# THE INFLUENCE OF DIFFERENT FEEDING TECHNOLOGIES ON RUMEN PH AND SOME MICROBIOLOGICAL PARAMETERS OF DAIRY COWS

# DAŽĀDAS ĒDINĀŠANAS TEHNOLOĢIJAS IETEKME UZ PIENA GOVJU SPUREKĻA PH UN MIKROBIOLOĢISKIEM RĀDĪTĀJIEM

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**ABSTRACT**

The aim of the research was to study the effect of different feeding technologies on rumen pH and some microbiological parameters of dairy cows.

The investigations were carried out during the indoor period with 12 Lithuanian Black&White cows fed balanced ration of the same composition. Taking into consideration feeding technologies were formed two groups of dairy cows: for the control group food was given as separate components; for the experimental – as a chopped mixer of forages prepared by a van-mixer „OptiMix". Concentrates for both groups of cows were given individually. Samples of rumen fluid were taken 3 hours after morning feeding every tenth day. The duration of the experiment was 40 days. The following parameters were studied: pH (by a potenciometric method); total count of lactobacillus, total count of enterobacteria (by a plate method), total count of bifidobacteria (by a method of limitary dilutions).

It was defined during the experiment that in cases of different feeding technologies rumen pH in all stages of the experiment was higher by 1.4 – 7.2% ($P>0.05$), total count of lactobacillus lower by 0.3–5.8% ($P>0.05$) if to compare to the control group of cows. Total count of enterobacteria in both groups of cows changed in the limits of 1.7 and 4.3% ($P>0.05$). Prevailing species in the relationship between lactobacillus and enterobacteria remained lactobacillus. The defined growth of bifidobacteria in all stages of the experiment was $10^7$ as in the experimental so in the control groups. It can be concluded from the results of the experiment that different feeding technologies had no significant effect on rumen pH and qualitative composition of rumen microflora. Statistically reliable differences were not found.

**KEY WORDS:** cows, rumen, „Mix"-forages, pH, lactobacillus, bifidobacteria, enterobacteria.

## INTRODUCTION

The unique ecosystem of microorganisms in the rumen of ruminants and other parts of the digestive system is formed in dependence on animal physiological state, forage composition, their structure, quality and the environment (4, 5, 12, 22, 24). Quality of the microbiological processes, dissociation of food components and its assimilation greatly depends on the microbial count, their variety and interaction (6, 7, 9, 16). Rumen microflora is characterized by a quite great number of lactic acid bacteria (*Lactobacillus* and *Bifidobacteria*) and lower number of enterobacteria, *Pseudomona*, *Enterococcus*, *Staphylococcus*, yeast fungi and *Clostridia*. With rumen development and changes of ration this number tends to decrease (10, 22, 30, 32, 33, 34). Lactic acid bacteria are very active in the fermentative processes and synthesis of biologically active substances. Producing lactic and other acids they maintain acidity of the environment, in which activity of conditionally pathogenic and putrefactive bacteria is suppressed, metabolic processes are optimized and positively effected resistance of the organism (28). Quite important role in the rumen is played by enterobacteria most of which belong to nonpathogenic species *Lactobacillus* and enterobacteria are mutually commensal species between which in normal conditions prevailing species is lactic acid bacteria (5). When cows are fed forage of poor quality microbiological processes in the rumen are disturbed, production quality becomes worse, neonate calves are weaker (8, 33).

In order to improve forage nutritional value and its assimilation in the organism forage is effected by various physical, chemical and biological factors (1, 2, 3, 19). It has been widely reported that chopped forage is easier accessible to microorganisms and this fact leads to higher activity of fermentative processes (14, 15, 18, 25, 26). It was defined in previous experiments that forage cut by a van-mixer „OptiMix“ positively effected reduction activity of bacteria in the rumen, count of infusoria, total amount of free fatty acids, glucose fermentation and forage organic matter digestibility (14, 15).

The aim of the research was to study the effect of forage preparation technologies on rumen pH, total count of lactobacillus, enterobacteria, their relation and count of bifidobacteria.

