d 28 after the inoculation.

Ruminal fluid, used for inoculating the experimental wethers, was obtained from 4 other sheep that had been fed a corn silage diet and had a normal protozoal population. The fluid was mixed and 100 ml portions centrifuged at 500 *g* for 3 min. The supernatant fraction of each portion was discarded and the protozoal fraction suspended in 100 ml of saline. Each wether was dosed with 100 ml of suspension *via* the rumen cannula.

Samples of ruminal fluid (100 ml) were obtained from each wether daily (between 10:00 and 11:00 h) *via* the rumen cannula, ≈ 1h after feeding. Samples were strained through 4 layers of cheesecloth and after measuring pH, the fluid was subdivided for analysis. One portion was immediately used for the determination of sulfide while the second portion was stored for other chemical analyses. The third portion was immediately centrifuged at 70 000 *g* for 30 min to obtain the soluble cell-free fraction.

Feed samples were freeze-dried, ground and the DM determined. Organic matter (OM) was calculated as DM loss after ashing at 550°C for 16 h. Acid detergent fiber (ADF) was estimated by the method of Goering and Van Soest (1970). Nitrogen (N) was determined by Kjeldahl digestion. α-Amino-N in the supernatant of rumen fluid and in hydrolyzed (6 N HCl) Gehrke *et al*, 1985) feed samples was measured by the ninhydrin method (Moore and Stein, 1954). Ammonia in ruminal fluid was determined by the phenol-hypo-

Table I. Composition of diets (dry matter basis).

Ingredient	*Diet*	
	Casein	*Soybean meal*
Composition (%)		
Corn silage	85.0	76.3
Casein	12.6	–
Soybean meal	–	21.3
Vitamin-mineral premix[a]	2.4	2.4
Chemical analysis		
Crude protein (%)	14.5	14.4
α-amino nitrogen (%)	1.55	1.57
Acid detergent fiber (%)	27.7	27.2
Ash (%)	7.0	6.5
Sulfur (%)	0.13	0.15
Copper (μg/g)	14.8	15.0

[a] The premix contained (g/kg) : 380 cobalt-iodized salt, 290 limestone, 197 biofos, 132 chromic oxide, 0.037 retinol, 0.005 cholecalciferol, 0.3 vitamin E and 1.3 (casein diet) or 0.9 (soybean meal diet) copper chloride ($CuCl_2 \cdot 2H_2O$).

chlorite reaction (Weatherburn, 1967). Determination of volatile fatty acids (VFA) in ruminal fluid was as described previously (Erfle *et al*, 1979). Sulfide in ruminal fluid was measured using a sulfide ion electrode (Khan *et al*, 1980). Counting of ciliate protozoa in ruminal fluid was as described previously (Veira *et al*, 1983).

Analysis of variances was used to test the statistical significance of differences between pooled means for d3–d0 and d9–d13 relative to inoculation of sheep with a mixed population of ciliate protozoa.

RESULTS

Protozoal numbers and pH

Microscopic examination of the ruminal fluid taken on the 4 consecutive days before the ruminal inoculation with protozoa confirmed the protozoa-free status of each wether. Following the inoculation, protozoal populations rapidly increased each day reaching a peak on d 8 for both the CA (199×10^4/ml) and SBM (166×10^4/ml) diets. However, after d 9 protozoal numbers decreased to new levels of $\approx 100 \times 10^4$/ml for wethers on both diets. Similarly, pH values gradually increased ($P < 0.01$) for both the CA and SBM fed wethers during the 8 d post-inoculation from an average of 5.8 and 6.0 respectively before the inoculation to 6.3 and 6.2, respectively after d 8 following the inoculation (table II).

Volatile fatty acids

The concentration of total VFA, in wethers on both diets, gradually decreased during the 9 d following the inoculation and remained approximately the same thereafter. It was significantly lower ($P < 0.01$) after d 9 following the inoculation than before the inoculation with protozoa (table III). The concentration of acetic acid was higher ($P < 0.05$) and isobutyric acid lower ($P < 0.01$) for wethers fed the SBM compared to those fed the CA diet. There were also lower ($P < 0.01$) molar proportions of isobutyric, isovaleric and valeric acids for wethers fed the SBM compared to those fed the CA diet. Faunation resulted in a decrease ($P < 0.01$) in the concentration of acetic and propionic acids and an increase ($P < 0.01$) in the concentration of isobutyric acid for wethers fed both diets. Acetic acid : propionic acid ratios were not affected by protozoa. Faunation also caused a decrease ($P < 0.01$) in the molar proportion of acetic acid and an increase ($P < 0.01$) in the proportion of isobutyric and butyric acids. None of the interactions between protein and protozoa were significant ($P > 0.05$).

