CHAPTER 5

GENERAL DISCUSSION

5.1 Research findings

The key finding in the present study was that while molasses blocks containing anti-protozoal detergent were effective in administering to sheep the daily dose of Alkanate that has been previously shown to defaunate animals by the drenching method, it was not effective in eliminating protozoa from the rumen of full-fed sheep. The simplest of practical approaches, that of simply supplying animals with detergent in a molasses block, does thus not appear to be effective, at least with the two detergents tested here.

It was found that the period of highest block intake coincided with the period of highest feed intake. Therefore the efficacy of the detergent would be reduced because of dilution effects of saliva and possibly through adsorption of detergent onto the high levels of fresh fibre in the rumen.

Further work on the efficacy of the anti-protozoal-molasses block demonstrated that this method of administration successfully defaunated sheep when the basal diet was withdrawn. Under these circumstances, protozoal numbers fell to zero in treated animals within 4 days. This result suggests that the efficacy of the antiprotozoal activity of detergent is influenced by the level of feed intake, presence of

Chapter 5 GENERAL DISCUSSION

fibre and nutrients in the rumen. In addition to variations in rumen dietary fibre, restricted roughage intake results in less salivation and possibly a reduction in rumen volume, therefore increasing the effective concentration of detergent in the rumen fluid. Feed restriction may also reduce rumen motility which in turn will reduce the outflow of rumen contents, consequently the detergent will remain in the rumen for a longer time. Feed restriction will limit the availability of nutrients for protozoa and thus reduce their rate of reproduction and possibly increase their susceptibility to detergent, resulting in a reduction in protozoal numbers (Figure 3.13). In order to clarify this situation, *in vitro* studies were carried out to determine the factors influencing the efficacy of Alkanate 3 SL₃ as an anti-protozoal agent.

Results from the *in vitro* studies de nonstrated that the age of the Alkanate detergent (6 years versus fresh) did not affect anti-protozoal performance. However, the efficacy of the Alkanate detergent was reduced significantly when fibre was added to the rumen fluid incubations. This reduction in effectiveness was probably due to the adsorption of the active molecules onto fibre (Wright and Curtis, 1976) which would effectively reduce the concentration of active molecules in the liquid phase. A large proportion of protozoal population live in the fluid phase (Van Soest, 1994).

5.2. Future research

Although the results from these studies indicate that molasses blocks containing antiprotozoal detergents did not control protozoal populations in the rumen, other known antiprotozoal agents should be tested before this method of administration is

Chapter 5 GENERAL DISCUSSION

abandoned. It is possible that the efficacy of other antiprotozoal agents may be less affected by the level of fibre in the rumen. Clearly the control of rumen protozoa with anti-protozoal molasses blocks has the potential for increasing ruminant production, particularly under conditions where the N-content of pasture or diet frequently limits animal production (Bird, 1982). Hence, the improvement of effectiveness of this antiprotozoal molasses block and the availability of a cheap, safe and effective antiprotozoal agent is now a major research priority.

In addition to *Enterolobium Cyclocarpum* leaves (Leng *et al.*, 1992; Bahaudin *et al.*, 1992: SetyaNingrat, 1994) a natural source of anti-protozoal agent has also been found in *Sapindus rarac* seeds (Thalib *et al.*,1994). These sources are readily available, particularly in the tropics (Allen and Allen, 1981), and further study of their efficacy when offered to ruminants in molasses blocks is now required. Two future research areas which require experimentation are :

- a. Studies on the efficacy of molasses blocks containing natural anti-protozoal agents such as products of *Enterolobium Sp.* or *Sapindus Sp.*
- b. Studies on the efficacy of natural anti-protozoal agents when administered to ruminants as a feed supplement (by feeding in a supplement).

If successful, the first method would have widespread application for increasing production in grazing animals, particularly under tropical condition. For this method to be workable, it is essential that studies of their efficacy be conducted, and in particular the extent to which those natural anti-protozoal agents bind onto feed

Chapter 5 GENERAL DISCUSSION

particles needs to be determined. Moreover, the relevant questions which need to be examined are as follow.