## MATERIAL AND METHODS

The experiment was carried out during the indoor period with Lithuanian Black&White cows at the Research Center of Digestive physiology and pathology and the Center of Practical training and experiments of the Lithuanian Veterinary Academy.

Taking into consideration feeding technologies were formed two groups of cows: control (n=6), – cows were fed uncut forage and experimental (n=6) – fed ration cut by a van-mixer „OptiMix“. The cows of the control and experimental groups were fed twice daily the same ration balanced according to the amount of crude protein and requirements of metabolizable energy on the basis of generally accepted in Lithuania standards (23). The ration for each cow contained: haylage of perennial grass (15 kg), maize silage (10 kg), silage of various grass (12 kg), hay (4 kg), straw (2 kg), saladine (10 kg), concentrates (averagely8 kg), mineral-vitamin supplements and licking salt. The ration per cow contained dry matter 21.4 kg, digestible protein 2204 g, sugar 2354 g, fiber 4.07 kg, calcium 145 g, phosphorus 109 g, carotine 1049 mg, salt 145 g. For the cows of the control group forage ration was given separately unmixed and uncut. The cows of the experimental group forage was given cut up to 2–3 cm mixed by a van-mixer „Opti Mix“. Concentrates for both groups of cows were given individually. Rumen fluid was sampled 3 hours after morning feeding on the 10; 20; 30; 40 days of the experiment from the caudoventral part of the rumen by a throat-oesophagus tube GDZ–1 (20). The experiments were carried out during 2 hours period at the microbiological laboratory. The dilutions were completed by a classic method up to $10^{-12}$. The following

investigations were carried out: pH by a potenciometric method; total count of lactobacillus, and count of enterobacteria by a plate method; count of bifidobacteria by a method of limited dilutions (31).

Total count of lactobacillus was defined by dissemination of separate rumen fluid dilutions on the surface of selective medium MRS („Liofilchem", Italy). The plates were incubated in microaerophylic conditions at the environment of 5–10 % $CO_2$, 37C$^o$ temperature for 48 hours. After the incubation on the agar were calculated typical colonies grown in the MRS agar. Preliminary identification of lactobacillus was carried out according to morphology of colonies, Gram reaction and activity of catalasis (27).

Count of enterobacteria was defined by dissemination of different dilutions of rumen fluid on the surface of Levin agar („Merk",Germany). The plates were incubated at aerobic conditions at 37C$^o$ temperature for 48 hours. The colonies were counted and the smears were evaluated microscopically.

Count of bifidobacteria was defined in semi-liquid medium of Blauroc (29), when dilutions of the rumen fluid were disseminated by a deep method and incubated at 37C$^o$ temperature for 48 hours. The growth of bifidobacteria was evaluated visually after incubation. From a deep layer of the column by a special pipette were isolated colonies with typical growth and evaluated microscopically.

The results of the experiments were evaluated by the method of statistical analysis (11). The data of lactobacillus and enterobacteria are presented as logarithmic expressions.

## RESULTS AND DISCUSSION

Using different feeding technologies it was defined that pH in the control and experimental groups fluctuated in the ranges of physiological norm (3, 12, 13, 21). Rumen pH at the experimental group of cows on the 10-th day of the experiment was – by 1.4 %; on the 20-th – 7.2 %; on the 30-th – 3.6 %; on the 40-th – 3.2 % higher if to compare to the control group of cows, however, statistically reliable differences were not observed (P>0,05; fig.1). As it was stated by F.Bargo et al., (2002); M. Maekawa, (2002), K.M., Krause et al., (2003) chewing of cut and uncut forage is not able to increase the amount of saliva, so only inconsiderable fluctuations of pH are observed. The results of the experiments only confirms the statement that rumen pH is independent on the way of feeding.

