Effect of an enzymatic-probiotic preparation on the microfauna of the rumen of the Russian Black Pied and Ayrshire cows

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Journal of Livestock Science (ISSN online 2277-6214) 13: 96-99
Received on 31/1/22; Accepted on 16/3/22; Published on 30/3//22
doi. 10.33259/JLivestSci.2022.96-99

Abstract

The main purpose of the research was to study the effect of "Rumit" enzyme-probiotic additive, obtained on the basis of reindeer rumen microorganisms (Rangifer tarandus), on the population and generic diversity of rumen protists of lactating Russian Black Pied and Ayrshire cows. In the conditions of one farm with the same feeding rations, control and experimental groups of two breeds of 15 animals each were formed. For 90 days, the experimental groups received 50 g per day of a probiotic preparation in addition to the main diet. At the beginning and at the end of the experiment, the contents of the rumen were selected for counting and determining the generic composition of the ciliate fauna. At the beginning of the experiment, the same density and uniform population of protists were determined in all groups. The use of the probiotic contributed to the growth of the rumen microfauna. In the experimental group of the Russian Black Pied cattle, the density of the ciliate fauna was 2 times higher than in the control group ($p>0.95$). In cows of the Ayrshire breed of the experimental group, in the cicatricial content, the population of ciliates exceeded this indicator of the control by 237.2 thousand ind./ml. Also, the use of probiotics led to the emergence of new genera of protists and their active reproduction. It was concluded that the drug "Rumit" had a positive effect on the density and generic composition of the ciliate fauna of the rumen of cows, which indicates the optimal parameters of its functioning and the state of health of animals in general.

Key-words: cow; Russian Black Pied cattle; Ayrshire breed; ciliate fauna; rumen; probiotic.

Introduction

Ruminants get the energy they need for growth mainly from microbial breakdown of plant biomass in the rumen. Several biotic and abiotic factors influence the effectiveness of fiber degradation, which may ultimately affect the productivity and health of animals (Comtet-Marre et al, 2018).

The symbiotic microbiota of the rumen is necessary for the digestion of plant fibers; it also influences the changes in the productivity and health indicators of ruminants (Li et al, 2019). Protists are essentially considered an indicator of animal health, as they are very sensitive to various external factors. The study of the microfauna population is a prerequisite for monitoring the digestive status of ruminants' proventriculus. The ciliates act as "orderlies" that absorb bacteria, including pathogenic ones; however, most authors recognize them as symbionts with a manifestation of mutualism. This is confirmed by numerous biochemical and physiological studies, according to which it is established that feed undergoes mechanical processing by the ciliates, which use hard-to-digest fiber for their nutrition and, thanks to active movement, create a kind of microcirculation of the environment. Inside the ciliates, we can see small particles of feed consumed by an animal. Ciliates loosen and grind the feed, as a result of which its surface increases, it becomes more accessible to the action of bacterial enzymes. While digesting proteins, starch, sugars and partially fiber, ciliates accumulate polysaccharides in their body. Their body protein has a high biological value. However, the importance of ciliates for ruminal digestion has not yet been sufficiently studied, which is due to the difficulties of studying them outside the body (Litonina et al, 2021a).

The effect of probiotic feeding has been extensively studied in poultry (broilers- Temiraev et al, 2020; layers- Churyumova et al, 2021), quails (Gaitov et al, 20210) and to some extent in lambs (Raghebian et al, 2017). But it is not studied elaborately in cows. In recent years, researchers have been paying close attention to the ability of microorganisms to decompose bacterial and fungal toxins to non-toxic compounds in order to develop highly effective biological products (Cho et al, 2009; Reddy et al, 2009). It is assumed that the effectiveness of biotransformation of toxins is provided by the high lability of metabolism in microorganisms: a huge variety of enzyme systems, synthesis of organic acids and other diverse compounds (Abrunhosa et al, 2014). According to a number of researchers, bacteria associated with deer rumen can actively detoxify usnic acid (Sundset et al, 2009; Luzina et al, 2016) and mycotoxins (Burkin et al, 2014) contained in the components of diets.

In this regard, "Biotrof+" Ltd screened highly effective isolates from the rumen content of *Rangifer tarandus* to create a collection of bacteria with cellulolytic and antimicrobial properties and with the ability to decompose mycotoxins, as the basis of highly effective drugs for reindeer husbandry and other livestock industries. Based on the conducted research, the biological preparation "Rumit" was created (Litonina et al, 2021b).

The purpose of the study: To identify the effect of the enzymatic-and-probiotic preparation "Rumit" obtained on the basis of microorganisms of the reindeer rumen (Rangifer tarandus) on the population and the generic diversity of protists in the rumen of lactating cows of the Russian Black Pied and Ayrshire breeds.

Material and Methods

To study the effect of the enzymatic-and-probiotic supplement on microbiota of the rumen, we conducted a scientific and economic experiment in the period from March 29 to July 19, 2021. The experiment was conducted at "Zarya" Ltd, located in Chagodoshchensky District of the Vologda Oblast (Russia); the duration of the experiment (preliminary and accounting periods) was 110 days.

At the livestock farm (N 59° 8' 50.7228" E 35° 35' 5.5572), we formed control and experimental groups of the Russian Black Pied and Ayrshire cows of 15 heads each. The experimental cows were in the phase of the beginning of lactation (milking); they were chosen by the method of balanced groups, taking into account their pedigree, number of the latest completed lactation (LCL) and milk yield during LCL, live weight, number of milking days and daily milk yield for the current lactation.

