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INFLUENCE OF THE COMPLEX ACTION OF SORBENTS AND PROTECTED PROTEIN ADDITIVES ON THE COURSE OF METABOLISM OF RUMEN MICROFLORA

Tetyana YELETSKAYA, CandBioSc, SR.,
https://orcid.org/0000-0001-8980-6972
Nataliya RUSKO, CandAgSc, https://orcid.org/0000-0003-2739-994X
Nataliya LYASHENKO, https://orcid.org/0009-0005-3629-9264
Galina PRUSOVA, CandAgSc, SR., https://orcid.org/0000-0002-2604-5720
Livestock farming institute of NAAS of Ukraine, Kharkiv, Ukraine

The effect of the combined action of sorbents (zeolite and polymer) and a protected protein additive (TEP mix) on the metabolism of microflora in the rumen was investigated in the conditions of the physiological yard on the black and spotted breed heifer with an established the rumen fistula. Changes in the composition of rumen microflora under the influence of additives and sorbents were studied, and the pH of rumen fluid was determined.

Changes in microbiological processes in the rumen of the experimental animal were detected, but these were not negative and did not disrupt the normal course of rumen digestion. Fluctuations in the average daily pH of the rumen content between experiments were insignificant. Changes in the enzymatic activity in the rumen were assessed when feeding sorbents and additives: when feeding zeolite, amylolytic activity, depending on the time of feeding, increased by 3.7 % before feeding ($p<0.01$), and after 3-4 hours decreased by 23.0 % ($p<0.05$); when feeding a diet with a polymer, amylase activity in the rumen was lower than in the control diet, both before and after feeding, which resulted in a decrease in the average daily indicator by 15.7 % ($p<0.05$). Adding a polymer to the diet did not cause significant fluctuations in the average daily values of cellulolytic activity in the rumen. On the diet with zeolite, changes in cellulolytic activity were also insignificant, but with an increase in amylolytic activity, a decrease in cellulolytic activity was observed and vice versa. An average daily increase in the number of ciliates (within physiologically normal limits) of 16.9 and 26.4 % was established for the first and second experiments, respectively, for the control. The average daily number of bacteria in animals that consumed diets with zeolite and polymer was approximately at the same level as the control indicators.

Thus, the studied sorbents and feeds with protected protein can be used in cow diets without harm to metabolic processes in the rumen. In the presence of low-quality concentrated grain feeds, unbalanced diets, it is possible not to prevent the breakdown of protein in these feeds, but to contribute to a significant increase in the synthesis of microbial protein, which has high biological value.

Keywords: protein protection, digestion, rumen, rumen microflora, sorbents

ВПЛИВ КОМПЛЕКСНОЇ ДІЇ СОРБЕНТІВ І ДОБАВОК ЗАХИЩЕНОГО БІЛКУ НА ПЕРЕБІГ МЕТАБОЛІЗМУ РУБЦЕВОЇ МІКРОФЛОРИ

Тетяна ЄЛЕЦЬКА, к. б. н., ст. досл., https://orcid.org/0000-0001-8980-6972
Наталія РУСЬКО, к. с.-г. н., https://orcid.org/0000-0001-8980-6972
Наталія ЛЯШЕНКО, https://orcid.org/0009-0005-3629-9264
Галина ПРУСОВА, к. с.-г. н., ст. досл., https://orcid.org/0000-0002-2604-5720
Інститут тваринництва НААН, Харків, Україна

В умовах фізіологічного двору на теличці чорно-рябої породи з встановленою рубцевою фістулою досліджено вплив сумісної дії сорбентів (цеоліту та полімеру) та добавки захищеного білку (ТЕП мікс) на метаболізм мікрофлори в рубці. Досліджено зміни складу рубцевої мікрофлори під впливом добавок та сорбентів, визначено pH рубцевої рідини.