Total count of lactobacillus in the rumen fluid of the experimental group of cows in all stages of the experiment remained lower than in the control group, however, fluctuated in the ranges of physiological norm. Total count of lactobacillus after 10 days of the experiment in the experimental group of cows was by 0.3 %; after 20 days – by 5.7 %, after 30 days – by 5.8 % and after 40 days – by 3.1 % lower if to compare to the control group of cows, but reliable differences were not stated (P>0.05; fig. 2.). As it was stated by V.V.Subotin (2004) total count of lactobacillus in the rumen of adult animals depends on the ration and can fluctuates in quite wide ranges.

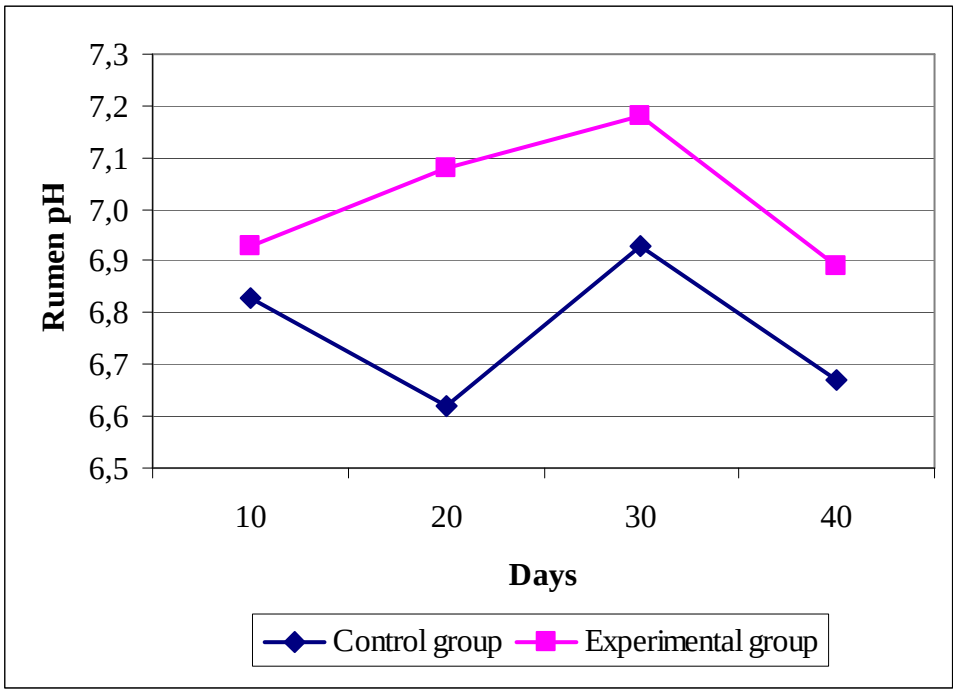


Figure 1.. **The influence of different feeding technologies on rumen pH**

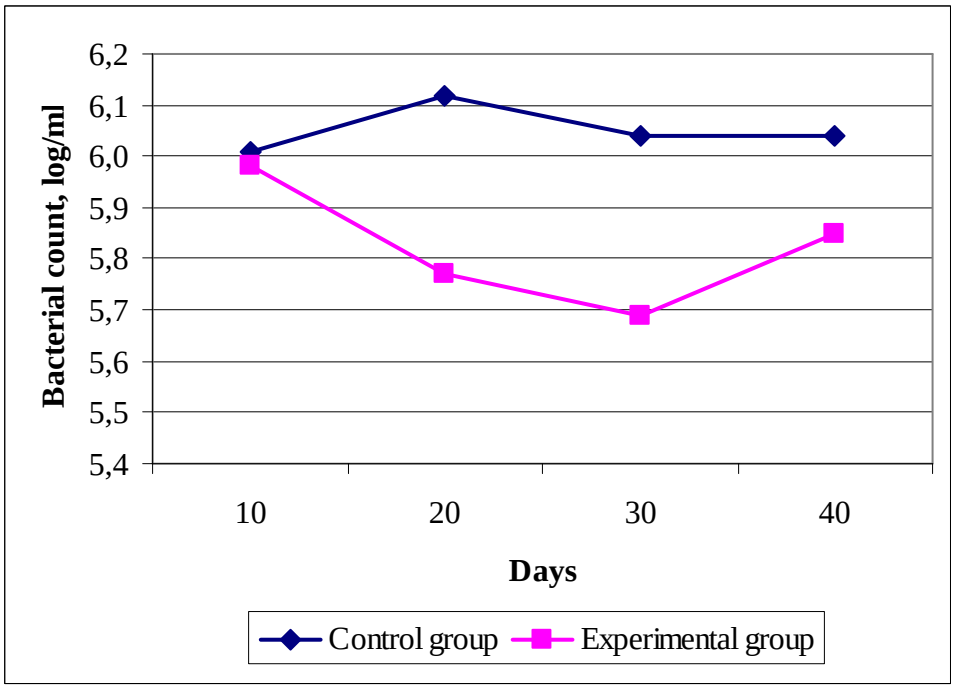


Figure 2.. **The influence of different feeding technologies on total count of lactobacillus in rumen**