In the process of production experiments, the probiotic Rumit ("Biotrof+" Ltd, Russia, 2019) was tested. The probiotic is safe for feeding cattle, contains no conditionally pathogenic or pathogenic micro-flora, including *Pseudomonas aeruginosa, Enterobacteriaceae, Staphylo-coccus aureus, Candida albicans.* The cows from the control groups received a usual ration, and the animals from the experimental groups additionally received 50 g of the studied probiotic "Rumit" per head per day during lunch feeding.

Sampling of the contents of the rumen was carried out with the help of a gag at the beginning and end of the experiment from five heads in each group according to the Kurilov technique (1972) 2–3 hours after feeding. The protists were counted with the use of Micmed-6 microscope (LOMO, Russia) in the Fuchs-Rosenthal chamber at the Laboratory for Bioeconomics and Sustainable Development, Vologda Research Center of the Russian Academy of Sciences. The definition of genera was carried out according to the definitive tables of ophtyoscolecidae (Dogel 1929). According to the work of Pesenko (1982), the population was considered as the ratio of the relative abundance of the genera included in it (pi) – the shares of individual genera in the total number of individuals of all genera.

All the manipulations with the experimental animals were performed according to the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Official Journal of the European Union L276/33, 2010).

Results and Discussion

The number and generic composition of microorganisms in the rumen of animals depends on a number of factors, among which the diet plays a primary role (Reddy et al, 2009; Li et al, 2019).

When studying the population and density of protists in the rumen of lactating cows of Russian Black Pied and Ayrshire breeds, no differences were found at the beginning of the experiment in the control and experimental groups, since these indicators had approximately the same values, and in the context of generic diversity, the dominance of the *Entodinium* genus was established.

When using "Rumit" feed additive in animal diets, an increase in the density of ciliate fauna was observed. The results of the studies are presented in Table 1.

At the end of the experiment, in the experimental groups of Black-and-White cows, the density of ciliates was higher by 143.1 thousand ind./ml, and in Ayrshire cows – by 237.2 thousand ind./ml, compared with the control groups ($p>0.95$). There have been changes in the generic diversity of microfauna under the influence of the studied preparation. In the rumen of the Russian Black Pied cattle of the experimental group, the *Isotricha* genus was identified, which is absent in the control group. The number of ciliates in the rumen of cows of the experimental group compared with the control group was two times higher for the genera *Entodinium* and *Diplodinium*, and three times – for *Dasytricha*.

Besides, the inclusion of the feed additive in the diets of animals of the experimental group of Ayrshire breed contributed to the emergence of a new genus, *Epidinium*, which has a relative abundance of 1.4%. Positive growth dynamics were shown by the ciliate genera *Entodinium, Diplodinium* and *Dasytricha*, their density in the rumen of the experimental group was higher than in the control group by 221.2 ($p>0.95$), 11.5 and 5.7 ($p>0.95$) thousand ind./ml, respectively.

Herbivorous animals have to obtain essential amino acids, which are contained only in animal proteins. The stomach of ruminants has several chambers to carry out this process (therefore, ruminants can be called "protest-eaters"); they use ciliates and other protozoa that reproduce in the rumen, reticulum and omasum. For example, representatives of the genus *Entodinium* are vital to ruminants, since they are mainly responsible for the breakdown of fiber and plant proteins contained in the grass being eaten, and are also able to control the number of bacteria and fungi. The destruction of fiber in the rumen is carried out with the help of cellulolytic activity of populations of microorganisms. This makes it possible for ruminants to feed on coarse fibrous feeds, which would be impossible without the ciliates (Li et al, 2019). We also established a positive relationship between the number of cellulolytic bacteria, which the animals received together with the additive, and the density of ciliate fauna.

The samples of lactating cows of experimental groups of both breeds have shown a sufficiently large species diversity of protists. Besides, they have also shown ciliates in the division stage, which indicates that the parameters of the functioning of the rumen are optimal, as well as the state of the animals' health in general

Normalization of microflora in the digestive tract increases the intensity of absorption of nutrients and the digestibility of feed, which in turn contributes to an increase in the productivity of cows.

Conclusion

Thus, the enzymatic-and-probiotic preparation "Rumit" has a positive effect on the population and the density of ciliate fauna in the rumen of lactating cows, creating more favorable conditions for the formation and growth of protists.

Table 1. Density of ciliates in the rumen of cows in 1 ml of rumen fluid (thousands/ml) ($M \pm m$, $n = 5$).

Genus ciliates	Breed			
	Russian Black Pied		Ayrshire	
	control group	experienced group	control group	experienced group
Entodinium	124.7±35.7	259.4±45.6*	159.3±48.6	380.5±76.5*
Diplodinium	6.3±1.5	12.8±3.4	9.3±2.6	20.8±4.7
Epidinium	3.1±0.6	3.1±1.9	-	5.8±2.0
Dasytricha	1.3±0.1	3.8±1.5	1.8±0.8	7.5±2.1*
Isotricha	-	1.3±0.01	-	-
Total	132.8±39.1	275.9±47.9*	170.3±51.3	407.5±81.1*

*p>0.95

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