



Effect of Dietary Supplements Farmatan TM and Pine Tree Energy on the Regulation of Ruminal Digestion and Microbiocenosis of Lactating Black and White Cows

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Received 30th January 2022; Accepted 10th June 2022; Available online 5th September 2022

Abstract: The study aimed to evaluate the effect of dietary supplements on ruminal digestion and intestinal microbiocenosis of lactating cows. The study was carried out under production conditions on three groups of new black-and-white heifers (10 heads each). Mean live weight of cows was 500±20 kg. The cows of the control group received a basic diet, which included haylage of perennial grasses, corn silage, legume hay, concentrate feed, and molasses. Cows of the experimental groups, in addition to the basal diet (BD), received the Farmatan TM supplement (consists of a balanced combination of tannins, essential oils of clove and cinnamon, sodium acetate, and organic zinc) at a dose of 40 g. head⁻¹ per day (experimental group 1) and a pine tree energy supplement at a dose of 150 g. head⁻¹ per day (experimental group 2). The supplement was mixed with concentrate feed and given once in the morning feeding. In the course of the study, the parameters of ruminal fermentation in experimental animals and the microorganisms of the rumen and the large intestine were studied. At the same time, it was found that the inclusion of the Farmatan TM feed additive (40 g per day) and pine tree energy supplement (150 g per day) in the diet of cows at the beginning of lactation contributed to the strengthening of enzymatic processes in the rumen, which was expressed in an increase in the formation of volatile fatty acids by 7.6 and 20.3%, an increase in the proportion of propionic acid and a slight decrease in the proportion of acetic and butyric acids. When using feed additives (Farmatan TM and pine tree energy supplement), a decrease in the content of *Clostridium* in the rumen of cows was noted by 26.3 and 30.3% in the 1st and 2nd experimental groups, respectively, compared with the control group. In cows of the experimental groups, a decrease in the content of molds and yeast-like fungi in the rumen was also noted. The results obtained allow recommending the studied additives (Farmatan TM and pine tree energy supplement) to enhance enzymatic processes in the rumen.

Keywords: *Clostridium*, Dietary supplement, Microbiocenosis, Ruminal digestion.

Introduction

It is not enough to rationally feed highly productive animals with traditional feeds, the quality of which leaves much to be desired, to offer biologically complete nutrition equal to their high metabolism for the fulfilment of their genetic potential. In this regard, an additional supply of macronutrients is required to mobilize morpho functional systems and organs when using ergotropic substances with directed hepatoprotective, anti-stress, and immune-modulating action in nutrition to prevent and correct disorders of digestive and metabolic processes (De Nardi *et al.*, 2016; Bogolyubova *et al.*, 2018).

The highly concentrated type of feeding during the newly calved period causes disturbances of rumen microbial and enzymatic processes, changing the balance in the symbiotic microflora towards an increase in pathogenic microorganisms and inhibition of cellulolytic ones. The resulting acidosis of the rumen leads to reduced consumption of feed, a decrease in feed digestibility, a decrease in the use of minerals, function defects of the mammary gland and reproductive organs (metritis, delayed afterbirth, abortions, infertility), limb diseases, and fatty liver (Liu *et al.*, 2019; Orton *et al.*, 2020).

An important role in the transformation and assimilation of nutrients in ruminants is played by the proventriculus, where under the influence of microflora (symbiotic digestion) and enzymes of the feed itself (autolytic digestion), complex digestive and metabolic processes occur. Rumen digestion affects the digestibility and utilization of feed nutrients in the rest of the gastrointestinal tract since

ruminants receive approximately 70% of nutrients and energy through the activity of microorganisms. Scholars are studying the possibilities of modulating processes in the rumen (Váradyová *et al.*, 2018). Rumen pH affects all aspects of rumen function in ruminants and the body as a whole. The reaction of the contents of the rumen, depending on the diet, can be slightly acidic or slightly alkaline, which is the most important condition for the development of microorganisms and the course of biochemical processes in the pancreas. Rumen acidification (decrease in pH level) negatively affects the vital activity of some microorganisms. The main consequence of lowering the pH below 6.0 is the extinction of cellulolytic activity, which is associated with a decrease in the ability to maintain pH inside the bacterial cell at such a low pH value. The pH values of the cicatricial contents below 5.0 units are detrimental to ciliated protozoa (Chiba, 2014).