Встановлено зміни мікробіологічних процесів в рубці дослідної тварини, але вони не мали негативного характеру та не порушували нормальний перебіг рубцевого травлення. Коливання середньодобового pH вмісту рубця між дослідами незначні. Оцінено зміни ферментативної активності в рубці при згодовуванні сорбентів та добавки: при згодовуванні цеоліту амілолітична активність в залежності від часу годівлі: до годівлі збільшувалася на 3,7 % ($p<0,01$), а через 3-4 години знижувалась на 23,0 % ($p<0,05$); за згодовування раціону з полімером – активність амілази у рубці була нижчою, ніж на контрольному раціоні, як до, так і після годівлі, що позначилося на зниженні середньодобового показника на 15,7 % ($p<0,05$). Додавання полімеру до раціону не викликало значних коливань середньодобових значень целюлозолітичної активності в рубці. На раціоні з цеолітом зміни целюлозолітичної активності також були незначними, але при збільшенні амілолітичної активності спостерігалося зниження целюлозолітичної і навпаки. Встановлено середньодобове збільшення числа інфузорій (у фізіологічно нормальних межах) на 16,9 та 26,4 % для першого та другого дослідів, відповідно контролю. Середньодобова кількість бактерій у тварин, які споживали раціони з цеолітом і полімером, перебувала приблизно на одному рівні з показниками контролю.

Таким чином, досліджувані сорбенти та корми з захищеним білком можуть бути використані в раціонах корів без шкоди для метаболічних процесів у рубці. За наявності низькоякісних концентрованих зернових кормів, незбалансованих раціонів, можна не запобігати розщепленню білка цих кормів, а сприяти значному збільшенню синтезу мікробного білка, який має високу біологічну цінність.

Ключові слова: захищеність протеїну, травлення, рубець, рубцева мікрофлора, сорбенти.

Introduction. The role of rumen microflora in digestive processes is multifaceted and irreplaceable, which is why, according to (Ørskov E.R., et al., 2012), two aspects should be taken into account when it comes to feeding ruminants: providing nutrients to rumen microorganisms and meeting the needs of the host animal itself. The microflora of the rumen consists of many microorganisms of different species: bacteria, infusoria, lower fungi (Amanzougarene Z. M., et al., 2022). Rumen bacteria are the most widespread group of microorganisms (1 ml of ruminal content contains from 107 to 1012 bacteria (Zhao G., 2019), and its species composition of bacteria is quite large - up to 150 species (F.G. Fabulyak et al., 1990). One of the most important biochemical processes carried out by

ruminant rumen bacteria is the breakdown of fiber, which is carried out by various microorganisms systematic groups and also by cellulolytic fungi (Allison M. J., 1970). Protozoa play an active role in the metabolism of carbohydrates (starch and cellulose breakdown), possessing protease and peptidase activity, and also synthesize proteins Infusoria have a lipid-synthesizing function, and also ferment carbohydrates to LHK (Chalmers M.J. et al., 1977). The protein of protozoa makes up 12% of the ruminal nitrogen content (Kushnir V.I., 1997). the population of microorganisms is a rather fragile ecosystem with complex trophic relationships, adapted to the conditions of the rumen and functioning according to the principle of a self-regulating system. The accumulation of biomass of bacteria and ciliates in the rumen occurs only under optimal conditions. The temperature from + 38 to 40 ºC and the pH of the environment, which is close to neutral (6.0 - 7.3) are considered normal (Eliman M. E., 1982).The relationship between rumen symbionts is currently considered to be a competition between protozoa and bacteria for food. The number and species composition of the rumen of animals is largely dependent on the composition of the diet. Non-nitrogenous substances usually increase the number of bacteria. Небілкові азотисті речовини зазвичай збільшують біомасу інфузорій, however, an increase in the number of bacteria is not always observed.

