Physiol. Res. 45: 117–123, 1996

The Level of Faunation of Rumen in Relation to Some Factors of Nitrogen metabolism

J. FEJEŠ, J. VÁRADY[1]

Institute of Animal Physiology, Slovak Academy of Sciences, and [1]University of Veterinary Medicine, Košice, Slovak Republic

Received July 13, 1995
Accepted January 2, 1996

Summary

Our experiments on metabolic processes in the rumen were performed on sixteen 5-month-old lambs divided into 4 groups (defaunated - D, totally refaunated - T, partially refaunated - P and intact - I). The absence or presence of protozoa in the rumen did not significantly affect the pH values. The greatest differences in NH_3 concentration in the rumen before feeding were found between the T and D group ($P<0.05$). The animals of the T and I groups had higher NH_3 concentrations than the D and P groups 1–5 hours after feeding ($P<0.05$ to $P<0.001$). Blood urea concentrations before and after feeding were significantly higher in the group I compared to the other groups ($P<0.05$ to $P<0.001$). Significant differences in the total nitrogen in rumen fluid were only found between groups D and I. The values of protozoan nitrogen in the rumen and their mutual relationship among the groups could be expressed by the following ratios: I>T>P>D. Proportions of the values of bacterial nitrogen followed in this order: D>P>T>I. The animals in group D had a significantly higher level of residual nitrogen than those in the other groups ($P<0.05$).

Key words
Defaunation – Rumen – pH – NH_3 – Urea – Nitrogen – Lambs

Introduction

The ruminal content in ruminants is a complex mixture of the consumed feed and microorganisms. The microbial biomass is represented by bacteria, protozoa and fungi. Bacteria are the major and essential part of the biomass. The relative number of protozoa in the rumen is lower than the number of bacteria but their proportion in the microbial volume is 40–80 % (Harrison and McAllan 1980). Bacteria and protozoa participate in the hydrolysis of proteins but they differ in the process of degradation. About 40 % of the ruminal bacteria are proteolytically active. Their proteases are located on the external side of the bacterial membrane and they are easily liberated. Thus the process of proteolysis occurs on the bacterial cell surface.

Ciliata actively participate in ruminal digestion (Amos and Akin 1978, Demeyer 1981). The effect of protozoa is especially notable in the case of digestion of insoluble proteins and bacterial proteins (Ushida and Jouany 1985, Ushida *et al.* 1984, 1986, Hino and Russell 1987, Wallace 1989). Although protozoa extensively participate in ruminal digestion, they are not essential for the host and they may be removed from the rumen (Hungate *et al.* 1971, Weller and Pilgrim 1974, Leng 1982, John and Ulyatt 1984) and they apparently do not contribute to postruminal nourishment.

The fact that ruminal protozoa do not have to be a part of the ruminal heterogeneous microbial population, evoked interest in their relevance for the process of digestion. A major proportion (50–80 %) of the protein entering the small intestine of ruminants is of microbial origin. During this transformation, significant recirculation of protein nitrogen between protozoa and bacteria occurs which could be classified from the host's side as energetic loss.

Defaunation therefore appears to be a suitable way of manipulating ruminal fermentation which can restrict the degradation of dietary proteins in the

rumen as well as their increased "bypassing" into the duodenum. This possibility was also supported by the findings of Bird and Leng (1978) and Bird *et al.* (1979) who observed a positive effect on the growth rate of defaunated animals.

Inspired by both positive and negative reactions to defaunation of ruminants in relation with the utilization of food found in the literature, we carried out some experiments in which we investigated the influence of defaunation, total or partial refaunation on some factors of nitrogen metabolism in male lambs.

Material and Methods

The experiment was carried out on sixteen 5-month-old lambs of (21.8 to 23.9 kg b.w.) with ruminal fistulas. Each animal was fed a diet of 600 g pasture hay, 300 g supplementary mixture for lambs and 100 g dried sugarbeet pulp. The daily ration was 850 g dry matter, containing 79.2 g digestibility N substances, 0.48 starch units and 197 g fibres per individual sheep per day. A mineral mixture was given according to the CSN-467070. The lambs had free access to water and salt. The animals were divided into four groups (D, T, P, I) and were fed twice daily. Every group consisted of four lambs. They were housed in separate pens which allowed no contact between animals.