- a) Will a molasses block containing natural anti-protozoal agent set sufficiently hard to ensure that sheep can achieve a suitable intake of natural antiprotozoal agent over a prolonged period ?
- b) Will sheep consume a molasses block cortaining natural anti-protozoal agent?
- c) Will the consumption of natural anti-protozoal agent contained in molasses block defaunate the rumen ?
- d) Will the consumption of a molasses block containing natural anti-protozoal agent have any adverse effects on the animal?

The second type of study suggested could lead to means of administering antiprotozoal agents in cut-and-carry and othe "intensive" systems.

References

- Abe, M. and Iriki, T. (1978). Effects of diet on the protozoa population in permeable continuous cultures of rumen contents. British J. Nutrition. 39:255-264.
- Abou Akkada, A.R. and El-shazly, K. (1964). Effect of absence of ciliate protozoa from the rumen on microbial activity and growth of lambs. Appl. Microbiology. 4:384-390.
- Abou Akkada, A.R. and El-shazly, K. (1965). Effect of presence or absence of rumen ciliate protozoa on some blood components, nitrogen retention and digestibility of food constituents in lambs. J. Agric. Sci. (Camb).64:251-259
- Abou Akkada, A.R., Bartley, E.E., Berube, R., Fina, L.R., Meyer, R.M., Hendricks,
 D. and Julius, F. (1968). Simple method to remove completely ciliate protozoa of adult ruminants. Appl. Microbiology. 16: 1475-1477.
- Abou Akkada, A.R., Eadie, J.M. and Howard, B.H. (1963). The biochemistry of rumen protozoa 7. The carbohydrases of polyplastron multivesiculatum. Biochem. J. 89: 268-272.
- Allen, O.N. and Allen, E.K. (1981). The Leguminosae: A Source Book of Characteristics, Uses and Nodulation. The University of Wisconsin Press. Madison.
- Bahaudin, R., Hendratno, C., Syamsi, A. and Aryanti. (1992). Study on the effect of *enterolobium* leaf on the rumen ecosystem of PE goat. In *:Aplikasi Isotop dan* Radiasi Dalam Bidang Pertanian, Peternakan dan Biologi. BATAN, Jakarta.
- Bauchop, T. (1977). In "Microbial Ecology of the Gut" (Ed. Clarke, R.T.J. and Bouchop, T.). Academic Press.
- Bauchop, T. (1989). Colonization of plan: fragments by protozoa and fungi. In:Nolan J.V., Leng, R.A. and Demeyer, D.I. (Eds.). *The Roles of Protozoa and Fungi in Ruminant Digestion*. Penambul Books, Armidale. pp.83-96.
- Becker, E.R. and Everett, R.C. (1930). Comparative growth of normal and infusoriafree lambs. Amer.J. Hyg. 11:362-370.
- Becker, E.R. and Hsuing, T.S. (1929). The method by which ruminants acquire their fauna of infusoria and remarks concerning experiments on the host-specificity of these protozoa. Proceedings of the National Academy of the United States of America. 15:684-695.