Increasing the productivity of ruminants, in particular cows during lactation, requires much larger amounts of energy and protein than the mechanism of ruminal symbiotic microflora can provide (Bogdanov G.O., 2013; Monteiro H.F. et al., 2021). Regulation of the intensity and direction of enzymatic processes in the rumen is possible by feeding, introduction of additives of biologically active substances and inhibitors. Additives of sorbents in the diet of cows absorb a certain amount of ammonia and retain it for some time in the rumen, which increases the probability of its inclusion in the composition of microbial protein in the process of slow digestion of crude fiber. Increasing the intake of the protected protein component under the conditions of simultaneous control of the content of additional energy protected from destruction in the rumen allows to increase the level of protein assimilation in periods of greatest need for the body of lactating cows, without negative consequences in terms of metabolic disorders. Currently, agents with adsorption properties are widely used in animal husbandry: inorganic natural materials (zeolites, bentonites, activated carbon), synthetic polymers (polyacrylamide - in various modifications, etc.) (I. I. Grabovenskyi et al., 1984; A. D. Goetsch, et al., 1985). Zeolite is a good absorbent for many organic and inorganic substances (macro- and microelements, vitamins, enzymes, amino acids, etc.) (Lorenz M. et al., 2014). Polyacrylamide gel, which has a spongy structure with a large surface, is most often used for enzyme immobilization. It has been established that the digestibility of feed nutrients in ruminants can be increased due to biologically active substances that create favorable conditions for the growth and development of microflora in the rumen, which leads to an increase in digestibility (Attwood G. T. et al., 1996).

Based on the above, more thorough studies of the complex effect of additives and sorbents on the digestive processes in the rumen of cows are expedient, which will allow to find out the mechanisms of increasing the efficiency of assimilation of nutrients by animals without the risk of their loss as a result of ammonia toxicosis and ketosis, which is definitely relevant and has significant social significance for agricultural producers.

Purpose of the work. To establish the effect of protected protein additives and sorbents on the patterns of metabolism of the scar microflora.

Materials and methods. In the conditions of the physiological yard, a surgical operation was performed to establish a fistula on the rumen of a heifer of the black and spotted breed. The animals received the basic diet (BD), which consisted of hay, silage, fodder molasses and compound feed. Combined feed consisted of wheat, barley, sunflower

meal, wheat bran and mineral additives. In the experimental diets, 50 % of the compound feed was changed to a bypass product (TEP mix). Also, two types of sorbents were added to the diet: 4 % zeolite (first experiment), 0.3 % polymer "Solunate" in the compound feed (second experiment).

Each experimental period lasted 21-28 days. The study d scheme is presented in Table 1.

Table 1

Experiment scheme

Diets	Periods, days			
	Egalitarian, 21	**1-й Egalitarian, 28**	**Egalitarian, 21**	**2-й Egalitarian, 28**
	BD	BD + Zeolite	BD	BD + Polymer

At the beginning of each period, chemical analysis of diet feed samples was performed: dry matter (DM), ash (A), crude protein (CP), crude fat (CF), crude fiber (CF), nitrogen-free extractives (NFE), and mineral composition were determined.

Table 2

Theoretical feed consumption by the experimental animal, kg

Feed	Control (BD)	BD+ Zeolite	BD+ Polymer
Forage hay, kg	5,0	5,0	5,0
Corn silage, kg	30	30	30
Feed molasses, kg	1,5	1,5	1,5
Compound feed, kg	8,0	8,0	8,0

Table 3

Actual feed consumption by the experimental animal, kg

Feed	Control (BD)	BD+ Zeolite	BD+ Polymer
Forage hay, kg	4,25	4,02	4,36
Corn silage, kg	28,16	29,85	29,15
Feed molasses, kg	1,5	1,5	1,5
Compound feed, kg	8,0	8,0	8,0

To assess the direction of rumen digestion processes when feeding diets with different sorbents, samples of the specified feed ingredients were incubated in bags with the following parameters: bag size: 7.0 cm by 5.5 cm; average tissue pore diameter 102 μm; sample quantity 5 g and incubation time (15 min for the zero sample and 6 h for the control sample). The course of rumen digestion was indirectly assessed by the difference in the nutrient content of feed samples with and without the sorbent, which were incubated in the rumen environment. The results were processed by the method of variational statistics using the Student's test.

Research results. When feeding diets with zeolite and polymer, according to the experimental data, the digestibility of dry and organic matter differed little from the digestibility of these substances in the control experiment (Table 4). Crude protein digestibility decreased by 35 and 29 %, respectively.