Ammonia nitrogen is the main source of nitrogen for microbial growth and microbial protein synthesis in the rumen. An increase in its content in the rumen of animals can cause a more intensive growth of the microbial mass, which is a valuable source of essential amino acids for the host organism (Mackie *et al.*, 2022).

Most of the carbohydrates that enter the ruminant digestive tract undergo bacterial fermentation already in the rumen. As a result of this process, volatile fatty acids (VFA) are formed – a highly valuable energy-plastic material from which lipoproteins and carbon

skeletons of almost all amino acids are synthesized.

In this regard, the use of feed additives in animal nutrition in order to create optimal conditions in the rumen for the growth and development of microorganisms and enhance enzymatic processes is an urgent task of science and practice.

The use of biologically active substances to regulate the processes of digestion and metabolism is one of the ways to increase the adaptive capabilities of the animal organism and realize the genetically determined productivity potential.

FarmatanTM (manufacturer: Tannin Sevnica, Slovenia) is a feed additive in the form of powder for ruminants, which is a balanced combination of ellagitannins (sweet chestnut wood extract) with essential clove and cinnamon oils, sodium acetate, and organic zinc. It has antioxidant potential, helps against stress, and protects against colibacteriosis, salmonellosis, and clostridiosis. Tannins are the main active ingredient of Farmatan TM. Tannins are plant polyphenols that are found in almost all plants and protect them from viruses, fungi, and bacteria (Al-Khafaji *et al.*, 2021). They increase the amount of undegradable protein in the rumen (bypass protein) by inhibiting the action of the urease enzyme (Min & Solaiman, 2018).

Essential oils reduce the synthesis of prostaglandins, which is the main factor in the reduction of somatic cells in milk (Mousavi *et al.*, 2018).

Clove extract (eugenol), getting into the rumen, interferes with the synthesis of volatile fatty acids (VFAs) during the fermentation of feed carbohydrates. Due to this component, the

ratio of synthesized products changes towards the accumulation of propionate and partially butyrate because of a decrease in the formation of acetic acid (acetate), which is more beneficial for the cow in energy terms (Hu *et al.*, 2018).

Cinnamon extract (Cinnamaldehyde), like tannins, when ingested into the rumen, prevents the lysis of protein peptides by microbial enzymes and does not allow feed proteins to be completely broken down to amino acids and ammonia in the rumen (Appolloni *et al.*, 2021).

Sodium acetate promotes the synthesis of fat in the pancreas, the formation, and secretion of bile acids, enhances the absorption of fats from the intestine, activates the synthesis of amino acids and plasma proteins, while the presence of sodium ions stimulates the function of the liver, kidneys, and intestinal mucosa improves electrolyte balance and regulates the acid-base balance of the body (Yu *et al.*, 2022).

Organic zinc provides excellent protection to breast tissues, reducing the risks of infection in it and preventing parakeratosis. Besides, it improves the condition of hair, hooves, joints, and udders, and improves reproductive performance (Váradyová *et al.*, 2018).

PTES produced by Khiminvest LLC STC (Nizhny Novgorod) is a mixture of natural ingredients containing propylene glycol, sugar, pine tree extract, activated charcoal, flaxseed, and table salt.

The components of the studied additive are selected according to the duration of fermentation, due to which a prolonged energy effect is achieved. When one finishes breaking down, the other is at the peak of breaking down.

First, sugars are broken down (fermented), which is a trigger for the breakdown of other components. The breakdown of sugars provides propionic acid, an important source of energy for the rumen epithelium, which improves the development of the villi and the absorption of nutrients.