- Bergen, W.G. and Yokoyama, M.T. (1977). Productive limits to rumen fermentation. J.Animal Sci. 46: 573-584.
- Bergen, W.G., Purser, D.B. and Cline, J.H. (1968). Determination of limiting amino acid of rumen isolated microbial protein fed to rats. J. Dairy Sci. 51:1698-1700.
- Bird, S.H. (1982). Studies on the relatonship between rumen protozoa and production in sheep and cattle. Phd. University of New England, Armidale.
- Bird, S.H. (1989). Production from ciliate-free ruminants. In:Nolan, J.V., Leng, R.A. and Demeyer, D.I. (Eds.). The Roles of Protozoa and Fungi in Ruminant Digestion. Penambul Bocks, Armidale. pp.233-246.
- Bird, S.H. (1991). Role of protozoa in relation to the nutrition of the host Animal. In: Ho, Y.W., Wong, H.K. Abdullah, N. and Tajuddin, Z.A. (Eds.). Recent Advances on the Nutrition of Herbivores.
- Bird, S.H. (1996). Communication University of New England.
- Bird, S.H. and Leng, R.A. (1978). The effects of defaunation of the rumen on the growth of cattle on low-protein high-energy diets. British J. Nutrition. 40:163-167.
- Bird, S.H. and Leng, R.A. (1985). Productivity responses to eliminating protozoa from the rumen of sheep. In: Leng, R.A., Barker, J.S.F., Adams, D.B. and Hutchinson. (Eds.). Biotechnology and Recombinant DNA Technology in the Animal Production Industries-Reviews in Rural Science 6. University of New England, Armidale. pp. 109-117.
- Bird, S.H., Baigen, D.R., Dixon, R. and Leng, R.A. (1978). Ruminal protozoa and growth of lambs. Proc.Aust.Soc.Anim.Prod.12:137-145
- Bird, S.H., Hill, M.K. and Leng, R.A. (1979). The effect of defaunation of the rumen on the growth of lambs on low-protein high-energy diets. British J. Nutrition 42: 81-87.
- Bird,S.H. and Leng, R.A. (1984). Further studies on the effects of the presence or absence of protozoa in the rumen on live-weight gain and wool growth of sheep.
 British J. Nutrition. 52: 607-611.
- Bird, S.H., Romulo, B. and Leng, R.A. (1994). Effect of lucerne supplementation and defaunation on feed intake, digestibility, N retention and productivity of sheep fed straw based diets. Animal Feed Sci. and Technology. 45:119-129.

- Blackburn, T.H. and Hobson, P.N. (1960). Proteolysis in the sheep rumen by whole and fractionated rumen contents. J. Gen. microbilogy 22:272-289.
- Blaxter, K.L. and Czerkawski, J.W. (1966). Modification of methane production of the sheep by supplementation of its diet. J. Sci. Food Agric. 17:417-423
- Boyne, A.W., Eadie, J.M. and Raitt, K. (1957). The development and testing of a method of counting rumen ciliate protozoa. J. Gen. Microbiology. 17:414-423.
- Bryant, M.P. (1970). In "Dukes Physiology of Domestic Animals". 8th ed. Carstock Publ. Assoc., New York.
- Bryant, M.P. (1979). Microbial methane production. Theoretical aspects. J. Animal Sci. 48:193-201.
- Bryant, M.P. and Small, N. (1960). Observations on the ruminal microorganisms of isolated and inoculated calves. J. Dairy Science. 43:654-667.
- Burggraaf, W. and Leng, R.A. (1980). Antiprotozoal effects of surfactant detergents in the rumen of sheep. N.Z. J. of Agricultural Research. 23: 287-291.
- Chalupa, W. (1977). Manipulating Rumen Fermentation. J. Animal Sci. 46:pp.585-599.
- Church, D.C. (1976). Digestive physiology and nutrition of ruminants, vol1, 2nd Ed. Corvallis, Oregon.
- Church, D.C. (1979). Digestive Physiology and Nutrition of Ruminants. Vol.1. Digestive Physiology. O and B books Inc., Corvallis.
- Clarke, R.T.J. (1977). Protozoa in the rumen ecosystem. In: Clarke, R.T.J. and Bauchop, T. (Eds.). *Microbial Ecology of the Gut*. Academic press, Inc., New York. pp.251-275.
- Clarke, R.T.J. and Reid, C.S.W. (1974). Foamy bloat of cattle. J. Dairy Science. 57:753-785.
- Coleman, G.S. (1964). The metabolism of Eschericia coli and other bacteria by Entodinium Caudatum. J. Gen. Microbilogy 37:209-223.
- Coleman, G.S. (1969). The metabolism of starch, maltose, glucose and some other sugars by the rumen ciliate entodinium caudatum. J. Gen. Microbiology. 57: 303-332.
- Coleman, G.S. (1975). Interrelationship between rumen ciliate protozoa and bacteria.
 In : Mc Donald, I.W. and Warner A.C.I. (Eds.). *Digestion and Metabolism in The Ruminant*. University of New England, Armidale, Australia. pp. 149-164.