Nitrogen

Faunation decreased ($P < 0.05$) the total-N and non-ammonia-N (NAN) concentration (table II) in ruminal fluid of wethers on both diets. The decrease in wethers fed the CA diet corresponded with the increasing population of ruminal protozoa and reached a peak on d 9 (fig 1). In wethers fed the SBM diet the concentration of total-N and NAN decreased rapidly during the first 5 d following the protozoal inoculation and remained approximately the same throughout the rest of the experiment. However, the effects of protein or of interaction between protein and protozoa were not signifi-

Table II. Mean pH and concentrations of nitrogen (mg/100 ml), copper (μg/ml) and sulfide (μg/ml) in rumen fluid during 4 d before (fauna-free) and 9–13 d after (faunated) inoculation with ciliate protozoa of wethers fed corn silage diet supplemented with casein or soybean meal

	Casein		*Soybean meal*		*Standard error*	*Statistical significance*		
	Fauna-free	*Faunated*	*Fauna-free*	*Faunated*		*Protein*	*Protozoa*	*Interaction*
pH	5.83	6.35	6.00	6.20	0.092	NS	$P < 0.01$	$P < 0.05$
Total nitrogen	183	151	189	168	11.4	NS	$P < 0.01$	NS
Non-ammonia nitrogen	161	129	176	149	11.8	NS	$P < 0.01$	NS
Ammonia nitrogen	22.2	22.6	12.9	19.2	1.69	$P < 0.01$	$P < 0.05$	$P < 0.05$
Soluble nitrogen	31.5	37.7	25.6	48.9	4.88	NS	$P < 0.01$	NS
Free α-amino nitrogen	2.75	3.58	2.66	7.92	1.010	$P < 0.01$	$P < 0.01$	$P < 0.01$
Soluble copper	0.153	0.162	0.146	0.110	0.0160	NS	$P < 0.05$	$P < 0.05$
Sulfide	4.10	3.78	3.92	4.81	0.354	NS	$P < 0.05$	$P < 0.05$

NS : not significant ($P > 0.05$).

Table III. Mean concentrations and molar proportions of volatile fatty acids in rumen fluid during 4 d before (fauna-free) and 9 – 13 d after (faunated) inoculation with ciliate protozoa of sheep fed corn silage diet supplemented with casein or soybean meal.

	Casein		*Soybean meal*		*Standard error*	*Statistical significance*		
	Fauna-free	*Faunated*	*Fauna-free*	*Faunated*		*Protein*	*Protozoa*	*Interaction*
Concentration (mmol/l)								
Acetic	51.70	43.73	58.94	47.97	3.271	$P < 0.05$	$P < 0.01$	NS
Propionic	17.86	14.35	19.36	16.33	1.101	NS	$P < 0.01$	NS
Isobutyric	1.13	1.25	0.71	0.90	0.057	$P < 0.01$	$P < 0.01$	NS
Butyric	6.79	6.95	7.94	8.12	0.818	NS	NS	NS
Isovaleric	2.18	2.09	1.80	1.99	0.316	NS	NS	NS
Valeric	1.34	1.20	1.19	1.02	0.123	NS	NS	NS
Total	81.00	69.57	89.94	76.33	5.162	NS	$P < 0.01$	NS
Molar proportion (%)								
Acetic	63.76	61.66	65.50	62.61	0.861	NS	$P < 0.01$	NS
Propionic	22.08	21.30	21.58	21.69	0.912	NS	NS	NS
Isobutyric	1.41	1.87	0.80	1.20	0.089	$P < 0.01$	$P < 0.01$	NS
Butyric	8.37	10.27	8.78	10.55	0.501	NS	$P < 0.01$	NS
Isovaleric	2.70	3.11	1.99	2.55	0.319	$P < 0.01$	NS	NS
Valeric	1.67	1.79	1.36	2.33	0.144	$P < 0.01$	NS	NS
Acetic: propionic	2.94	2.94	3.05	2.90	0.147	NS	NS	NS

NS : not significant ($P > 0.05$).

