Influence of Probiotic Feed Additives on Rumen Microflora of Cattle

Luboš Zábranský[1], Veronika Hadačová[1], Miloslav Šoch[1], Bohuslav Čermák[1], Anna Poborská[1], Luboš Smutný[2], Iveta Novotná[1], Petr Smolík[1], Tomáš Frejlach[1], Anna Šimková[1], Kateřina Švejdová[1]

[1]*University of South Bohemia in Czech Budejovice, Faculty of Agriculture, Department of zootechnical sciences, Studentská 1668, 370 05, České Budějovice, Czech Republic*
[2]*AGROSOFT Tábor, s.r.o, Údolní 2930, 390 02 Tábor, Czech Republic*

Abstract
Feed digestibility is very important for milk production. This is related to the functional status of rumen, which is influenced mainly by the amount of feed intake and its quality. The aim of the study was to determine how probiotic supplements affect rumen microflora in cannulated cows. Feed supplements were given to cannulated cows in the experimental stable and changes in the number of microorganisms in the rumen were monitored. They were found positive trends in the growing number of protozoa in the rumen, but not statistically significant effect of probiotics on the development of the rumen microflora ($P > 0.05$).

Keywords: rumen, probiotics, protozoa, feed supplements

1. Introduction

Successful nutrition of ruminants depends on settlement and maintaining a healthy rumen and digestive system. Preservation of a high productivity and an increase of product quality of individuals is connected with the use of highly concentrated food that has a negative effect on the rumen environment [1]. Ruminants rely on symbiosis with rumen microbiota. Microorganisms produce for the host proteins, vitamins and organic acids with short chain [2]. Stable intraruminal environment is also related to the fluctuation of pH, the concentration of fatty acids with short chain (SCFA) and ammonia in the ruminal fluid. SCFA are mainly produced from microbial fermentation and they stimulate the development of the ruminal epithelium, which results in improvement of the structure and absorptive capacity [3]. After ingestion probiotic organisms can modulate the balance and activity of gastrointestinal microflora, whose role is essential for intestinal homeostasis [4]. It has also been shown at probiotics that they increase the protection against toxins produced by pathogenic bacteria, and inhibit the growth and proliferation of pathogenic microorganisms [5].

2. Materials and methods

We used two adult cows breeds Aberdeen-Angus with an implemented permanent rumen cannula (13 cm) for evaluating the effects of probiotics family *Bifidobacteriunm sp.* (107 g-1). The average body weight during the experiment was in the first animal was 799 ± 7.1 kg , is the second animal 594 ± 9 kg. Probiotics *Bifidobacterium sp.* were administered in lyophilized form of 2 g each, stirred in 100 ml of drinking water and applied through the fistula into the rumen. The animals

* Corresponding author: Luboš Zábranský, tel.: +420387772568, zabransky@zf.jcu.cz

were fed twice a day (6:00 and 15:00). The basal diet consisted of hay and water (ad libitum intake). Samples of rumen fluid were collected in three periods 3 times a week, every Monday, Wednesday and Friday always three hours after the morning feeding. The concrete collection was conducted through the rumen cannula by a tube probe connected to vacuum hand pump, according to [6]. Rumen fluid was immediately transported to the laboratory. For subsequent laboratory analysis the samples were filtered through gauze. Rumen fluid was used to determine the pH, the analysis of nitrogen compounds, VFA and for determining the number of ciliates.

Determination of protozoa content – the quantity of protozoa in the rumen fluid was determined by counting in a Bürker chamber after staining and dilution of samples with 0.1% methylene blue in a ratio of 1:10. To determine the total number of protozoa in 1 ml of rumen fluid, the found number was multiplied by a factor 10,000, comprising a chamber factor and dilution index.

3. Results and discussion

Totally 60 samples of rumen fluid were collected from each individual. The amount of ciliates differed significantly only between individuals. There was a noticeable decline in the amount of ciliates in the first experimental animal, while in animal 2, the average amount of ciliates slightly increased. The results of testing of individual variable values towards treatment (attempt, control) with a fixed effect of an individual were insignificantly $P = 0.4811$. According to the literature the rumen fluid consists of about 106 protozoa [7]. Protozoa numbers in this experiment were lower because of feeding animals only by hay. The same conclusion reached in his study also [8]. The quantity of rumen microflora is also determined by the size of rumen, it is to a large extent determined by the inner surface of forestomach [9, 10]. Protozoa are an integral group affecting the digestion of cellulose; their presence increases the digestibility of cellulose up to 20% [11, 12]. For this reason, the increase of this microflora is desirable. However, with the growth of protozoa, the composition of other rumen microorganisms also changes. Protozoa use bacteria as a food source. Their absorption and subsequent lysis reduce the amount of many strains [13-15]. An increased production of hydrogen molecules also means an increase in methanogenic bacteria that live in symbiosis with protozoa [16, 17]. In the overall assessment of protozoa importance we must also point their detoxifying role, especially the degradation of fungal toxins [18, 19].

4. Conclusions

The increase of protozoa is related to the change of other microbial populations, especially with the amount of cellulolytic bacteria significantly involved in the fermentation processes in the rumen. In this part of the experiment we did not manage to prove statistically the effect of *Bifidobacterium sp.* to an increase of the number of protozoa in the rumen fluid. The average number of monitored infuzoria in a millilitre of rumen fluid fluctuated around values of 292 ± 64 thousand in the control and 312 ± 75 000 after application of *Bifidobacterium sp.* Determination the number of protozoa in the rumen fluid in cannulated cows is only a part of a broader experiment, where are currently recorded, evaluated and statistically processed the data regarding the effect of *Bifidobacterium sp.* on rumen pH, levels of fatty acids, protein content and determination of NDF. In conclusion, we can state that the *Bifidobacterium sp.* had a positive effect on increasing the number of ciliates in the rumen fluid, but statistically significant effect was not proved.