- Coleman, G.S. (1975). The interrelationships between rumen ciliate protozoa and bacteria. In : McDonald,I.W. and Warner,A.C.I. (eds.) Digestion and metabolism in the ruminant. University of New England. Armidale. pp.150-164.
- Coleman, G.S. (1978). The metabolism of cellulose, glucose and starch by the rumen ciliate protozoon *Eudiplodinium maggii*. J.Gen. Microbiology. 107:p.359-366.
- Coleman, G.S. (1979). The role of rumen protozoa in the metabolism of ruminants given tropical feeds. Tropical Animal Production. 4:199-213.
- Coleman, G.S. (1983). The cellulolytic activity of thirteen species of rumen Entodiniomorphid protozoa. J. Protozoal. 30:36A.
- Coleman, G.S. (1985). The cellulose content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. J. Agric. Sci. (Camb). 104:349-360.
- Coleman, G.S. (1989). Protozoal-bacterial interaction in the rumen. In: Nolan J.V., Leng, R.A. and Demeyer, D.I. (Eds.) *The Roles of Protozoa and Fungi in Ruminant Digestion*. Penambul Books, Armidale. pp.13-27.
- Czerkawski, J.W. (1972). Manupilation of rumen fermentation. Proc. Nutr. Soc.31:125-139.
- Czerkawski, J.W. (1986). An introduction to rumen studies. Pergamon press. Oxford.
- Demeyer, D.I. (1981). Rumen microbes and digestion of plant cell walls. Agriculture and Environment. 6:295-337.
- Demeyer, D.I. and Van Nevel, C.J. (1979). Effect of defaunation on the metabolism of rumen micro-organisms. British J Nutrition. 42:515-167.
- Eadie, J.M. (1962a). The Development of Rumen Microbial Population in Lambs and Calves under Various Conditions of Management. J.Gen.Microbiology. 29:563-578.
- Eadie, J.M. (1962b). Inter-Relationships between certain rumen ciliate protozoa. J. Gen. Microbiology. 29: 579-588.
- Eadie, J.M. (1967). Studies on the ecology of certain rumen ciliate protozoa. J. Gen. Microbiology. 49: 175-194.

- Eadie, J.M. and Gill, J.C. (1971). The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. British J. Nutrition. 26: 155-167.
- Eadie, J.M. and Hobson, P.N. (1962). Effect of the presence or absence of rumen ciliate protozoa on the total rumen bacterial count in lambs. Nature 193:503-505.
- Eadie, J.M., and Mann, S.O. (1970). Development of the rumen microbial population
 : High starch diets and instability. In: Philipson A.T. P. (Ed.) Physiology of digestion and metabolism in the Ruminant. Oriel Press, Newcastle Upon Tyne. pp 335-347.
- Finlay, B.J., Esteban,G., Clarke, K.J., Williams, A.G., Embley, T.M., Hirt, R.P. (1994). Some rumen ciliates have endosymbiotic methanogens.
 FEMS Microbiology Letters. 117:157-162.
- Forster, R.J. (1989). The effects of manipulation of rumen protozoa on rumen fermentation and productivity of sheep. Phd. Thesis. Departement of Biochemistry, Microbiology and Nutrition. The University of New England. Armidale.
- Giesecke, D. (1970). Physiology of digestion and metabolism in the ruminant. In: A.T.Phillipson (Ed.). Oriel press, Newcastle upon Tyne. pp.306-318.
- Harrison and McAllan, (1980). Factors affecting microbial growth yields in the reticulorumen. In: Ruckebush, Y. and Thivend, P. (Eds.) Digestive Physiology and Metabolism in Ruminants. Press, Lancaster. pp 205-226
- Hendratno, C., Nolan, J.V. and Leng, R.A (1991). The importance of urea-molasses multinutrient blocks for ruminant production in Indonesia. In: Isotope and related Techniques in animal Production and Health, Proceedings of a Symposium in Vienna, 15-16 April 1991. IAEA, Vienna. pp 157-169.
- Hungate, R.E. (1966). The Rumen and Its Microbes. Academic Press, Ltd. NY.
- Hungate, R.E. (1969). A Roll Tube Method for the cultivation of strict anaerobes. In: Norris, J.R., Ribbon, P.W. (Eds.). Methods in Microbiology. Academic Press, London. pp. 117-132.
- Hungate, R.E. (1975). The rumen microbial ecosystem. Johnston, R.F., Frank, P.W. and Michener, C.D. (Ed.). Ann. Rev. Ecol. Syst. 6:39-66.