Table 4

Digestibility of nutrients in the rumen of diets, %

Indicators	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
Dry matter	51,4±1,27	49,1±1,85	50,1±1,51
Organic matter	58,5±0,44	56,7±1,30	56,8±1,93
Crude protein	13,7±0,46	8,9±2,16	9,7±1,15
Crude fat	-28,3±17,56	-10,0±2,77	-30,7±5,89
Crude fiber	69,6±1,02	70,1±1,78	70,0±1,82
Crude ash	-50,0±10,29	-57,5±10,24	-51,9±4,90
NFE	64,3±2,77	65,7±1,34	64,71±1,96

The study of the complex effect of protected protein supplements and sorbents on the patterns of the course of metabolism of scar microflora showed a probable decrease in the concentration of hydrogen ions in scar fluid in dynamics (Table 5).

Table 5

pH in rumen content, average daily and in dynamics

Sampling time, hours	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
7:00 (before feeding)	6,8±0,15	7,0±0,06	7,0±0,13
11:00 (after 3 hours)	6,2±0,07	6,2±0,05	6,1±0,09
13:00 (after 5 hours)	6,1±0,05	6,2±0,18*	6,2±0,09*
Average daily concentration	6,3±0,04	6,5±0,07	6,4±0,05

*Note: * - probability of difference p <0.05.*

Organic matter of feed, including proteins and carbohydrates, entering the rumen, is largely broken down by microorganisms. The bulk of nutrients in plant feeds used by ruminants are carbohydrates. Microorganisms of the rumen are able to break down and use as a source of energy large amounts of polysaccharides, such as starch, cellulose and other structural carbohydrates to the stage of monosaccharides. Indicators of the course of microbiological processes in the rumen during the experiment are presented in tables 6 - 9. When determining the amylolytic activity in dynamics (table 6) during feeding the

experimental diets, some decrease was noted. It should be noted that zeolite reduced amylase activity to 28.6 U/ml immediately after feeding the diet (3–5 hours after feeding), and the polymer contributed to a decrease in the amylolytic activity of the rumen microflora both before and after feeding ($p < 0.05$). On the contrary, before feeding, the amylolytic activity of the rumen contents was significantly higher on the diet with zeolite, compared to the control diet, ($P < 0.01$), which suggests both a direct and an indirect effect of zeolite on this indicator.

Table 6

Amylolytic activity in dynamics and average daily, units/ml

Sampling time, hours	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
7:00 (before feeding)	45,3±1,03	50,2±1,45**	41,8±1,81
11:00 (after 3 hours)	43,8±1,82	32,5±1,63	38,6±3,02
13:00 (after 5 hours)	36,8±2,15	28,6±3,25*	31,8±3,18
Average /day	42,3±2,30	36,3±1,55	36,9±1,95*

*Note. * - probability of difference p <0.05; ** - probability of difference p <0.01.*

The cellulolytic activity of the rumen microflora underwent some changes depending on the type of additives fed in the diet. Thus, during the period of the most intensive fermentation (3-5 hours after feeding), the cellulolytic activity in animals receiving zeolite was higher than the control values by 9-34% (Table 7). During the increase in amylolytic activity, a decrease in cellulolytic activity is observed, and vice versa (Tables 6, 7).

Table 7

Cellulolytic activity in dynamics and average daily, %

Sampling time, hours	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
7:00 (before feeding)	22,5±1,30	20,5±0,76*	18,3±0,48*
11:00 (after 3 hours)	12,6±0,91	13,8±2,01*	11,9±1,6
13:00 (after 5 hours)	12,5±1,14	16,8±2,12	14,9±0,55
Average /day	16,8±0,18	16,8±1,22	15,3±0,85

*Note. * - probability of difference p <0.05.*

In animals fed a diet with polymer, 3 hours after feeding, lower cellulolytic activity was noted compared to control indicators - by 2.5 %. No significant differences in the average daily cellulolytic activity were found in different periods of the experiment.

It is known that ciliates are sensitive to changes in feeding conditions. When the rumen pH decreases to 5.0 and below, during starvation and a number of pathological conditions of the antrums , the number of ciliates decreases until they completely disappear. According to studies of the number of ciliates in the rumen fluid, an average daily increase in their number (within physiologically normal limits) was noted in the experimental

groups receiving compound feeds with zeolite and polymer, which indicates a positive effect of additives on their development (Table 8).