In the first period, the twelve animals of groups D, T and P were defaunated with Manoxol OT. Before the application of Manoxol OT the animals fasted. In the fourty-eighth and seventy-second hour of the fasting the animals were intraruminally given 0.1 g Manoxol per kg body weight. The dose of Manoxol was dissolved in 50 ml distilled water heated up to 39 °C. The success of defaunation was regularly controlled. After 21 days of defaunation the animals of the first group (D) were defaunated. The second group (T - totally defaunated lambs) was refaunated by 200 ml ruminal content per lamb, gained from the intact (I) group. The defaunated animals of the third group (P - partially refaunated) were inoculated with a monoculture of protozoa of *Epidinium ecaudatum caudatum* in the amount of 2100 individuals per animal. *Epidinium ecaudatum caudatum* was isolated by Coleman's method (1978). In the course of the subsequent three weeks the state of defaunation or refaunation was checked. After the period of adaptation, we took samples at hourly intervals before feeding up to the 5 hours after feeding.

The pH values in the ruminal contents were measured with a pH-meter (Radelkis) and the ammonia content was assessed by the micromethod of Conway and O'Malley (1942). The level of total, protozoal, bacterial and residual nitrogen in the rumen content was determined in the filtered ruminal fluid.

To obtain the protozoal suspension we centrifuged the ruminal fluid at 200x*g* for 2 min (1350 rpm). We washed the sediment with McDougall's solution and repeated the centrifugation until the vegetable residues had been removed entirely. The protozoal sediment was used for the measurements. The bacterial nitrogen fraction was taken from the ruminal fluid after centrifugation at 1000x*g* (3000 rpm) for 2 min. The supernatant was collected and centrifuged at 20 000x*g* for 60 min. The sediment was used for measuring the bacterial nitrogen fraction and the supernatant for assessing the residual nitrogen. The values of nitrogen were measured by an automatic nitrogen analyzer Macro N FOSS-Heraeus.

The results were expressed as means ± S.E.M. The statistical significance was assessed by Student's t-test.

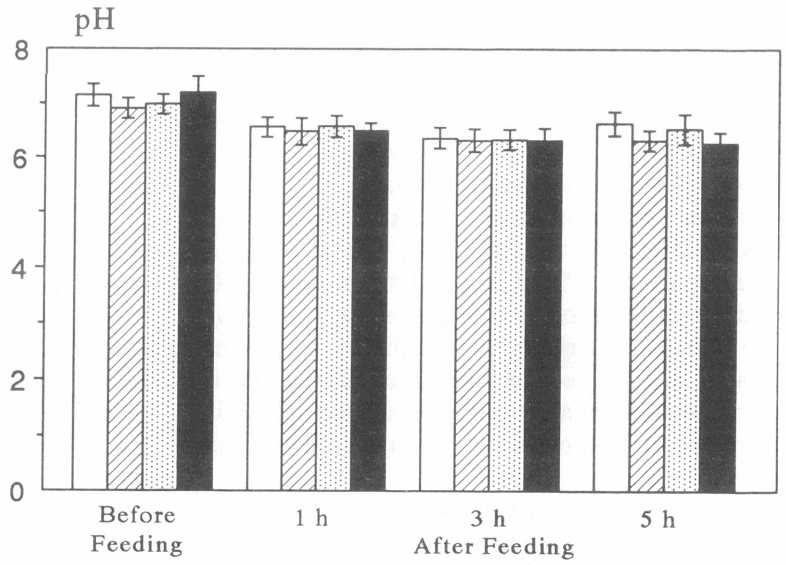

Fig. 1
The pH of rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Data are means ± S.E.M.