- Hungate, R.E.(1960). Symposium: selected topics in microbial ecology. I. Microbial ecology of the rumen. Bacteriological Reviews. 24:353-364.
- Jouany, J.P. (1989). Effects of diets populations of rumen protozoa in relation to fibre digestion in: Nolan J.V., Leng,R.A. and Demeyer,D.I. (Eds.) *The Roles of Protozoa and Fungi in Rummant Digestion*. Penambul Books, Armidale, Australia.pp. 59-74.
- Jouany, J.P., Demeyer, D.I., Gain, J. (1988). Effect of defaunating the rumen. Animal Feed Science Technology. 21:229-265.
- Jouany, J.P., Zinab, B., Senaud, J., Groliere, C.A., Grain, J. and Thivend, P. (1981).
 Role of the rumen ciliate protozoa *Potyplastron Multivesiculatum*, *Entodinium sp.* and *Isotricha Prostoma* in the digestion of a mixed diet in sheep.
 Reproduction, Nutrition, Developpement 21: 871-884.
- Jouany, J.P. and Senaud, J. (1979). Role of rumen protozoa in the digestion of food cellulosic materials. Annales de Recherches Veterinaires. 10:261-263.
- Jouany, J.P. and Senaud, J.(1982). Influence des cilies du rumen sur la digestion de differents glucides chez le mouton. I. utilisation des glucides parietaux (celluose et hemicelluloses) et de l'amidon. Repod. Nutr. Dev. 22:735-741
- Kayouli, C., Demeyer, D.I., Van Nevel, C.J., Dendooven, R. (1983/1984). Effect of defaunation on straw digestion in sacco and on particle retention in the rumen.
 Animal Feed Science Technology. 10:165-172.
- Knox, M. (1995). The use of Medicated Blocks to control nematode parasites of ruminants. In : Rowe, J.B. and Nolan, J.V. (Eds.). Recent Advances in Animal Nutrition in Australia 1995. University of New England, Armidale. pp116-121.
- Kobayashi T. and Itabashi H. (1986). Effect of intra-ruminal VFA infusion on the protozoal populations of the rumen. Bulletin National Institute of Animal Industry. 44:47-54.
- Kurihara, Y., Eadie, J.M., Hobson, P.N. and Mann, S.O.(1968). Relationship between bacteria and ciliate protozoa in the sheep rumen. J. Gen. Microbiology. 51:267-288.
- Kurihara, Y., Takeshi, T., Shibata, F. (1978). Relationship between bacteria and ciliate protozoa in the sheep fed on a purified diet. J. Agric. Sci. 90:373-381.
- Leng, R.A. (1976). From plant to animal protein. In : Sutherland, T.M., McWilliam, J.R. and. Leng, R.A. (Eds.). University of New England, Armidale.