Total count of enterobacteria in the experimental group of cows after 10 and 30 days was respectively by 2.2 % and 1.7 % lower, after 20 and 40 days – by 4.3 %, and 4.1 %, higher if to compare to the control group of cows, however, these differences were statistically unreliable (P>0,05; fig. 3).

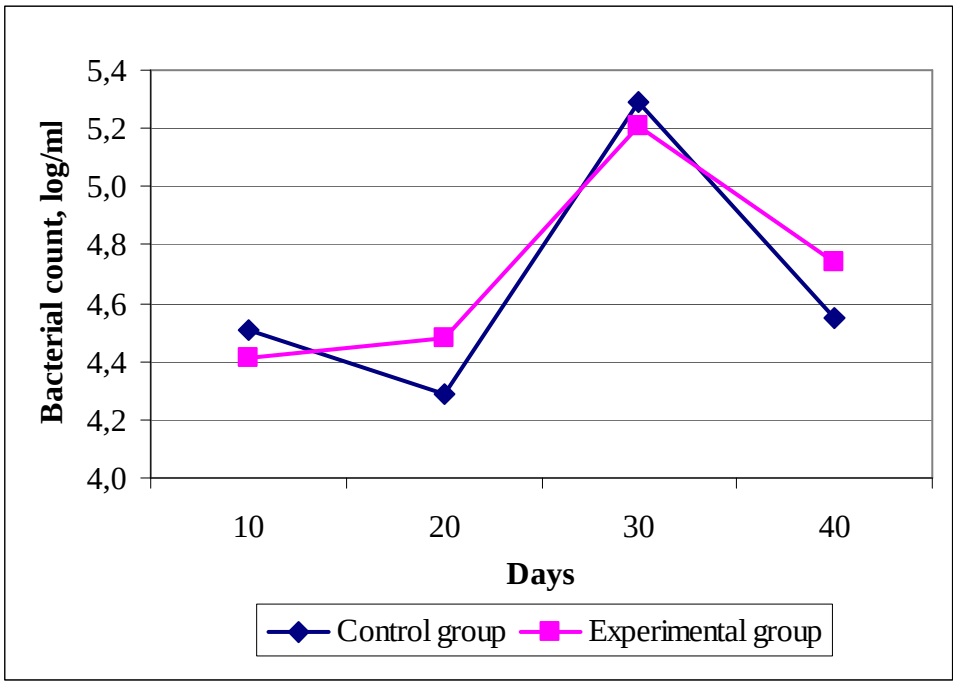


Figure 3. **The influence of different feeding technologies on total count of enterobacteria in rumen**

Total count of enterobacteria between the control and experimental groups differed insignificantly.

Analyzing the relationship between lactobacillus and enterobacteria it was revealed that as in the control so in the experimental groups prevailed lactobacillus. The control group contained higher number of lactobacillus: after 10 days – by 25 %; after 20 days – by 30 %, after 30 days – by 12 % and after 40 days – by 25 % while in the experimental group these numbers were respectively 27 %, 23 %, 9 % and 18 %. This fact is confirmed by literature data as well: lactobacillus are always prevailing in the digestive system of healthy ruminants (5, 32).