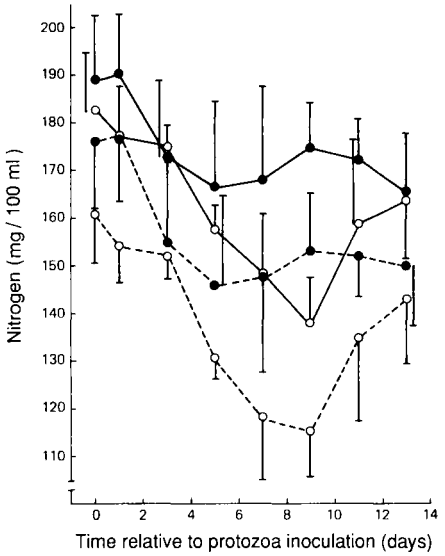

Fig 1. Total (-) and non-ammonia (---) nitrogen in ruminal fluid of fauna-free wethers fed a corn silage diet containing casein (o) or soybean meal (•) as protein supplement before and after inoculation with ciliate protozoa. Vertical bars are standard errors.

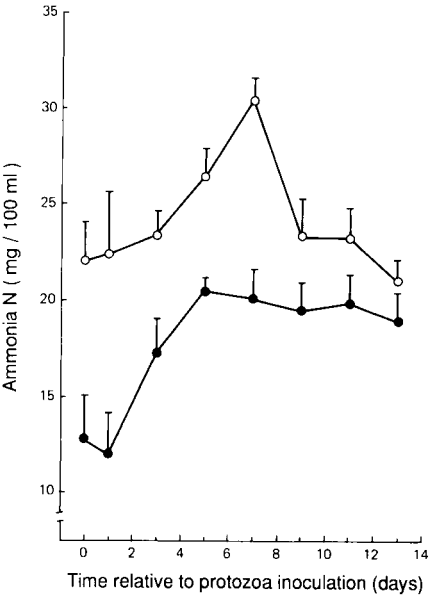

Fig 2. Ammonia-nitrogen in ruminal fluid of fauna-free wethers fed a corn silage diet containing casein (o) or soybean meal (•) as protein supplement before and after inoculation with ciliate protozoa. Vertical bars are standard errors.

cant ($P > 0.05$). The decreases in total-N and NAN concentrations corresponded to increases in ammonia-N concentrations (fig 2). The ammonia-N concentrations in ruminal fluid were higher ($P < 0.01$) for wethers fed the CA diet than for those fed the SBM diet (table II). For wethers fed the CA diet, the concentration increased from 22.2 mg/100 ml at d 0 to a peak value of 30.2 mg/100 ml on d 7, and decreased to 21.1 mg/100 ml by d 13 (fig 2). However, for wethers fed the SBM diet, the ammonia-N concentration increased from 12.9 mg/100 ml at d 0 to 20.5 mg/100 ml by d 5, and remained at approximately the same level until d 13. This resulted in significant effects ($P < 0.05$) of both protozoa and protein × protozoa interaction (table II).

The concentration of N and free α-amino-N (fig 3) in the soluble cell-free fraction of ruminal fluid of wethers fed the CA diet increased only slightly during the first 3 d following the inoculation with ciliate protozoa. They remained at approximately the same level thereafter. However, for wethers fed the SBM diet, these parameters substantially increased during the initial 7 d post-inoculation, and then stabilized between d 7 and d 13. There were significant effects ($P < 0.01$) of both protein and protozoa and of interaction between protein and protozoa on the concentrations of free α-amino-N in the soluble fraction of

ruminal fluid (table II). However, while the effect of protozoa on the concentration of soluble N was significant ($P < 0.01$) the effects of protein or of interaction between protein and protozoa were not.

Copper and sulfide

The concentration of Cu in the soluble, cell-free fraction of ruminal fluid (fig 4) was initially (d 0) similar for wethers fed the CA diet (0.153 μg/ml) and for those fed the SBM diet (0.146 μg/ml). The concentration decreased for both diets during the 1st d after the inoculation with protozoa, but during the following 4 d it further declined for wethers fed the SBM diet while it increased to approximately pre-inoculation level for those fed the CA diet. Although there were some changes in the Cu concentration after d 5 for both diets, it generally remained at approximately pre-inoculation level for wethers fed the CA diet (between 0.147–0.177 μg/ml) and much below the pre-inoculation level for those fed the SBM diet (between 0.097–0.117 μg/ml). The effects of protozoa and of interaction between protein and protozoa (table II) were significant ($P < 0.05$) but the effect of protein was not.