Results and Discussion

The pH values of the ruminal fluid during the period of observation are depicted in Figure 1. In a number of reports (Conrad *et al.* 1958, Luther *et al.* 1966, Whitelaw *et al.* 1972, Jouany and Senaud 1982) it is mentioned that the pH of ruminal content is lower in ciliate-free animals than in faunated animals. In our experiments on young rams fed a diet consisting of 60 % quality pasture hay, 30 % supplementary mixture for lamb breeding and 10 % dried sugarbeet slices, we did not observed any statistical difference in pH values either before feeding or during a period of 1 to 5 hours after the feeding among the particular groups. It is not possible to state the definite reason for the insignificant changes of pH values which we encountered during the experiments. We should consider the actual nutritional conditions and the few weeks' adaptation of the ruminal bacterial population to the feeding regime, which probably play a crucial role both in the defaunated and partially refaunated animals. Rowe *et al.* (1981) and Veira *et al.* (1984) observed similar changes in the pH values which were, however, different under conditions of continual feeding where the degradation of organic matter had the same course concerning the ciliate-free and faunated lambs.

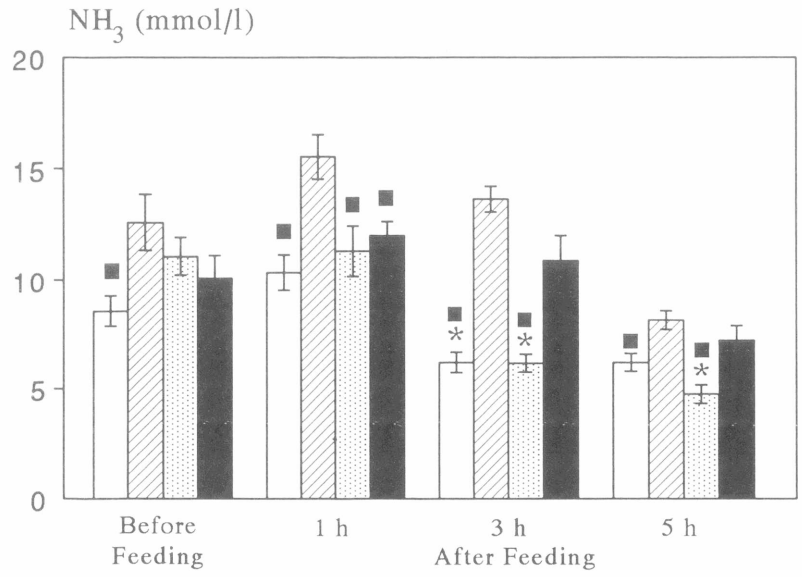

Fig. 2
Concentration of NH_3 in the rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: intact lambs – asterisk, totally refaunated lambs – full square.

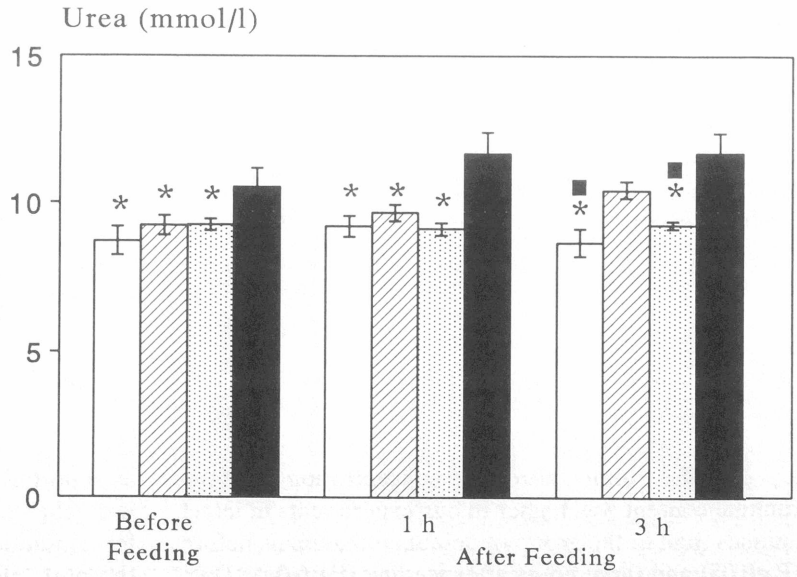

Fig. 3
Concentration of urea in the blood in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: intact lambs – asterisk, totally refaunated lambs – full square.