- Leng, R.A. (1982). Modification of rumen fermentation. In: Hacker, J.B. (Ed.).
 Nutritional Limits to Animal Production from Pastures. Farnham Royal:
 Commonwealth Agricultural Bureaux.
- Leng, R.A. (1991). Improving ruminant production and reducing methane emissions from ruminants by strategic supplementation. WPA/400/1-91/004.
- Leng, R.A. and Nolan, J.V. (1984). Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.
- Leng, R.A., Bird, S.H., Klieve, A., Choo, B.S., Ball, F.M., Asefa, P., Mudgal, V.D., Chaudhry, U.B., Haryono, S.U., and Hendratno, N. (1992). The potential for forage supplements to manipulate rumen protozoa to enhance protein to energy ratio in ruminants fed on poor quality forages. *In Legume trees and other fodder trees as protein sources for livestock*. Animal Production and Health Paper No. 102, FAO, Rome.
- Leng, R.A., Gill, M., Kempton, T.J., Rowe, J.B., Nolan, J.V., Stachiw,S.J. and Preston, T.R. (1981). Kinetics of large ciliate protozoa in the rumen of cattle given sugar cane diets. British J. Nutrition. 46:371-384.
- Leng,R.A., Bird,S.H. and Burggraaf,W.(1980). The role of rumen protozoa in the nutrition of ruminants. In: D.J. Farrell (Ed.). Recent Advances In Animal Nutrition 1980. University of New England. Armidale. Australia.
- Lindsay, J.R. and Hogan, J.P. (1972). Digestion of two legumes and rumen bacterial growth in defaunated sheep. Aust. J. Agric Res. 23:321-330.
- Mackie, R.I., Gilchrist, F.M.C., Roberts, A.M., Hannah, P.E. and Schwartz, H.M. (1978). Microbiological and chemical changes in the rumen during stepwise adaptation of sheep to high concentration diets. J. Agric. Sci. (Camb.) 90 : 241-254.
- Males, J.R. and Purser, D.B. (1970). Relationship between rumen ammonia levels and the microbial population and volatile fatty acid proportions in faunated and defaunated sheep. Appl. Microbiology. 19:485-490.
- McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D. (1988). Animal nutrition 4th edition. Longman Scientific and Technical, Harlow, P.151.
- McNaught, M.L., Owen, E.C., Henry K.M. and Kon, S.K. (1954). The utilization of non-protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins

of preparations of dried rumen bacteria, rumen protozoa and brewer's yeast for rats. Biochem. J. 56:151-156.

- Mendoza, G.D., Britton, R.A., and Stock R A. (1993). Influence of ruminal protozoa on site and extent of starch digestion and ruminal fermentation. J. Animal Sci. 71: 1572-1578.
- Miltimore and Mc Arthur (1962). Relationship between *in vivo* rumen gas composition and feed efficiency of calves. Nature 196: 288-289.
- Newbold, C.J., Chamberlain, D.G., Williams, A.G. (1986). The effects of defaunation on the metabolism of lactic acid in the rumen. J. Sci. Food Agric. 37:1083-1090.
- Nolan, J.V. (1989). Implication of protozoa and fungi for the protein nutrition of ruminants in: Nolan J.V., Leng, R.A. and Demeyer, D.I. (Eds.). *The Roles of Protozoa and Fungi in Ruminant Digestion*. Penambul Books, Armidale, Australia. pp.211-221.
- Nolan, J.V., Norton, B.W., and Leng, R.A. (1976). Further studies of the dynamics of nitrogen metabolism in sheep. British J. Nutrition. 35:127-147.
- Nour, A.M., Abou Akkada A.R., El-shazly K., Naga, M.A., Borhami, B.E. and Abaza, M.A. (1979). Effect of increased levels of urea in the diet on ruminal protozoal counts in four ruminant species. J. Animal Sci. 49:1300-1305.
- Ogimoto Keiji and Imai Soichi (1981). Atlas of rumen microbiology. Japan Scientific societies press, Tokyo.
- Orpin C.G. and Letcher, A.J.(1978). Some factors controlling the attachment of the rumen holotrich protozoa *Isotricha intestinalis* and Isotricha prostoma to plant particles in-vitro. J. Gen. Microbiology. 106:33-40.
- Orpin, C.G. (1977c). Studies on the defaunation of the ovine rumen using Dioctyl sodium sulphosuccinate. J. of Applied Bacteriology. 43:309-318.
- Orpin, C.G. and Letcher A.J. (1983/1984). Effect of absence of ciliate protozoa on rumen fluid volume, flow rate and bacterial populations in sheep. Animal feed science and technology. 10:145153.
- Orpin, C.G. (1977a). Invasion of plant tissue in the rumen by the Flagellate *Neocallimastix Frontalis*. J. Gen. Microbiology. 98:423-430.
- Orpin, C.G. (1977b). The rumen Flagellate *Piromonas Communis* : its life-history and invasion of plant material in the rumen. J. Gen. Microbiology. 99:107-117.