After the sugars, propylene glycol is fermented. It is almost completely digested in the rumen. Fermentation takes place in just a few hours into propionic acid. Propionic acid through the walls of the rumen enters the liver, where it is processed into glucose.

The coniferous extract is a natural vitamin carrier. In addition, the needles contain carotene, chlorophyll, xanthophyll, and other substances that play a role in the metabolism and synthesis of several new vitamins in the body.

Sugar for ruminants is important for the normal functioning of the microflora of the rumen. Activated carbon has a high adsorption ability and is capable of retaining various substances, gases, and liquids on its surface (Yıldızlı *et al.*, 2021).

Flax seeds swell in the gastrointestinal tract, forming mucous solutions that have dietary properties (Oomah, 2020).

Table salt serves to maintain water balance and acid-base balance in the blood.

Our objective was to study the features of pre-pancreatic digestion based on the analysis of indicators of enzymatic processes in the rumen of experimental cows based on the analysis of ruminal fermentation, the microbiological aspects of the contents of the rumen and the large intestine under the action of a complex of biologically active substances.

Materials & Methods

The general scheme of the study

The studies were conducted under production conditions on three groups of new black-and-white heifers with a live weight of 500 ± 20 kg (10 heads each) with a preliminary (equalization) period (10 days) selected for productivity and lactation in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe, 1986). The cows of the control group received a silage-haylage concentrate basic diet (BD). Cows of the experimental groups, in addition to the BD, received the Farmatan TM supplement at a dose of 40 g. head^{-1} per day (experimental group 1) and a pine tree energy supplement (PTES) at a dose of 150 g. head^{-1} per day (experimental group 2). The supplement was mixed with concentrate feed and given once in the morning feeding.

The duration of the scientific and economic experiment was 60 days. The animals of the experimental and control groups were kept in the same room, in the same zoohygienic conditions, tethered.

Methods

In the course of the study, we studied the following indicators:

- 1- Parameters of ruminal fermentation in experimental animals (pH, VFA, ammonia concentration);
- 2- The microbiological landscape of the contents of the rumen and the large intestine.

To characterize ruminal digestion in animals ($n=5$), at the end of the experiment, samples of the rumen contents were taken using an

esophageal probe 3 hours after feeding to determine the parameters of ruminal fermentation and microbiological landscape.

Faecal samples for the analysis of microbiocenosis of the large intestine were obtained during the act of forced defecation in 3 animals from each group after the end of feeding them supplements. The analysis was carried out in the Samara Regional Veterinary Laboratory. The sample delivery time (at $t \sim 2-4^{\circ}\text{C}$) to the laboratory did not exceed 1.5 hours from the moment of collection.

Microbiological analysis of the contents of the cow rumen was carried out by inoculation successive tenfold dilutions into accumulative and differential diagnostic media by deep (1.0 ml) and surface (0.2 ml) methods, followed by counting the number of colony-forming units (CFU/g or ml) (Plyashchenko & Sidorov, 1979). Species identification of microorganisms was carried out by assessment of morphology and microscopy results of colonies grown on microbiological differential diagnostic media; (Federal Budget Institution of Science State Scientific Center (FBIS SSC) of Applied Microbiology and Biotechnology, Moscow region, and HiMedia, India) and panels of Analytical profile index (API) test systems (BioMerieux, France).

Determination of the number of microorganisms during inoculation by the cup method and was calculated by the formula:

$$N = m / (V * d)$$

where m is the arithmetic mean of colonies cultured on two Petri dishes; V is the volume of inoculant introduced into each cup, and cm^3 ; d is the dilution coefficient.

In the ruminal fluid: pH was determined by Aquilon pH meter, the VFA content was

determined by steam distillation on the Markham apparatus, and the ammonia content by micro diffusion method in Conway cups (Kondrakhin, 2004).

The data obtained in the experiment were processed using methods of the analysis of variance (ANOVA). At the same time, the following values were calculated: the arithmetic means (M), the mean square error (m), and the indicator of significant difference (P -value). The results of the studies were considered highly reliable at $P < 0.001$ and reliable at $P < 0.01$ and $P < 0.05$. At $P < 0.1$, but $P > 0.05$, there was a tendency to the reliability of the data obtained. At $P > 0.1$, the difference was considered unreliable.