The different proportions of the protozoal population were markedly reflected in the NH_3 concentration in the ruminal fluid (Fig. 2) between the D and T groups in the period before the feeding ($P<0.05$). The NH_3 concentration was distinctly lower in groups D and P compared to the T and I groups,

during the observation periods, where the significance varied from $P<0.05$ to $P<0.001$. The lowered level of ammonia in the rumen is the most frequently observed effect of defaunation in general (Coleman 1980, Demeyer 1981, Veira *et al.* 1983, Kayouli *et al.* 1984). Leng and Nolan (1984) considered the increased recycling of microbial protein in the rumen, the number of bacteria utilizing ammonia and the extent of degradation of dietary proteins as the probable reasons for this situation. Demeyer and Van Nevel (1979) proved that protozoa do not change the entire spectrum of the bacterial population *in vitro*. The absence of protozoa was considered to lower the overall synthesis of microbial proteins in the rumen. Ushida *et al.* (1986) observed significant differences in the NH_3 levels between defaunated or faunated animals in relation to the volume of the highly degradable proteins in contrast to the insoluble proteins. The animals used in our experiments were fed a diet of the same composition – 30 % of the feeding dose was composed of a 300 g supplementary mixture for lambs. The mixture contained: 17 % wheat bran, 16 % rye bran, 9 % extracted sunflower meal, 25 % barley, 25 % oat, 2.5 % urea. The supplementary mixture contributed to more than 53 % of the total amount of digestible nitrogen substances in the diet.

The presence of protozoa (Schinchi *et al.* 1986, Wallace *et al.* 1987) increases the proteolytical activity of the microbial population. Their significance is emphasized by the turnover of the insoluble and bacterial proteins (Hins and Russell 1987, Wallace and McPherson 1987). The protozoan ability to metabolize urea is not clear. Onodera *et al.* (1977), came to the conclusion that protozoa are not ureolytical and their ureolytical activity is dependent on bacteria. Some authors (Lewis *et al.* 1957, Juhász 1959, McIntyre 1970) reported that the urea concentration in the blood positively correlated with the amount of consumed digestible nitrogen-substances and the NH_3 concentration in the rumen. As has been shown in our experiment (Fig. 3), the level of urea in the blood was always higher in the group of intact animals compared to the other groups during the observation period. The values of urea concentration in blood withdrawn from the jugular vein in the totally refaunated group were significantly higher ($P<0.05$) than in the D and P groups three hours after feeding. Since the animals of all groups in our experiment were fed a diet with identical amounts of digestible nitrogen-substances, we suppose that the composition of the ruminal microbial population is an important factor influencing the level of urea in the blood. The presence or absence of protozoa in the rumen may have a direct influence on blood urea levels. Our finding confirms the assumption of Leng and Nolan (1984) about the increased recycling of microbial protein in the rumen during the presence of protozoa.