- Phillipson, A.T. (1955). Advances in veterinary Science, vol.II, p.238. New York: Academic Press.
- Pilgrim, A.F., Gray, F.V., Weller, R.A. and Belling, C.B. (1970). Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. British J. Nutrition. 24:589-598
- Potter, E.L. and Dehority, B.A. (1973). Effects of changes in feed level, starvation, and level of feed after starvation upon the concentration of rumen protozoa in the ovine. Appl. Microbiology. pp. 692-698.
- Preston, T.R. and Leng, R.A. (1987). Matching Livestock Production Systems to Available Resources. Penambul Books, Armidale.
- Preston, T.R. and Willis, M.B. (1970). Intensive Beef Production. Pergamon Press, Oxford. pp 76-84.
- Punia, B.S. Leibholz, J. Faichney, G.J. (1987). The role of rumen protozoa in the utilization of paspalum (Paspalum dilatum) hay by cattle. British J. Nutrition 57:395-406.
- Purser, D,B. and Moir, R.J. (1966). Variations in rumen volume and associated effects as factors influencing metabolism and protozoa concentrations in the rumen of sheep. J. Animal Sci. 25: 1116-1122.
- Purser, D.B. and Moir, R.J. (1959). Ruminal flora studies in the sheep. IX. The effect of pH on the ciliate population of the rumen *in vivo*. Australian J. of Agricultural Research. 10:555-564.
- Romulo, B., Bird, S.H., and Leng, R.A. (1989). Effects of defaunation and protein supplementation on intake, digestibility, N retention and fungal numbers in sheep fed straw-based diets in: Nolan J.V., Leng, R.A. and Demeyer, D.I. (Eds.) *The Roles of Protozoa and Fungi in Ruminant Digestion*. Penambul Books, Armidale. pp.285-288.
- Rung, G., Sekiguchi, M., Imai, S. (1986). Changes in rumen ciliates and in rumen fluid caused by excessive brewer's grain feeding and restauration of them. Japanese J. of Zootechnical science. 57:887-894.
- Satapathy, N. And Purser, D.B. (1967). Protozoa, bacterial, and volatile fatty acid changes associated with feeding tylosin. Appl. Microbiol. 15:1417-1421.

- Senaud, J., Jouany, J.P., Lassalas, B., and Bohatier, J. (1995). Digestion of a dehydrated lucerne/barley diet (70:30) in defaunated, *Isotricha*-monoinoculated and mixed-fauna-inoculated rumen in sheep. Reprod. Nutr. Dev 35:249-266.
- Setia Ningrat, R.W.S. (1994). A preliminary study of the chemistry and toxicology of a natural antiprotozoal agent. Master Γhesis. University of New England. Armidale.
- Slyter, L.L., Oltjen, R.R., Kern, D.L., and Blank, F.C. (1970). Influence of type and level of grain and diethylstillbestrol on the rumen microbial populations of steers fed all-concentrate diets. J. Animal science 31 : 996-1002.
- Soetanto, H. (1986). Studies on the role of rumen-anaerobic fungi and protozoa in fibre digestion. M. Rur. Sci. Thesis.
- Thalib, A., Widiawati, Y., Hamid, H., Suherman, D., Sabrani, M. (1995). The effects of saponins from Sapindus Rarac fruit on rumen microbes and host animal growth. Annales De Zootechnic. 44(Suppl 1): 161.
- Thomas, P.C. (1973). Microbial protein synthesis. Proc. Nutr. Soc.32:85-91.
- Ushida, K. and Jouany, J.P. (1985). Effect of protozoa on rumen protein degradation in sheep. Reproduction, Nutrition, Developpement. 25:1075-1081.
- Ushida, K., Jouany, J.P. and Demeyer, D.I. (1991). Effects of presence or absence of rumen protozoa on the efficiency of utilization of concentrate and fibrous feeds.
 In: T. Tsuda, Y. Sasaki, R. Kawashima (Eds.) *Physiological Aspect of Digestion and Metabolism in Ruminants*. Academic Press, New York. pp. 625-654.
- Ushida, K., Jouany, J.P., Lassalas, B. and Thivend, P.(1984). Protozoal contribution to nitrogen digestion in sheep. Can. J. Animal Science. 64 (suppl):20-21.
- Ushida, K., Kaneko, T., Kojima, Y. (1987). Effect of presence of large entodiniomorph protozoa on the rumen bacterial flora, fauna composition of small entodinia and in vitro cellulolysis and xylanalysis. Jpn J. Zootech. Sci. 58: 893-902.
- Valdez, R.E., Alvarez, F.J., Ferreiro, H.M., Guerra, F., Lopez, J., Preigo, A.,
 Blackburn, T.H., Leng, R.A. and Preston, T.R. (1977). Rumen function in cattle
 given sugar cane. Tropical Animal Production. 2:260-272.
- Van Soest, P.J. (1994) Nutritional Ecology of the Ruminant 2nd edition. Comstock Publishing associates Cornell University Press.