Results & Discussion

With a general tendency to decrease the pH of the rumen content after feeding, characteristic of animals in the experimental groups, a non-significant decrease in the level of ammonium nitrogen formation was observed of cows receiving Farmatan and PTES supplements (by 6.44-16.7%), with a non-significant higher concentration of VFA by 7.6 and 20.3%, in experimental groups 1 and 2, respectively (Table 1).

This finding suggests that carbohydrate hydrolysis in experimental animals is more intensive. When evaluating at the molar ratios of different volatile fatty acids, there is a modest increase in propionic acid and a slight decrease in acetic and butyric acids in experimental cows. Our findings are in line with those of other researchers, who found that feed additives containing glycerol, propylene glycol, and other similar ingredients improve the metabolic state of animals (Klebaniuk *et al.*, 2016; Harahap *et al.*, 2022).

Table (1): The evolution of ruminal metabolism indices (Mean± SD, n=3).

Indicator	Group			Reference values (Kondrakhin, 2004)
	Control (BD)	experimental 1 (BD + Farmatan TM)	experimental 2 (BD +PTES)	
pH	6.97±0.44 ^a	6.83±1.04 ^a	6.66±0.31 ^a	6.2-7.3
VFA (mM.100 ml ⁻¹)	7.88±0.18 ^a	8.48±0.47 ^a	9.48±0.90 ^b	5.0-15.0
Acetate, %	65.2±1.3 ^a	60.3±0.8 ^b	61.5±0.8 ^b	55-75
Propionate, %	22.0±0.9 ^a	24.4±0.7 ^a	25.2±0.7 ^b	15-25
Butyrate, %	14.0±0.7 ^a	12.8±0.6 ^a	11.9±0.6 ^a	10-17
Ammonia nitrogen (mg.100 ml ⁻¹)	11.65±1.02 ^a	10.90±1.75 ^a	9.70±1.04 ^a	6.5-35.0

The differences compared to the control are statistically significant at b- P<0.05.

The use of Farmatan and PTES supplements had no negative effect on the composition of the biocenosis of experimental animals' rumens, according to comparative characteristics of the main groups of microorganisms found in the rumen contents (Table 2). On the contrary, the content of lactobacilli and the quantity of mesophyll aerobic and optional-anaerobic microorganisms (QMA&OAMO) were higher (P<0.05) when the studied feed additives were introduced into the diets.

The positive effects of supplements include a decrease of *Clostridium* content in the rumen of cows by 26.3 and 30.3% in the 1st and 2nd experimental groups, respectively, compared with the control group (table, 2). In the experimental groups, a decrease in the content of mold and yeast-like fungi in the rumen was also noted.

The small intestine contains a relatively small number of microorganisms. In this part of the intestine, enterococci, *E. coli*, acidophilic and spore bacteria, actinomycetes, yeast, etc. are most often resistant to the action of bile.

The large intestine is the richest in microorganisms. Its main inhabitants are enterobacteria, enterococci, thermophiles, acidophiles, spore bacteria, actinomycetes, yeast, molds, a large number of putrefactive, and some pathogenic anaerobes (*Cl. sporogenes*, *Cl. nutrificus*, *Cl. perfringens*, *Cl. tetani*, *F. necrophorum*). 1 g of herbivore excrement can contain up to 3.5 billion different microorganisms. The microbial mass is about 40% of the dry matter of feces (Xiao *et al.*, 2017).

In the large intestine, complex microbiological processes associated with the breakdown of fibre, pectin substances, and starch occur. The microflora of the gastrointestinal tract is usually divided into obligate (lactic acid bacteria, *E. coli*, enterococci, *Cl. perfringens*, *Cl. sporogenes*, etc.), which has adapted to the conditions of this environment and has become its permanent inhabitant, and facultative, varying depending on the type of feed and water (Zhang *et al.*, 2018).