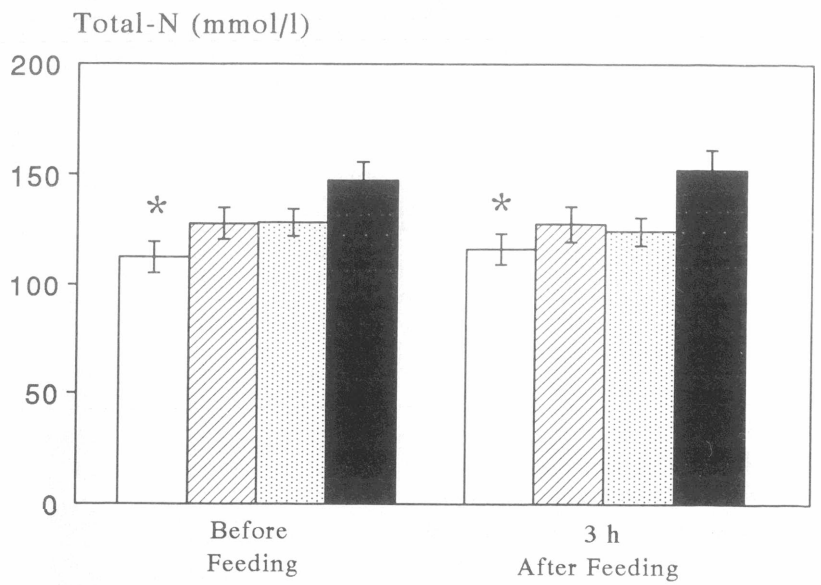

Fig. 4
Concentration of total nitrogen in the rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: intact lambs – asterisk.

The total nitrogen concentration in the ruminal content was higher in our experiments in intact animals than in the group of defaunated animals before ($P<0.05$) and three hours after feeding ($P<0.001$) (Fig. 4). The total nitrogen concentration in groups T and P was higher than in the D group but was lower than in the I group. Nevertheless, these differences were not statistically significant. The defaunation of ruminants, as reported by Kreuzer *et al.* (1986) and Veira (1986), was the cause of changes not only in ruminal fermentation but also in intermediary metabolism of the host animal. A generally accepted opinion is that the degradation of dietary proteins in the defaunated rumen is lower than in the faunated one, especially in the case when the dietary source contains insoluble proteins.

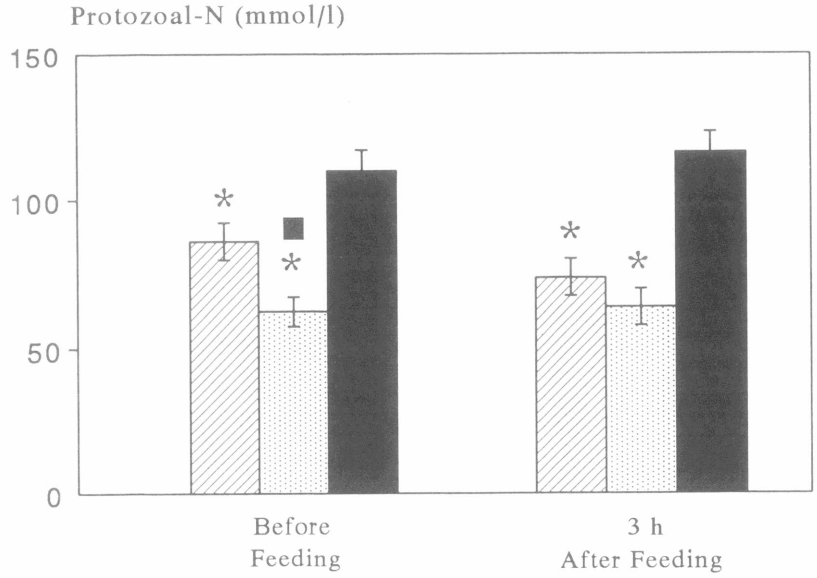

Fig. 5
Concentration of protozoal nitrogen in rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: intact lambs – asterisk, totally refaunated lambs – full square.