- Veira, D.M. (1986). The role of ciliate protozoa in nutrition of the ruminant. Journal of Animal Sci. 63:1547-1560.
- Veira, D.M., Ivan, M. and Jui, P.Y.(1983). Rumen ciliate protozoa: Effects on digestion in the stomach of sheep. J. Dairy Science 66:1015-1022.
- Veira, D.M., Ivan, M. and Jui, P.Y.(1984). The effect of ciliate protozoa on the flow of amino acids from the stomach of sheep. Can. J. Animal Science.64(suppl):22.
- Walker, G.J. and Hope, P.M. (1964). Degradation of starch Granules by some Amylolytic Bacteria from the rumen of sheep. Biochem. J. 90:398-408.
- Wallace, R.J., Broderick, G.A. and Brammall, M.L. (1987). Microbial protein and peptide metabolism in rumen fluid from faunated and ciliate-free sheep. British J. of Nutrition 58:87-93
- Warner, A.C.I. (1965). In "Physiology of Digestion in the Ruminant" (Ed. R.W. Dougherty et. al.), Butlerworths, Washington. P 346.
- Weller, R.A. and Pilgrim, A.F. (1974). Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continous in vitro fermentation system.British J. Nutrition. 32:341-351.
- Weller, R.A., Gray F.V. and Pilgrim, A.F. (1958). The conversion of plant nitrogen to microbial nitrogen in the rumen of the sheep. British J. Nutrition. 12: 421-429.
- Whitelaw, F.G., Eadie, J.M., Bruce, L.A. and Shand, W.J. (1984). Methane formation in faunated and ciliate-free cattle and its relationship with rumen Volatile fatty acid proportions. British J. of Nutrition. 52: 261-275.
- Whitelaw, F.G., Eadie, M.J., Mann, S.O., Reid, R.S.(1972). Some effects of rumen ciliate protozoa in cattle given restricted amounts of a barley diet. British J.Nutrition. 24:425-437.
- Willard, F.L. and Kodras, R. (1967). Survey of chemical compounds tested *in vitro* against rumen protozoa for possible control of bloat. Appl.Microbilogy.15:1014-1019.
- William, A.G. and Harefoot, G.G. (1976). Factors Affecting the uptake and metabolism of soluble carbohydrates by the rumen ciliate *Dasytricha ruminantium* isolated from ovine rumen contents by filtration. J. Gen Microbiology 96:125-136.

Williams, A.G. (1989). Metabolic activities of rumen protozoa in: Nolan J.V.,
Leng, R.A. and Demeyer, D.I. (Eds.). *The Roles of Protozoa and Fungi in Ruminant Digestion*. Penambul Books, Armidale, Australia. p.97-126.

Williams, A.G. and Coleman, G.S. (1991). The Rumen Protozoa. Springer-Verlag.

- Williams, A.G. and Strachan, N.M. (1984). The distribution of polysaccharidedegrading enzymes in the bovine rumen digesta ecosystem. Current Microbiology. 10:215-220.
- Wright, D.E. and Curtis, M.W. (1976). Bloat in cattle XLII. The action of surface active chemcals on ciliated protozoa. N.Z.J. Agric. Res., 19: 19-23.
- Yokoyama, M.T. and Johnson, K.A. (1988). Microbiology of the rumen and intestine.
 In : Church, D.C. (Eds.). *The Ruminant Animal Digestive Physiology and Nutrition*. A Reston Book, Englewood Cliffs, New Jersey.