This influence could occur after increasing the number of experimental cannulated cows, by increasing the number of samplings and by extending the time period of the experiment.

Acknowledgements

This study was created with the contribution of projects NAZV QJ1210144, NAZV QJ1530058 and NAZV QJ1210375.

References

1. Wencelová, M., Váradyová, Z., Mihaliková, K., Čobanová, K., Plachá, I., Pristaš, P., Jalč, D., Kišidayová, S., Rumen fermentation pattern, lipid metabolism and the microbial community of sheep fed a high-concentrate diet supplemented with a mix of

medicinal plants. Small Rumin. Res. 2015, 125, 64–72, doi:10.1016/j.smallrumres.2015.01.028
2. Pinloche, E., McEwan, N., Marden, J.-P., Bayourthe, C., Auclair, E., Newbold, C.J., The Effects of a Probiotic Yeast on the Bacterial Diversity and Population Structure in the Rumen of Cattle. PLoS One 2013, doi:10.1371/journal.pone.0067824
3. Lesmeister, K. E., Heinrichs, A. J., Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal dairy calves. *J. Dairy Sci.* 2004, 87, 3439–3450
4. Ewaschuk, J. B, Naylor, J. M, Zello G. A., Lactobacillus rhamnosus strain GG is a potential probiotic for calves, Can J Vet Res, 2004, 68, 249-258
5. Guerra-Ordaz, A. A., Molist, F., Hermes, R.G., Gómez de Segura, A., La Ragione, R.M., Woodward, M.J., Tchorzewska, M. a., Collins, J.W., Pérez, J.F., Martín-Orúe, S.M., Effect of inclusion of lactulose and Lactobacillus plantarum on the intestinal environment and performance of piglets at weaning. Anim. Feed Sci. Technol. 2013, 185, 160–168
6. Hofírek, B., Dvořák, R., Němeček, L., Doležal, R., Pospíšil, Z., Cattle diseases. Czech buiatrick company, 2009
7. Saleem, F., Bouatra, S., Guo, A. C., Psychogios, N., Mandal, R., Dunn, S. M., Ametaj, B. N., Wishard, D. S., The Bovine Ruminal Fluid Metabolome. Metabolomics, 2012, 9, 360-378
8. Carberry, C. A., Kenny, D. A., Han, S., Mccabe, M. S., Waters, S. M., Effect of Phenotypic Residual Feed Intake and Dietary Forage Content on the Rumen Microbial Community of Beef Cattle, Appl. Environ. Microbiol. 2012, 78(14), 4949-4958
9. Bartoš, S. (1987): Microbiology and biochemistry of digestion in the rumen, Academia, 1987, pp. 183
10. Abubakr, A. R., Alimon, A. R., Yaakub, H., Abdullah, N., Ivan, M., Digestibility, rumen protozoa, and ruminal fermentation in goats receiving dietary palm oil by-products, Journal of the Saudi Society of Agricultural Sciences, 2013, 12, 147–154
11. Yoder, R. D., Trenkle A., Burrougs, W., Influence of Rumen Protozoa and Bacteria upon Cellulose Digestion In Vitro. J. Anim. Sci. 1996, 25, 609-612
12. Michalowski, T., Rumen protozoa in the domestic ruminant in Holzapfel, W. H., Naughton, P. J.: Microbial Ecology of Growing Animals: Biology of Growing Animals, Elsevier Health Sciences, 2005, 504
13. Jarvis, B. D. W., Lysis of viable rumen bacteria in bovine rumen fluid. Appl. Microbiol. 1988, 16, 714-723
14. Meyer, J. H. F., Mackie, R. I., Microbiological Evaluation of the Intraruminal In Sacculus Digestion Technique. Appl. Environ. Microbiol. 1986, 51(3), 622-629
15. Ivan, M., Daynellde, M. S., Mahadevan, S., Hidiroglou, M., Effect of bentonite on wool growth and nitrogen metabolism in fauna free and faunated sheep. J. Anim. Sci. 1992, 70, 3194-3202
16. Lloyd, D., Williams, A. G., Amann, R., Hayes, A. J., Durrant, L., Ralphs, J. R., Intracellular prokaryotes in rumen ciliate protozoa: detection by confocal laser scanning microscopy after in situ hybridization with fluorescent 16S rRNA probes. Europ. J. Protist. 1996, 32, 523–531
17. Das, S., Adhya, T. K., Dynamics of methanogenesis and methanotrophy in tropical paddy soils as influenced by elevated CO2 and temperature interaction, Soil Biol. Biochem. 2012, 47, 36-45
18. Xiao, H., Marquardt, R. R., Frohlich, A. A., Phillips, G. H., Vitti, T. G., Effect of a hay and a grain diet on the rate of hydrolysis of ochratoxin A in the rumen of sheep. J. Anim. Sci. 1991, 69, 3706-3714
19. Mobashar, M., Hummel, J., Blank, R., Südekum, K-H., Ochratoxin A in Ruminants. A Review on Its Degradation by Gut Microbes and Effects on Animals. Toxins, 2010, 2, 809-839.