The Study of Diversity of Ciliate Protozoa in Ghizel Sheep Fed in Pasture and Nourished by Dried Grape by-Product

Taghizadeh Akbar, Mahbob Soltan Ali, Zarrini Golamreza, Besharati Maghsoud and Ansari Adel

1Department of Animal Science, Faculty of Agriculture, University of Tabriz, Iran
2Center of Excellence for Biodiversity, University of Tabriz, Iran
3Department of Animal Sciences, Faculty of Natural science, University of Tabriz, Iran
4Payame Noor University of Benis, Shabestar, Iran

Abstract: Problem statement: Ciliate protozoa are one of the normal microorganisms that found in rumen of both domestic and wild ruminants. Several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. The aim of the present study was to determine the rumen ciliates protozoa diversity in Ghizel sheep of East Azerbaijan province and evaluate effects of alfalfa replacement by multiple level of dried grape by-product (0, 15, 30 and 45% of diet) on ciliate protozoa population.

Approach: In the first experiment, samples of rumen fluids were collected from 16 mature sheep. Selected sheep were belonged to 4 pure herds of Ghizel sheep in east Azerbaijan province. In the second experiment, sixteen mature Ghizel wether sheep of live weight 34 kg (±1.5) were used. Data obtained from study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure and treatment means were compared by the Duncan test.

Results: In experiment 1, different geographical locations affect total number of rumen ciliated protozoa (p<0.05), Entodinium spp. and Dasytricha spp. In experiment 2, the concentrations of Entodinium spp., Diplodinium spp., Holotricha spp. and Opharyoscolex species were higher when 15% dried grape by-product (DGB) was included in the diet. With increasing DGB in diets the number of Epidinium spp. and Euodiploidinium spp. reduced in treatments 2 (15% of diet replaced by DGB) and 3 (30% of diet replaced by DGB) (p<0.05). Conclusion: The different geographical locations affected total number of rumen ciliated protozoa, Entodinium spp. and Dasytricha spp. The concentration of Diplodinium was observed to increase when sheep were fed with dried grape by-product.

Key words: Ciliate protozoa, dried grape by-product, East Azarbaijan, Ghizel sheep

INTRODUCTION

The microbial populations in the rumen consist mainly of bacteria, protozoa and fungi that involved in the digestion of feed in the rumen. Hydrolysis of lignocelluloses feeds in the rumen is accomplished by the collective effort of bacteria, protozoa and fungi. Studies on defaunated animals have shown that exclusion of protozoa from the rumen has a beneficial effect on the growth rate, wool growth and feed conversion efficiency of animals under certain feeding conditions[1-3]. Intrinsic factors such as the physiological status of the host (age, health, reproductive period, lactation, social behavior, feeding habits and competition among micro-organisms) influence the diversity of microfauna and the total number of ciliates. Yet above all, extrinsic factors, mainly food, determine the limits of the variability of microfauna. The food factor comprises the chemical composition of the diet, the amount given, the physical nature of the food, the number of meals and the time intervals between meals. Ciliate protozoa are one of the normal microorganisms that found in rumen of both domestic and wild ruminants. Several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. These include type and amount of feed consumed[6], pH, turnover rate, frequency of feeding and feed level[7]. In animals that have been subjected to any kind of feed related stress, such as starvation or rumen acidosis, rumen ciliates may be eliminated[8]. Dogiell Dogiel Noirot-Thimothe (1959,1960) (cited by Ogimoto and Imai[9]) examined the problem of the specificity of the ciliates towards their hosts in a wide comparison of the fauna...
Ophryoscolecidae present in species of various ruminants occupying distinct geographical areas of various sizes. Ciliates follow definite geographical distributions. Some species are attached to one family of ruminants such as Caloscolex camelis with Camelus dromedarius, whereas others display a very wide dispersal. These differences in dispersal are probably due to the selection exercised by the food substrate chosen by the host, but inter specific competition is also involved. Some species therefore became dominant and cosmopolitan, whereas others are only represented in small numbers over a small area of distribution. Among domestic ruminants, the microfauna also vary depending on the geographical distribution of its hosts.

Ciliate protozoa play a diverse role in the ruminal metabolism of nutrients. To improve the efficiency of feed protein utilization, considerable effort has been made to find a means of total elimination of protozoa from the rumen (defaunation), but a practical defaunation technique has not been established. A massive reduction in the rumen protozoa population (reduced fauna) by chemical drenching of experimental animals has been found to improve milk production. However, such a method to produce reduced fauna is not practical for use in ruminant production.

Hristov et al. tested a large number of substances in vitro and of those examined, tannins, saponin-based plant extracts and linoleic acid were particularly effective at reducing protozoal numbers. These bioactive compounds lowered numbers of protozoa without specifically inhibiting the activity of bacterial populations.