The concentration of sulfide in ruminal fluid (fig 4) was initially (d 0) similar for wethers fed the CA diet (4.10 μg/ml) and for those fed the SBM diet (3.92 μg/ml). For wethers fed the CA diet, the concentration increased marginally during the 1st d after the inoculation with protozoa but declined to below the initial value by d 3, and stayed at this level for the remainder of the experiment. The concentration of sulfide for wethers fed the SBM diet increased to a peak value of 5.53 μg/ml on d 7. The con-

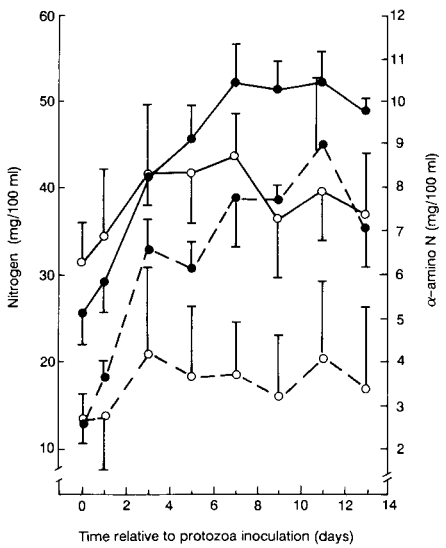

Fig 3. Nitrogen (-) and α-amino nitrogen (--) in soluble, cell-free fraction of ruminal fluid of fauna-free wethers fed a corn silage diet containing casein (o) or soybean meal (•) as protein supplement before and after inoculation with ciliate protozoa. Vertical bars are standard errors.

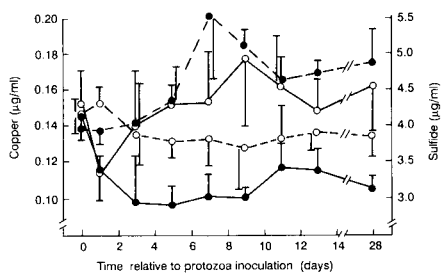

Fig 4. Sulfide in ruminal fluid (--) and copper in soluble, cell-free fraction of ruminal fluid (–) of fauna-free wethers fed a corn silage diet containing casein (o) or soybean meal (•) as protein supplement before and after inoculation with ciliate protozoa. Vertical bars are standard errors.

centration then decreased to 4.61 and 4.93 μg/ml on d 11 and d 28, respectively. The concentrations of sulfide in ruminal fluid (table II) were significantly affected ($P < 0.05$) by protozoa and by interaction between protein and protozoa but not by protein ($P > 0.05$).

DISCUSSION

Following the inoculation of fauna-free wethers with a mixed population of ciliate protozoa there was a rapid growth in protozoal numbers during the first 8 d for both the CA and SBM diet, a pattern similar to that previously found in this laboratory (Veira *et al*, 1984). This growth was associated with a rapid increase in the concentration of ammonia-N and a concomitant decrease in the concentrations of total-N and NAN (mainly for the CA diet), the latter probably due to increased bacterial turnover (Demeyer and Van Nevel, 1979). There was also a decrease in production of total VFA for both diets but the acetic : propionic ratio was not affected by protozoa. Protozoa increased the production of VFA when diets were deficient in S (Hegarty, 1989). This was not the case in the present experiment.

In *in vitro* incubations, 88 % of rumen bacterial protein turnover was due to ruminal protozoa, and the rate of turnover of bacterial protein in fauna-free sheep was only 6.4 % of that in faunated sheep (Wallace and McPherson, 1987). Protozoa can engulf large numbers of bacteria each minute (Coleman, 1975) and release the bacterial cellular contents into the surrounding medium (Coleman, 1964, 1975; Denholm and Ling, 1984). However, it should be noted that in the present experiment the decrease in the concentrations of total-N and NAN was much smaller for wethers fed the SBM diet than for those fed the CA diet, and the concentrations for the SBM diet remained almost constant after 5 d following the inoculation with protozoa. In contrast, the concentration of total-N and NAN for wethers fed the CA diet increased to new levels after d 9, as a consequence of decreased protozoal numbers. A reverse pattern to that of total-N and NAN was obtained for the concentration of ammonia-N. Also, there was virtually no effect of protozoal population on the concentrations of soluble-N, free α-amino-N and sulfide for wethers fed the CA diet, while there was a substantial increase in these parameters due to faunation of wethers fed the SBM diet. The above evidence suggests that there are differences in protozoal metabolism of CA and SBM, probably due to differences in ruminal solubility and physical characteristics of 2 protein supplements. The present *in vivo* observation is supported by recent *in vitro* experiments in which a consistent negative relationship between ciliate cell numbers and protein solubility was found (Michalowski, 1989). Also, protozoa were much more efficient at taking up bacteria-sized particles than soluble compounds or very small particles (Onodera and Kandatsu, 1970; Coleman and Sandford, 1979; Hino and Russell, 1987), and the extent of ingestion of bacteria by protozoa increased while the growth of protozoa decreased with increasing casein solubility (Michalowski, 1989). It ap-