Table 8

The ciliates number, in dynamics and average daily, thousand/ml

Sampling time, hours	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
7:00 (before feeding)	143,8±35,46	218,8±18,31	208,3±25,07
11:00 (after 3 hours)	122,5±19,22	145,0±14,72*	158,3±12,85
13:00 (after 5 hours)	157,5±19,79	152,5±22,91	167,8±16,10
Average /day	145,7±20,05	170,4±12,04	184,1±10,37

*Note. * - probability of difference p <0.05.*

A significant decrease in the total number of bacteria in the rumen contents before feeding was observed only in the diet with polymer. After feeding, the situation changed, and this indicator in animals on diets with zeolite and polymer was approximately at the same level as the control indicators (Table 9).

Table 9

The bacteria number, in dynamics and daily average, billion/ml

Sampling time, hours	Diets		
	Control (BD)	BD+ Zeolite	BD+ Polymer
7:00 (before feeding)	10,6±0,26	10,6±0,82	8,5±0,16*
11:00 (after 3 hours)	9,3±0,18	8,9±0,74	8,2±0,25
13:00 (after 5 hours)	7,9±0,15	8,3±0,46	7,6±0,36
Average /day	8,7±0,23	8,8±0,82	8,5±0,46

*Note. * - probability of difference p <0.05.*

Discussion. In the study of the digestibility of nutrients in the rumen when feeding diets with protected protein and sorbents, it was found that the total digestibility of dry matter and organic matter changed slightly. At the same time, it should be noted as a positive point that prevents nitrogen loss, a decrease in the digestibility of crude protein in experimental cows on diets with zeolite and polymer, which is consistent with the data (Martin M., 2014). The inclusion of sorbents in the diet did not lead to significant changes in the digestibility of crude fiber, this indicator was at the level of control values, and the digestibility of nitrogen-free extractives did not change significantly. Negative values of the digestibility of crude fat and crude ash are characteristic of ruminants. The increase in the amount of crude fat in the rumen content, apparently, may indirectly indicate the normal development of the rumen microflora, since it is microorganisms that synthesize fatty acids from non-lipid components of the feed (Wu Z. et al., 1991; Yu Y. et al., 2025).

Apparently, partial sorption of hydrogen ions by zeolite and polymer occurred in the rumen, due to which a more favorable pH level was maintained for the rumen

microflora and the action of bacterial enzymes, in particular proteinases and peptidases, the activity of which largely depends on the pH value. It is known that the optimal reaction for bacterial proteinases is pH about 7.0. The optimum action of peptidases is in the range of pH from 5.5 to 7.0, which depends on the type of bacteria that produce the enzyme (Bach A. et al., 2005). In our studies, the average daily pH of the rumen content of cows did not differ between periods. Changes in cellulolytic activity are probably associated with consistently higher rumen pH values, which favorably affected cellulolysis. A rumen pH level below 6.2 significantly inhibits the development of cellulolytic microorganisms (Atikah I. N., et al., 2021). Similar changes are consistent with the indicators of amylolytic activity in the rumen in animals receiving zeolite. In our opinion, some average daily decrease in amylolytic activity in the rumen, noted when feeding diets containing additives, should be considered rather a positive point. Starch is hydrolyzed very intensively under normal conditions, while a decrease in pH is noted, which does not always positively affect the activity of bacterial enzymes and the survival of some microorganisms. Reducing the fermentolysis of starch also allows for an increase in its entry into the intestine, where it is digested by the body's digestive enzymes to glucose, which helps to provide the body with glucose without enzymatic losses. In this case, theoretically, the consumption of glucogenic amino acids used in the process of gluconeogenesis should decrease. Regarding the opposite changes in amylolytic and cellulolytic activity (Tables 6, 7), it is known that starch in the diet can to some extent inhibit the digestibility of fiber in the rumen. The reasons for this phenomenon may be a decrease in pH below the optimum for the action of cellulases, or competition between amylolytic and cellulolytic organisms in the rumen.