Table (2): Microbiological indicators of the cow rumen content, (\log^{-10} , CFU.g⁻¹, n=3, Mean± SD).

Indicator	Group		
	Control (BD)	experimental 1 BD + (Farmatan TM 40 g. head ⁻¹ per day)	experimental 2 (BD + PTES 150 g. head ⁻¹ per day)
Lactobacillus	6.89±0.21 ^a	7.31±0.20 ^a	7.13±0.53 ^a
Clostridia	5.02±0.34 ^a	3.70±0.19 ^c	3.50±0.30 ^c
QMA&OAMO	6.97±0.24 ^a	7.55±0.36 ^a	7.87±0.46 ^a
Lactose-positive groups of E. coli	5.04±0.05 ^a	4.73±0.52 ^a	4.87±0.42 ^a
Lactose-negative groups of E. coli	5.52±0.49 ^a	5.77±0.77 ^a	6.10±0.14 ^a
Mold	5.00±0.04 ^a	4.34±0.37 ^a	4.48±0.28 ^a
Yeast-like fungi	2.99±0.99 ^a	2.78±0.06 ^a	2.55±0.44 ^a

The differences compared to the control are statistically significant at c - $P < 0.01$.

We have studied the effectiveness of the use of nutritional supplements (Farmatan TM and PTES) in the diets of dairy cows.

Faecal samples for the analysis of microbiocenosis of the large intestine were obtained during the act of forced defecation in three animals from each group after the end of feeding supplements.

The results of quantitative content of microbiocenosis of the large intestine in cows of the control and experimental groups are presented in table (3).

Analysis of the quantitative content of various representatives of the microbiocenosis of the large intestine in experimental cows showed that the content of bifidobacteria, lactobacilli, typical *Escherichia*, and *Candida* fungi in animals of the experimental groups did not change significantly compared to the control group (Table 3).

It should be highlighted that the experimental groups' cows had 22.1 percent (experimental 1) and 20.8 percent (experimental 2) fewer Enterococci than the control group (experimental 2). Compared to the control group, the experimental cows had a considerable drop in the quantity of *Clostridium* in the contents of the large intestine, and opportunistic enterobacteria *Citrobacter freundii* and *Citrobacter diversus* were not discovered at all. Lactose-negative, hemolytic, pathogenic enterobacteria, bacteria of the genus *Proteus*, *Staphylococcus aureus*, and non-fermenting bacteria were absent in the contents of the large intestine of cows of the control and experimental groups.

Table (3): The quantitative content of various representatives of the microbiocenosis of the large intestine in cows, (CFU log.r⁻¹, n=3).

Indicator	Group		
	Control (BD)	experimental 1	experimental 2
Bifidobacteria	8.00 ± 0.12 ^a	9.30 ± 0.25 ^a	8.45 ± 0.27 ^a
<i>Lactobacillus</i>	7.54 ± 0.02 ^a	8.40 ± 0.98 ^a	7.98 ± 0.32 ^a
Escherichia: typical	4.39 ± 0.02 ^a	4.20 ± 0.35 ^a	4.40 ± 0.40 ^a
Other opportunistic enterobacteria:			
<i>Acinetobacter</i> spp.	2.53 ± 2.33 ^a	2.33 ± 0.37 ^a	2.33 ± 0.43 ^a
<i>Citrobacter freundii</i>	2.93 ± 2.33 ^a	0 ^c	0 ^c
<i>Citrobacter diversus</i>	2.23 ± 2.33 ^a	0 ^c	0 ^c
Enterococci	7.57 ± 0.33 ^a	5.90 ± 0.03 ^b	6.00 ± 0.20 ^b
Clostridia	4.00 ± 0.36 ^a	2.60 ± 0.22 ^c	2.47 ± 0.30 ^c
Candida fungi	3.53 ± 1.76 ^a	4.00 ± 1.38 ^a	4.05 ± 1.60 ^a

The differences compared to the control are statistically significant at b- P<0.05, c- P<0.01.