pears, therefore, that the limitation of protozoa in utilizing casein (Hino and Russell, 1987; Michalowski, 1989) in the present experiment was responsible for the lack of major changes in the concentrations of ammonia-N, soluble-N, free α-amino-N and sulfide in the ruminal fluid of wethers fed the CA diet. However, the ability of ruminal protozoa to utilize SBM (Hino and Russell, 1987) resulted in the increased concentrations of soluble-N and free α-amino-N in the ruminal fluid following the protozoal inoculation of wethers fed the SBM diet. Bacterial metabolism of amino acids released by protozoal ingestion of SBM was probably responsible for the increased concentrations of ammonia-N and sulfide in the ruminal fluid of these wethers.

Before the inoculation with protozoa the concentration of ammonia-N in ruminal fluid of wethers fed the CA diet was approximately twice as high as that in the fluid of wethers fed the SBM diet, while the concentrations of sulfide were almost the same for both diets. Also, the concentrations of ammonia-N in ruminal fluid of wethers fed the CA diet increased following the inoculation with protozoa, while that of sulfide slightly decreased. Therefore, there was no similarity in the concentration of ammonia-N with that of sulfide for the CA diet. A similarity between these 2 concentations would be expected if amino acids are metabolized by ruminal bacteria and N released into the ammonia pool while S (from sulfur amino acids) is reduced to sulfide (Hume and Bird, 1970). Such similarity was obtained for the SBM diet. However, bacterial protein when degraded by protozoa is mostly excreted as peptides (Denholm and Ling, 1985) which are readily utilized by remaining bacteria, and the microbial protein turned-over by protozoal metabolism is not degraded beyond peptides (or free amino acids), while ammonia-N is liberated from bacterial nucleic acid (Hegarty, 1989). Therefore, microbial S and N are not degraded to their inorganic forms to an equal extent, because all cell S is in protein (Bird, 1973; Czerkowski, 1976), and may be recycled in peptide or amino acid form, while nucleic acid degradation will liberate ammonia-N but not sulfide. This would mainly apply to the CA diet in the present experiment.

The above evidence indicates that protozoa ingest SBM and thereby release inorganic and organic S. Both S forms are rapidly reduced by ruminal bacteria to sulfide and have a similar negative effect on Cu bioavailability (Suttle, 1974). However CA is poorly metabolized by protozoa; therefore, the protozoal requirement for N is to a large extent derived from an engulfment of ruminal bacteria which does not result in a significant production of sulfide (Hegarty, 1989). This difference in sulfide production explains why protozoa decrease both the solubility of dietary Cu in the stomach and the concentration of Cu in the liver of sheep fed SBM supplements (Ivan *et al*, 1986; Ivan, 1988, 1989), and why protozoa do not have an effect on dietary Cu metabolism when sheep are fed CA (Ivan, 1989) or urea (Ivan and Veira, 1982) supplements.

The results of the present experiment support our previous hypothesis that the protozoal effect on the

bioavailability of dietary Cu is associated with the increase in the availability of ruminal S (Ivan *et al*, 1986). This hypothesis has been substantiated by Australian researchers(Hegarty *et al*, 1989).

REFERENCES

Bird RP (1973) Sulphur metabolism and excretion studies in ruminants. XII. Nitrogen and sulphur composition of ruminal bacteria. *Aust J Biol Sci* 26, 1429-1434

Buckley DJ, Viera DM, Nicholls CF, Ivan M, St Amour G (1985) An automated device for controlled feeding of sheep in cages or pens. *Can J Anim Sci* 65, 273-277

Coleman GS (1964) The metabolism of *Escherichia coli* and other bacteria by *Entodinium caudatum. J. Gen Microbiol* 37, 209-223

Coleman GS (1975) The interrelationship between rumen ciliate protozoa and bacteria. *In : Digestion and Metabolism in the Ruminant* (McDonald IW, Warner ACI, eds) University of New England Publishing Unit, Armidale, Australia, 149-164

Coleman GS, Sandford DC (1979) The engulfment and digestion of mixed rumen bacteria and individual bacterial species by single and mixed species of rumen ciliate protozoa grown *in vitro*. *J Agric Sci* 92, 729-742