The aim of the present study was to determine the rumen ciliates diversity in Ghizel sheep of East Azerbaijan province and evaluate effects of alfalfa replacement by multiple level of dried grape by-product on ciliate protozoa population.

**MATERIALS AND METHODS**

**Experiment 1:** In the first experiment, samples of rumen fluids were collected from 16 mature sheep for studying of protozoa diversity in east Azerbaijan province. Selected sheep were belonged to 4 pure herds of Ghizel sheep in east Azerbaijan province. From each herds 4 sheep separated that they were approximately in same weight, age and condition. Rumen fluids were achieved by using stomach tube for counting protozoa.

**Experiment 2 (In vivo study):** Sixteen mature Ghizel sheep of live weight 34 kg (±1.5) were used. The animals were allocated individually in boxes with free-access to salt block and water. Four diets were used, one as basal diet (alfalfa) and the rest as experimental diets (DGB with alfalfa). The sheep were fed twice daily, at 0900 and 1600. For the first week, sheep received alfalfa for ad libitum intake. The amounts of consumed and refused feeds for every sheep were recorded. For the second and third week, DGB replaced 0, 15, 30, or 45% of the alfalfa DM. On the last day at 2 h after feeding, the digesta samples collected and were bulked for counting of rumen ciliate protozoa, VFA and NH₃-N analyses.

**Dried grape by-product:** Dried grape by-product was obtained from raisin production factories of Tabriz, Iran. The DGB that was collected contained grape cluster stems and rejected raisins.

**Chemical composition:** Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30) and crude protein (CP, method ID 984.13) were determined by procedures of AOAC. The NDF and ADF concentrations were determined using the methods of Van Soest et al. without sodium sulphite. Total Phenolics (TP) were measured using the Folin Ciocalteau method. Total Tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteau reagent. Tannic acid was used as the standard to express the amount of TP and TT.

Total numbers and generic composition of ciliate protozoa were determined according to the procedures described by Dehority.

**Statistical analysis:** Data obtained from study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure and treatment means were compared by the Duncan test.

**RESULTS**

**Experiment 1:** In this study, 7 species of rumen ciliated protozoa were detected, identified and counted. Five species were belonged to the entodinomorphid family and they were Entodinium, Epidinium, Diplodinium, Eudiplodinium and Ophryoscolex. The other species were Isotricha and Dasytricha and belonged to Holoticha family.

The concentration ciliated protozoa in rumen of 16 experimental Ghizel sheep of East Azerbaijan of Iran ranged from $8.1 \times 10^5$ to $56.81 \times 10^5$ mL⁻¹ (Table 1).

Based on analyzed data, all species of protozoa that expected to be counted in the samples were observed only in the samples of Osko area and samples of other areas had some of protozoa species.
**DISCUSSION**

Differences in protozoan populations due occur both among different ruminant species and different geographical locations\(^a\). This study indicated that different geographical locations affect total number of...
rumen ciliated protozoa, *Entodinium* spp. and *Dasytrisha* spp.

The concentration of protozoa in ruminal contents generally increases with the addition of concentrates to roughage diets\(^{[20-22]}\) and the results of present study (Table 3) were in convenient with above reports.

The concentrations of VFA and NH\(_3\)-N in the control treatment were greater than in the other treatments. The difference in VFA and NH\(_3\)-N concentrations between treatments may have occurred because of a lower rate of fermentation (inhibition of microbial activity) as a result of tannin content.

Priolo et al.\(^{[23]}\) reported the greater ruminal ammonia and a VFA concentration in PEG-vs. tannin-fed sheep indicates more rapid ruminal fermentation when PEG was given.

It seems that in addition to the lowering of ruminal pH, which occurs in all animals as a result of feeding high-concentrate diets, several other factors are involved in defaunation. These factors could include rate of feed consumption, rate of passage and salivary production. From the present study, these other factors seem to vary between individual animals and may be the determining factors of whether protozoa survive in the rumen.

**CONCLUSION**

The different geographical locations affect total number of rumen ciliated protozoa, *Entodinium* spp. and *Dasytrisha* spp. The concentration of Diplodinium was observed to increase when sheep were fed with dried grape by-product.

**ACKNOWLEDGEMENT**

The researcher thanks Center of Excellence for Biodiversity, University of Tabriz, Iran for funding of this research.

**REFERENCES**

1. Bird, S.H. and R.A. Leng, 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. Br. J. Nutr., 52: 607-611. DOI: 10.1079/BJN19840127

2. Bird, S.H., B. Romulo and R.A. Leng, 1994. Effects of lucerne supplementation and defaunation on feed intake, digestibility, N retention and productivity of sheep fed straw based diets. Anim. Feed Sci. Technol., 45: 119-129. http://cat.inist.fr/?aModele=afficheN&cpsidt=4001329

3. Demeyer, D.I., 1992. Biotechnology and the quality of animal products in sustainable agriculture. J. Applied Anim. Res., 1: 65-80.