Thus, the inclusion of the nutritional supplements Farmatan TM and PTES in the diets of dairy cows improved the microbiocenosis of their large intestine, as evidenced by a reduction in *Clostridium* in the microbiocenosis of their large intestine and the absence of *Citrobacter freundii* and *Citrobacter diversus* in control animals.

Our data are consistent with the work of other researchers who have studied the phytotherapeutic properties of tannins on ruminants. Thus, Bonelli *et al.* (2018) administered tannins isolated from chestnuts to newborn calves to reduce the duration of diarrhea. Huws *et al.* (2018) proposed methods for managing the rumen microbiome, including with the help of tannins, which can improve the productivity of ruminants while reducing environmental impact. Moreover, the introduction of tannins into the diet can change

the quality of products from ruminants (Girard *et al.*, 2016).

Conclusion

Based on the conducted studies, it can be concluded that the inclusion of the feed additive Farmatan TM (40 g per day) and PTES (150 g per day) in the diet of cows at the beginning of lactation contributed to the strengthening of enzymatic processes in the rumen, which was expressed in an increase in the formation of VFA by 7.6 and 20.3%, an increase in the proportion of propionic acid and a certain decrease in the proportion of acetic and butyric acids.

When compared to the control group, the content of *Clostridium* in the rumen of experimental groups 1 and 2 decreased by 26.3 and 30.3%, respectively.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

All ethical guidelines related to animal care issued by national and international organizations were implemented in this report.

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تأثير المكملات الغذائية فارماتان TM ومكمل طاقة شجرة الصنوبر على تنظيم الهضم في الكرش والنمو الميكروبي للأبقار السوداء والبيضاء المرضعة

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المستخلص: هدفت الدراسة إلى تقييم تأثير المكملات الغذائية على الهضم في الكرش والميكروبات المعوية للأبقار المرضعة. أجريت الدراسة على ثلاث مجموعات من الأبقار البيضاء والسوداء (10 رؤوس لكل منها) بعد الولادة. غذيت أبقار مجموعة السيطرة على عليقة أساسية، والذي تضمن دريس الأعشاب المعمرة ، وسيلاج الذرة ، وتبن البقوليات ، والأعلاف المركزة ، والملاس. وغذيت أبقار المجموعات التجريبية ، بالإضافة إلى العليقة الأساسية (BD) ، مكمل Farmatan TM بجرعة 40 غم/الرأس⁻¹ يوميا (المجموعة التجريبية 1) ومكمل طاقة شجرة الصنوبر بجرعة 150 غم. تم خلط الإضافات مع الأعلاف المركزة وأعطيت مرة واحدة في الوجبة الصباحية. وفي سياق الدراسة ، تمت دراسة معايير التخمر في الكرش وطبيعية الأحياء المجهرية في الكرش والأمعاء الغليظة. في الوقت نفسه، وجد أن إضافة Farmatan TM (40 غم في اليوم) ومكمل طاقة شجرة الصنوبر (150 غم يوميا) في النظام الغذائي للأبقار في بداية الرضاعة ساهم في تعزيز العمليات الأنزيمية في الكرش، والتي تم التعبير عنها في زيادة في إنتاج الأحماض الدهنية الطيارة بنسبة 7.6 و 20.3 % ، زيادة في نسبة حمض البروبيونيك وانخفاض طفيف في نسبة أحماض الخليك والبيوتيريك عند استخدام إضافات الأعلاف (Farmatan TM وملحق طاقة شجرة الصنوبر) ، لوحظ انخفاض في محتوى الكلوستريديوم في الكرش من الأبقار بنسبة 26.3 و 30.3 % في المجموعتين التجريبية 1 و 2 ، على التوالي ، مقارنة بمجموعة السيطرة. في أبقار المجموعات التجريبية ، لوحظ أيضا انخفاض في محتوى الأعفان والفطريات الشبيهة بالخميرة في الكرش.

الكلمات المفتاحية: كلوستريديوم، مكمل غذائي، ميكروبيوسينوسيس، هضم الكرش.