Regarding the characteristics of the state of the microfauna in the rumen at different feeding rations, the results obtained, in our opinion, are explained by the following reasons: the fractional nature of the components of the microbial population of the rumen: a free-living suspension of organisms, microorganisms that have attached to feed particles, and a population of microorganisms that is associated with the rumen wall. The free-living suspension of organisms is the organisms of the rumen fluid. This population is not very specific and contains impurities of organisms that have freed themselves from particles that were previously attached. This fraction is very active in terms of metabolism. The consumed feed falls precisely into this fraction. Microorganisms that have attached to feed particles. It should be emphasized that the main part of the fermentation of roughage is carried out by organisms that adhere to feed particles. This fraction includes both microorganisms that are weakly associated with feed particles and microorganisms that are strongly associated with feed particles. It is in this fraction that the most intensive digestion of fiber occurs. The population of microorganisms associated with the rumen wall includes bacteria that adhere to the rumen epithelium. They are specific and have proteolytic and ureolytic activity (Lobo R. R. et al., 2023). The attachment of microbes to feed particles plays an important role, as it provides close contact with the substrate and increases the time of microbes in the rumen and thereby promotes their survival. There is a constant exchange between the fractions of microorganisms. The total number of ciliates is counted in a certain volume of rumen fluid. When determining the number of bacteria, the rumen contents filtered through several layers of gauze are used. According to (Ørskov E.R., 2002; Ørskov E.R., 1992), it is necessary to distinguish the components of the rumen fluid. The food mass in the rumen is poorly homogenized. Therefore, the content of certain investigated components of the rumen contents and rumen metabolites at different levels may be different. When taking the rumen contents, as a rule, only the liquid fraction of the rumen contents, with free-living microorganisms, enters the Bunsen flask.

Thus, under the conditions of the experiment, the animals studied did not show any disruption of the normal course of microbiological processes in the complex stomach, and

the changes observed were not negative in nature and did not cause physiologically abnormal changes in rumen digestion. However, undoubtedly, the information obtained requires further detailed study using additional microbiological studies. It is obvious that in the presence of concentrated grain feeds of low quality and unbalanced diets, it may be more profitable and rational not to prevent the breakdown of crude protein of these feeds, but to increase the synthesis of microbial protein, which has high biological value. However, its practical use in feeding cows requires further studies on different diets, in combination with different feeds and especially concentrates, taking into account the impact on milk productivity and quality of dairy products.

Conclusions:

1. The effect of the complex action of sorbents and protected protein additives on microbiological processes in the rumen was established: in experimental animals, no disruption of the normal course of microbiological processes was noted, and the changes were not negative in nature and did not cause physiologically abnormal changes in rumen digestion.

2. It was determined that in experimental animals, both on the control and experimental diets, the apparent digestibility of dry matter and organic matter of the experimental diets (with zeolite and polymer) differed little. At the same time, a decrease in the digestibility of crude protein in experimental cows on diets with zeolite and polymer was established by 35 % and 29 %, respectively. The digestibility of crude fiber and nitrogen-free extractives did not change significantly when sorbents were included in the diet, while crude fat and crude ash had negative values.

3. Changes in enzymatic activity in the rumen were assessed: amylolytic activity when feeding zeolite depending on the time of feeding - before feeding increased by 3.7 % ($p<0.01$), and after 3-4 hours decreased by 23.0 % ($p<0.05$); changes in cellulolytic activity were insignificant, but with an increase in amylolytic activity, a decrease in cellulolytic activity was observed and vice versa.

4. It was determined that when feeding a diet with a polymer, amylolytic activity in the rumen was lower than in the control diet, both before and after feeding, which caused a decrease in the average daily rate by 15.7 % ($p<0.05$). Fluctuations in the average daily values of cellulolytic activity in the rumen were insignificant.

5. An average daily increase in the number of ciliates (within physiologically normal limits) of 16.9 and 26.4 % was found in diets with zeolite and polymer, respectively, in the control. The average daily number of bacteria in animals consuming diets with additives was approximately at the same level as in the control. Fluctuations in the average daily pH of the rumen content between the study periods are insignificant.

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