Czerkowski JW (1976) Chemical composition of microbial matter in the rumen. *J Sci Food Agric* 27, 621-632

Demeyer DI, Van Nevel CJ (1979) Effect of defaunation on the metabolism of rumen micro-organisms. *Br J Nutr* 42, 515-524

Denholm AM, Ling JR (1984) *In vitro* metabolism of bacterial cell wall by rumen protozoa. *Can J Anim Sci* 64 (suppl), 18-19

Denholm AM, Ling JR (1985) Release of diaminopimelic acid from bacterial cell walls by rumen protozoa. *Proc Nutr Soc* 44, 142A

Erfle JD, Mahadevan S, Sauer FD (1979) Effect of diet quality on adenosine-5′-triphosphate concentration and adenylate energy charge of rumen microbes from fistulated cows. *J Dairy Sci* 62, 284-291

Gehrke CW, Wall LL Sr, Absheer JS, Kaiser FE, Zumwalt RW (1985) Sample preparation for chromatography of amino acids : acid hydrolysis of proteins. *J Assoc Off Anal Chem* 68, 811-821

Goering GH, Van Soest PJ (1970) *Forage fiber analyses (apparatus, reagents, procedures, and some applications).* Agric Handbook 379. ARS, USDA, Washington, DC, pp 20

Hegarty RS (1989) Effects of protozoa on sulphur and nitrogen availability and the composition of bacteria in the rumen of sheep. Ph D Thesis, University of New England, Armidale, Australia

Hegarty RS, Nolan JV, Leng RA (1989) Evidence for protozoa influencing rumen sulphur availability. *In : The Roles of Protozoa and Fungi in Ruminant Digestion* (Nolan JV, Leng RA, Demeyer DI, eds) Penambul Books, Armidale, Australia, 305-306

Hino T, Russell JB (1987) Relative contributions of ruminal bacteria and protozoa to the degradation of protein *in vitro*. *J Anim Sci* 64, 261-270

Hume ID, Bird PR (1970) Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulphur. *Aust J Agric Res* 21, 315-322

Ivan M (1988) Effect of faunation on ruminal solubility and liver content of copper in sheep fed low or high copper diets. *J Anim Sci* 66, 1496-1501

Ivan M (1989) Effects of faunation and type of dietary protein on gastric solubility and liver content of copper in sheep. *J Anim Sci* 67, 3028-3035

Ivan M, Hidiroglou M (1980) The Ottawa plastic metabolism cage for sheep. *Can J Anim Sci* 60, 539-541

Ivan M, Veira DM (1982) Duodenal flow and soluble proportions of zinc, manganese, copper and iron in the rumen fluid and duodenal digesta of faunated and defaunated sheep. *Can J Anim Sci* 62, 979-982

Ivan M, Veira DM, Kelleher CA (1986) The alleviation of chronic copper toxicity in sheep by ciliate protozoa. *Br J Nutr* 55, 361-367

Khan SU, Morris GF, Hidiroglou M (1980) Rapid estimation of sulphide in rumen and blood with a sulphide-specific ion electrode. *Microchem J* 25, 388-395

Michalowski T (1989) Importance of protein solubility and nature of dietary nitrogen for the gowth of rumen ciliates *in vitro*. *In : The Roles of Protozoa and Fungi in Ruminant Digestion* (Nolan JV, Leng RA, Demeyer DI, eds) Penambul Books, Armidale, Australia, 223-231

Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J Biol Chem* 211, 907-913

Onodera R, Kandatsu M (1970) Amino acid and protein metabolism of rumen ciliate protozoa. IV. Metabolism of casein. *Jpn J Zootech Sci* 41, 307-313

Suttle NF (1974) Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. *Br J Nutr* 32, 559-568

Veira DM (1986) The role of ciliate protozoa in nutrition of the ruminant. *J Anim Sci* 63, 1547-1560

Veira DM, Ivan M, Jui PY (1983) Rumen ciliate protozoa : effects on digestion in the stomach of sheep. *J Dairy Sci* 66, 1015-1022

Veira DM, Ivan M, Jui PY (1984) The effect of ciliate protozoa on the flow of amino acids from the stomach of sheep. *Can J Anim Sci* 64 (suppl), 22-23

Wallace RJ, McPherson CA (1987) Factors affecting the rate of breakdown of bacterial protein in rumen fluid. *Br J Nutr* 58, 313-323

Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem* 39, 971-974