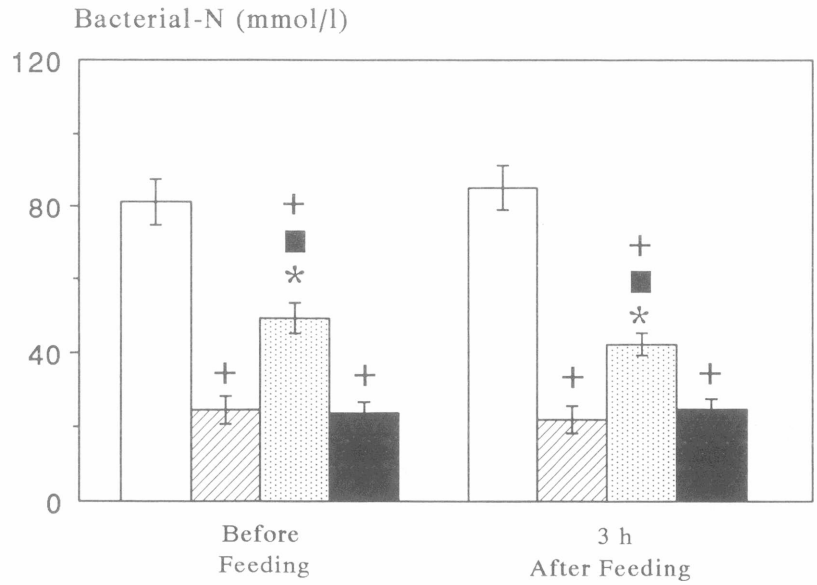

Fig. 6
Concentration of bacterial nitrogen in rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: intact lambs – asterisk, totally refaunated lambs – full square, defaunated lambs – cross.

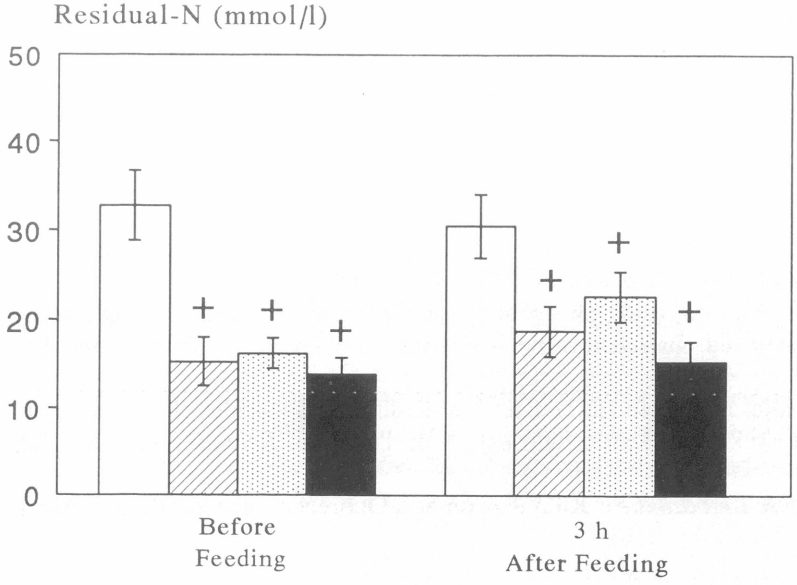

Fig. 7
Concentration of residual nitrogen in the rumen contents in lambs. Defaunated lambs (open columns), totally refaunated lambs (hatched columns), partially refaunated lambs (dotted columns) and intact lambs (full columns). Significantly different ($p<0.05$) from: defaunated lambs – cross.

Eadie and Gill (1971), discovered significantly higher concentrations of ruminal ammonia in faunated animals but they did not see any differences in total nitrogen or all the fractions of non-protein nitrogen. Unlike the above cited authors we found certain significant differences in the values of total, protozoan, bacterial and residual nitrogen. These differences in the values of nitrogen fractions observed during our experiments may be generalized to a certain extent due to the different proportions of the ruminal microflora or the presence of protozoa in the rumen. Among the other factors which might quantitatively or qualitatively, directly or indirectly influence the level of nitrogen metabolites in the rumen of defaunated animals we could include such factors as age of the animals, their physiological status, way of feeding, composition of the diet as well as the method of inducing defaunation or total or partial refaunation.

Defaunation is one of the various possibilities of manipulation with ruminal fermentation. In spite of the fact that in some instances there was a positive influence of defaunation on the growth rate during low protein and high energy diets (Bird *et al.* 1979). On the other hand, Van Soest (1982), observed increased microbial nitrogen synthesis in the rumen of fauna-free sheep fed on purified diets. The reasons for the controversial results published in the literature are probably in the adaptation and regulatory mechanisms of the host and in the routes of their utilization depending on the physiological status in the actual nutritional environment, while the composition of the diet, as our findings have already indicated, is one of the dominant factors.