The method of limited dilutions for the investigation of bifidobacteria is not exact enough, so statistical reliability was not calculated. However, this method demonstrated that the count of bifidobacteria in both groups of cows was $10^7$ and corresponded to the physiological norm (12).

The results of our experiments demonstrate that different technologies of forage preparation had no effect on the rumen parameters studied.

## CONCLUSIONS

1. Different technologies of forage preparation had no effect on rumen pH, total count of lactobacillus, enterobacteria and bifidobacteria.
2. The prevailing species between lactobacillus and enterobacteria in the rumen remained lactobacillus.

## REFERENCES

1. Bargo, F., Muller, L. D., Varga, G. A., Delahay, J. E., Cassidy, T. W. Ruminal digestion and fermentation of high producting dairy cows with three different feeding systems combing pasture and total mixed rations. - J. Dairy. Sci. 2002. 85 (11): 2964 -2973.

2. Bas, P., Archimède, H., Rouzeau, A., Sauvant, D. Fatty acid composition of mixed-rumen bacteria: effect of concentration and type of forage. - J. Dairy. Sci. 2003. 86 (9): 2940-2948.
3. Cunningham, J. G. Textbook of veterinary physiology. Second edition. - Michigan: W.b. Saunders Company, 1997.- 332-403 P.
4. Dehority, B. A. and Orpin, C. G. Development of, and natural fluctuations in, rumen microbial populations. The rumen microbial ecosystem. Hobson P. N. and Stewart C. S. Second edition. - London: Blackie academic & professional, 1997.- 196-235 P.
5. Hobson, R. H., Stewart, C. S. The rumen microbial ecosystem. - Blackie akademic professional London. Weinheim. Newyark. Tokyo. Melbourne. Madras. 1992.- 41-43 P.
6. Hutjens, M. F. Feed additives Veterinary Clinics of North America. - Food Animal Practice. 1991. 7: 525-540.
7. Yoon, I. K., Stern, M. D. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminant: A. review. - Austral. Asian J. Anim. Sci. 1995. 8: 533-55.
8. Jatkauskas, J., Vrotniakienė, V. Žolinių pašarų konservavimas Lietuvoje: progresas ir prioritetai. Žolinių pašarų konservavimas – gera praktika ir naujos žinios. - Tarptautinio seminaro pranešimų medžiaga. Kaunas, 2002.- 5-10 P.
9. Jeroch, H., Drochner, W., Simon, O. Ernärung landwirtschaftlicher Nutztiere. - Stuttgart, 1999.- 125-133 P.
10. Jochmann, K., Lebzien, P., Flachowsky, G. Einfluss von Milchsäurebacterien als Siliermittel auf Pansenphysiologische Parameter, die Verdaulichkeit der Silagen sowie Leistung von Milchkühen. - Űbers. Tierernährg. 1998. 26: 123-155.
11. Juozaitienė, V., Kerzienė, S. Biometrija ir kompiuterinė duomenų analizė. - Kaunas, 2001. 115 P.
12. Kamra, D. N. Rumen microbial ecosystem. - Curent Science. 2005. 89 (1): 124-131.
13. Krause, K. M., Combs, D. K. Effects of forage size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows. - J. Dairy. Sci. 2003. 86 (4): 1382-1397.
14. Laugalis, J., Monkevičienė, I., Želvytė, R., Sederevičius, A., Ramanauskienė, J., Makauskas, S., Kantautaitė, J. Melžiamų karvių didžiojo prieskrandžio turinio rodiklių priklausomybė nuo šėrimo technologijų. - Veterinarija ir zootechnika. Lietuvos veterinarijos akademija, 2004. 25 (47): 12-15.
15. Laugalis, J., Želvytė, R., Ramanauskienė, J., Monkevičienė, I., Sederevičius, A., Makauskas, S., Kantautaitė, J. Melžiamų karvių, šeriamų skirtingomis technologijomis, didžiojo prieskrandžio turinio anaerobinės mikrofloros tyrimai. - Veterinarija ir zootechnika. Lietuvos veterinarijos akademija, 2004. 25 (47): 16-20.
16. Mackie, R. I., Aminov, R. I., White, B. A., McSween, C. S. Molecular ecology and diversity in gut microbial ecosystems. Ruminant physiology: digestion, meatabolism growth and reproduction. Edited by P. B. Cronje. - CABI publishing, 2000.- 59-99 P.
17. Maekawa, M., Blauchemin, K. A., Christensen D.A. Effect of concentrate level and feeding managment on chewing activities, saliva production and ruminal pH of lactating dairy cows. - J. Dairy. Sci. 2002. 85 (5): 1165-1175.
18. Monkevičienė, I., Sederevičius, A. The influence of some feeding factors on ruminal fermentation processes *in vitro*. - Proceedings of international Conference (Current Issues in Veterinary Medicine). Jelgava, 2000. 119-120.
19. Nagaraja, T. G., Newbold, C. J., Van Nevel, C. J., Demeyr, D. I. Manipulation of ruminal fermentation. The rumen microbial ecosystem. Hobson P.N., Stewart C.S. Second edition. - London: Blackie academic & profesional, 1997.- 523-632 P.
20. Sederevičius, A. Diagnostiniai ir gydomieji zondai galvijams. - Kaunas, 2000.- 3-9 P.