4. Ivan, M., M. De Dayrell, S. Mahadevan and M. Hidirogolou, 1992. Effect of bentonite on wool growth and nitrogen metabolism in fauna free and faunated sheep. J. Anim. Sci., 70: 3194-3202. http://www.ncbi.nlm.nih.gov/pubmed/1429295

5. Santra, A. and S.A. Karim, 2000. Growth performance of faunated and defaunated Malpura weaner lambs. Anim. Feed Sci. Technol., 86: 251-260. http://cat.inist.fr/?aModele=afficheN&cpsidt=1517886

6. Dehority, B.A., 1978. Specificity of rumen ciliate protozoa in cattle and sheep. J. Protozool., 25, 509-513. DOI: 10.1111/j.1550-7408.1978.tb04177.x

7. Franzolin, R. and B.A. Dehority, 1996. Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. J. Anim. Sci., 74: 2803-2809. http://www.ncbi.nlm.nih.gov/pubmed/8923195

8. Williams, A.G. and G.S. Coleman, 1991. The Rumen Protozoa. Springer-Verlag Inc., New York, USA, pp: 441. ISBN: 0387975489.

9. Ogimoto, K. and S. Imai, 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo.

10. Hegarty, R.S., 1999. Reducing rumen methane emission through elimination of rumen protozoa. Aust. J. Agric. Res., 50: 1321-1327. http://cat.inist.fr/?aModele=afficheN&cpsidt=1244258

11. Moate, P.J., 1989. Defaunation Increases Milk Yield of Dairy Cows. In: Recent Advances in Animal Nutrition in Australia, Farrell, D.J. (Ed.), University of New England Printery, Armidale, NSW, Australia, pp: 18A.

12. Hristov, A.N., M. Ivan, L. Neill and T.A. McAllister, 2003. Evaluation of several potential bioactive agents for reducing protozoal activity in vitro. Anim. Feed Sci. Technol., 105: 163-184. http://cat.inist.fr/?aModele=afficheN&cpsidt=14659504

13. Hristov, A.N., M. Ivan and T.A. McAllister, 2004. *In vitro* effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high-concentrate, barley-based diet. J. Anim. Sci., 82: 2693-2704. http://jas.fass.org/cgi/content/abstract/82/9/2693

14. Markham, R., 1942. A steam distillation apparatus suitable for micro-Kjeldahl analysis. Biochem. J., 36: 790.

15. AOAC., 1999. Official methods of analysis of AOAC international. AOAC international. Maryland, USA. http://journalseek.net/cgi-bin/journalseek/journalsearch.cgi?field=issn&query=1080-0344
16. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation on animal nutrition. J. Dairy Sci., 74: 3583-3597. http://jds.fass.org/cgi/content/short/74/10/3583

17. Makkar, H.P.S., 2000. Quantification of Tannins in Tree Foliage. FAO/IAEA Working Document. IAEA, Vienna, Austria. http://www-naweb.iaea.org/nafa/aph/public/pubd31022manual-tannin.pdf

18. Dehority, B.A., 1993. Laboratory Manual for Classification and Morphology of Rumen Ciliate Protozoa. CRC Press, Boca Raton, FL., ISBN: 0849348757, pp: 120.

19. SAS Inc., 2002. SAS User’s Guide: Statistics. Statistical Analysis Systems Institute Inc., Cary NC. ISBN: 0917382374.

20. Grubb, J.A. and B.A. Dehority, 1975. Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. Applied Environ. Microbiol., 30: 404-412. http://www.ncbi.nlm.nih.gov/pubmed/1180549

21. Varel, V.H. and B.A. Dehority, 1989. Ruminal cellulolytic bacteria and protozoa from bison, cattle-bison hybrids and cattle fed three alfalfa-corn diets. Applied Environ. Microbiol., 55: 148-153. http://www.ncbi.nlm.nih.gov/pubmed/2705767

22. De Semet, S., D.I. Demeyer and C.J. Van Nevel, 1992. Effect of defaunation and hay: Concentrate ratio on fermentation, fiber digestion and passage in the rumen of sheep. J. Anim. Feed Sci. Technol., 37: 333-344. http://cat.inist.fr/?aModele=afficheN&cpsidt=5487838

23. Priolo, A., G.C. Waghorn, M. Lanza, L. Biondi and P. Pennisi, 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. J. Anim. Sci., 78: 810-816. http://jas.fass.org/cgi/content/abstract/78/4/810