Acknowledgement

This research was supported by the funds from the Grant Agency for Science of the Slovak Academy of Sciences No. 2/1320/95.

References

AMOS H.E., AKIN D.E.: Rumen protozoal degradation of structurally intact forage tissues. *Appl. Environ. Microbial.* **36:** 513–522, 1978.

BIRD S.H., LENG R.A.: The effect of defaunation of the rumen on the growth of cattle on low-protein high-energy diets. *Br. J. Nutr.* **40:** 163–167, 1978.

BIRD S.H., HILL M.K., LENG R.A.: The effects of defaunation of the rumen on the growth of lambs on low-protein high-energy diets. *Br. J. Nutr.* **42:** 81–87, 1979.

COLEMAN G.S.: In: *Rumen Entodiniomorphid Protozoa, Methods of Cultivating Parasites in Vitro.* A.E.R. TAYLOR, J.R. BARKER (eds), Academic Press, London 1978, pp. 39–54.

COLEMAN G. S.: Rumen ciliate protozoa. *Adv. Parasitol.* **18:** 121–163, 1980.

CONRAD H.R., HIBBS J W., FRANK N.: High roughage system for raising calves based on early development of rumen function. IX. Effect of rumen inoculations and chlortetracycline on rumen function of calves fed high roughage pellets. *J. Dairy Sci.* **41:** 1248–1255, 1958.

CONWAY E. J., O'MALLEY F.: Microdiffusion methods and urea using buffered absorbents (Revised methods for ranges greater than 10 g N). *Biochem. J.* **36:** 655–661, 1942.

DEMEYER D.I.: Rumen microbes and digestion of plant cell walls. *Agric. Environ.* **6:** 295–337, 1981.

DEMEYER D.I., VAN NEVEL C.J.: Effect of defaunation on the metabolism of rumen microorganisms. *Br. J. Nutr.* **42:** 515–524, 1979.

EADIE J. M., GILL J. C.: The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. *Br. J. Nutr.* **26:** 155–167, 1971.

HARRISON D. G., MCALLAN A. B.: Factors affecting microbial growth yields in the reticulorumen. In: *Digestive Physiology and Metabolism in Ruminants.* Y. RUCKEBUSH, P. THIVEND (eds), MTP Press, Lancaster, 1980, pp. 205–226.

HINO T., RUSSELL J.B.: Relative contribution of ruminal bacteria and protozoa on the degradation of protein in vitro. *J. Anim. Sci.* **64:** 261–270, 1987.

HUNGATE R.E., REICHL J., PRINS R.: Parameters of rumen fermentation in a continuously fed sheep: evidence of a microbial rumination pool. *Appl. Microbiol.* **22:** 1104–1113, 1971.

JOHN A., ULYATT M. J.: Measurement of protozoa, using phosphatidyl choline, and of bacteria, using nucleic acids, in the duodenal digesta of sheep fed chaffed lucerne hay (Medicago sativa L.) diets. *J. Agr. Sci. (Cambr.)* **102:** 33–44, 1984.

JOUANY J. P., SENAUD J.: Influences des cilies du rumen sur la digestion de differents glucides chez le mouton. I. Utilization des glucides parietaux (cellulose, hemicelluloses) et de l'amidon. *Reprod. Nutr. Dev.* **22:** 735–752, 1982.

JUHÁSZ B.: *A Nitrogén Transzformázio Kérdésé Kérödzökben.* Különlenyomat a Debreceni mezögazdasági. Akad. Évkönyvébl. 1959.

KAYOULI C., DEMEYER D.I., VAN NEVEL C J., DENDOOREN R.: Effect of defaunation on straw digestion in sacco and on particle retention in the rumen. *Anim. Feed Sci. Technol.* **10:** 165–172, 1984.

KREUZER M., KIRGESSNER M., MÜLLER H.L.: Effect of defaunation on the loss of energy in whethers feed different quantities of cellulose and normal or steam flaked maize starch. *Anim. Feed Sci. Technol.* **16:** 233–341, 1986.