21. Sederevičius, A. Gyvūnų organizmo skysčių fiziologiniai rodikliai. - Kaunas, 2004.- 56-61 P.
22. Stewart, C. S. Lactic acid bacteria in the rumen. In The Lactic Acid Bacteria. The Lactic Acid Bacteria in Health and Disease. Ed. Wood J.B.B. - New York: Elsevier, 1992.- 49-68 P.
23. Tarvydas, V., Bendikas, P., Mankevičius, R., Uchockis, V. Šėrimo normos, pašarų struktūra ir sukaupimas galvijams. - Vilnius, 1995.- 27 P.
24. Van Gylswyk, N. O. Enumeration and presumptive identification of some functional groups of bacteria in the rumen of dairy cows fed grass silage – based diets. - FEMS Microbiology ecology, Uppsala, 1990. 73: 243-254.
25. Varga, G. A., Kolver, E. S. Microbial and animal limitations to fiber digestion and utilization. - J.Nutr. 1997. 127: 819-823.
26. Želvytė, R. Subklinikinės acidozės diagnostika, fermentacinių procesų reguliacija ir optimizacija karvių didžiojo prieskrandžio turinyje. Kand. disertacija. - Kaunas, 2000.- 153 P.
27. Баникова, Л. А., Королева, Н. С., Семенихина, В .Ф. Микробиологические основы молочного производства. - Москва. Агропромиздат, 1987.- 322-333 Р.
28. Данилевская, Н. В., Субботин, В. В., Вашурин, О. А. Лактобифиол для стимуляции продуктивности дойных коров. - Ветеринария. 2003. 2: 50-54.
29. Квасников, Е. Н., Нестеренко, О. А. Молочнокислые бактерии и пути их использования. - Москва: Наука, 1975.- 388 Р.
30. Кондрахин, И. П. Диспепсия новорожденных телят - успехи проблемы. - Ветеринария. 2003. 1: 39-43.
31. Медицинская микробиология. (ред. Покровский, В. И., Поздеев, О. К.). - Москва: ГЭОТАР Медицина, 1999.- 76 Р.
32. Сидоров, М. А., Субботин, В. В., Данилевская, Н. В. Нормальная микрофлора животных и ее коррекция пробиотиками. - Ветеринаия. 2000. 11: 17-21.
33. Субботин, В. В., Сидоров, М. М. Основные элементы профилактики желудочно – кишечной патологии новорожденных животны. - Ветеринария. 2004. 1: 3-6.
34. Субботин, В. В. Профилактика желудочно – кишечных болезней новорожденных животных с симптомом диареи. - Ветеринария. 2001. 4: 3-7.