LENG R.A.: Dynamics of protozoa in the rumen of sheep. *Br. J. Nutr.* **48:** 399–408, 1982.

LENG R.A., NOLAN J.V.: Nitrogen metabolism in the rumen. *J. Anim. Sci.* **67:** 1072–1089, 1984.

LEWIS D., HILL K.J., ANNISON E.F.: Studies on the portal blood of sheep. 1. Absorption of ammonia from the rumen of the sheep. *Biochem. J.* **66:** 587–592, 1957.

LUTHER R.A., TRENKLE A., BURROUGHS W.: Influence of rumen protozoa on volatile acid production and ration digestibility in lambs. *J. Anim. Sci.* **25:** 1116–1122, 1966.

McINTYRE K.H.: The effects of increased nitrogen intakes on plasma urea nitrogen and rumen ammonia levels in sheep. *Aust. J. Agric. Res.* **21:** 501–507, 1970.

ONODERA R., NAKAGAVA Y., KANDATSU M.: Ureolytic activity of the washed suspension of rumen ciliate protozoa. *Agric. Biol. Chem.* **41:** 2177–2182, 1977.

ROWE J.B., DAWIES A., BROOME A.W.J.: Quantitative effects of defaunation on rumen fermentation and digestion in the sheep. *Proc. Nutr. Soc.* **40:** 49A, 1981.

SCHINCHI S., ITOH T., ABE M., KANDATSU M.: Effect of rumen ciliate protozoa fauna on the proteolytic activity of cell free rumen liquid. *Jpn. J. Zoot. Sci.* **57:** 89–96, 1986.

USHIDA K., JOUANY J.P.: Effect of protozoa on rumen protein degradation in sheep. *Reprod. Nutr. Develop.* **25:** 1075–1081, 1985.

USHIDA K., JOUANY J.P., LASSALAS B., THIVEND P.: Protozoal contribution to nitrogen digestion in sheep. *Can. J. Anim. Sci.* **64:** 20–21, 1984.

USHIDA K., JOUANY J.P., THIVEND P.: Role of rumen protozoa on nitrogen digestion in sheep given two isonitrogenous diets. *Br. J. Nutr.* **56:** 407–419, 1986.

VAN SOEST P.J.: *Nutritional Ecology of the Ruminant.* B. Books Inc., Corvallis, Oregon, 1982.

VEIRA D.M.: The role of ciliate protozoa in nutrition of the ruminant. *J. Anim. Sci.* **63:** 1547–1560, 1986.

VEIRA D.M., IVAN M., JUI P.Y.: Rumen ciliate protozoa: effect on digestion in the stomach of sheep. *J. Dairy Sci.* **66:** 1015–1022, 1983.

VEIRA D.M., IVAN M., JUI P.Y.: The effect of ciliate protozoa on the flow of amino acids from the stomach of sheep. *Can. J. Anim. Sci.* **64** (Suppl.): 22, 1984.

WALLACE R.J.: Ecology of rumen microorganisms: protein use. In: *Aspects of Digestive Physiology in Ruminants.* A. DOBSON, M.J. DOBSON (eds), Cornell University Press, Ithaca, 1988, pp. 99–122.

WALLACE R.J., McPHERSON C.A.: Factors affecting the rate of breakdown of bacterial protein in rumen fluid. *Br. J. Nutr.* **58:** 313–323, 1987.

WALLACE R.J., BRODERICK G.A., BRAMMALL, M.L.: Microbial protein and peptide metabolism in rumen fluid from faunated and ciliate-free sheep. *Br. J. Nutr.* **58:** 87–93, 1987.

WELLER R.A., PILGRIM, A.F.: Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. *Br. J. Nutr.* **32:** 341–351, 1974.

WHITELAW F.G., EADIE J.M., MANN S.O., REID R.S.: Some effects of rumen ciliate protozoa in cattle given restricted amounts of barley diet. *Br. J. Nutr.* **27:** 425–437, 1972.

Reprint Requests

Dr. J. Feješ, CSc., Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésova 4-6, 040 01 Košice, Slovak Republic.