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COMPARATIVE ASSESSMENT OF NATURAL FEED ADDITIVES FOR FUNCTIONAL EFFECTS ON THE DIGESTIVE PROCESSES IN THE RUMEN OF SHEEP (*Ovis aries*)

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Abstract

In ruminant husbandry, ergotropics, fungal cultures, modifiers, antioxidants, enzymes, and other feed additives with various biological properties help practitioners to create optimal conditions for ruminal microorganisms to grow and to digest various feed substrates of the diet. This paper first compares functional effects of bioactive natural substances (dihydroquercetin, organic iodine, micellar calcium, spirulina, and shungite) on the rumen digestion in Romanov sheep. The work aimed to investigate the peculiarities of the influence of natural feed additives different in their biological properties on fermentative and microbiological processes in the rumen of sheep. Dietary trials were performed in the conditions of the physiological yard, the Ernst Federal Science Center for Animal Husbandry, with Romanov sheep (*Ovis aries*). In 2014, 12 animals aged 4-5 months were assigned to four dietary treatments: a basal diet without supplementation (BD, the control animals), BD + shungite (0.3 % dry matter), BD + shungite (0.9 % dry matter), and BD + shungite (1.5 % dry matter). In 2018-2019, six fistulated sheep aged 2 years were fed according to two schemes. The first scheme of dietary treatment was as follows: BD for equalization, BD (control), dihydroquercetin (DHQ) (100 $mg \cdot head^{-1} \cdot day^{-1}$) + organic iodine (OI) (1.05 $mg \cdot head^{-1} \cdot day^{-1}$), BD for equalization, and DHQ (100 $mg \cdot head^{-1} \cdot day^{-1}$), each period lasted 14 days. In the second scheme, calcium carbonate micellate (M-Ca) injected into the rumen through a fistula (50 $\mu l \cdot head^{-1} \cdot day^{-1}$) was used instead of DHQ + OI, and spirulina (1.25 $g \cdot head^{-1} \cdot day^{-1}$, the dry powder mixed with 23.75 g filler) instead of DHQ. In all tests, at the end of each period, 3 hours after feeding, rumen contents were sampled to assess pH, redox potential (ORP), oxidization, total amount of volatile fatty acids (VFA), concentration of ammonia nitrogen, amylolytic activity, the biomass of ruminal protozoa and bacteria, and the microbial profiles. It was found that DHQ and spirulina have the most pronounced effect on ruminal pH, with a 12.7 and 9.5 % increase in the chyme pH, respectively. All dosages of shungite and M-Ca provide higher production of volatile fatty acids (VFA), the 20.6; 27.2 ($p < 0.05$), 22.8 and 6.6 % of the controls. DHQ + OI, DHQ and spirulina, in contrast, depress VFA production to 93.5, 81.2 and 81.8 % levels of the controls. The dietary additives, except for DHQ + OI, decrease the chyme level of ammonia, which was the greatest for DHQ and M-Ca, by 24.1 % at $p < 0.01$ and 26.3 %, respectively. Shungite also depresses the chyme ammonia concentration by 18.7 ($p < 0.05$), 14.9 ($p < 0.05$) and 9.4 % compared to the control. Dietary DHQ + OI, on the contrary, increases this indicator by 16.5 %. Dietary spirulina, M-Ca and DHQ + OI resulted in the 6.1, 1.7 and 1.1 % increase in the chyme amylolytic activity, respectively, the dietary shungite causes its significant decrease, by 16.8-19.9 %, while the influence of DHQ is the least with a 7.3 % decrease. It was shown that the rumen fermentation affects counts and species composition of the microbiota. The greatest increase in the chyme microbial biomass results from dietary shungite and depends on the mineral dosage. Dietary shungite increases the overall number of microorganisms 2 times ($p < 0.01$), by 47.4 % ($p < 0.05$) and 25.2 %, respectively, mainly due to infusoria, and changes the percentage ratio between infusoria and bacteria. DHQ + OI, DHQ and M-Ca also lead to an increase in the chyme microbial biomass but to a much lesser extent as compared to the shungite, i.e. to 14.6, 2.2 and 1.8 % of the control, respectively.

Dietary spirulina depresses the total number of microorganisms by 14.3 %. The study traces a positive relationship between the effects of the studied natural feed additives on the rumen microbiocenosis and the ammonia/VFA ratio of, which can characterize the rate of feed conversion to microbial protein.

Keywords: feed additives, shungite, dihydrouridine, organic iodine, spirulina, Romanov sheep, rumen, fermentation, chyme, amylase, volatile fatty acids, ammonia, microbiota

The rumen plays a primary role in the digestive system of ruminants. In this part of a compound stomach, due to microbiological fermentation, up to 80% of the needs for energy, 30-50% in protein, to a large extent in macro- and microelements and vitamins are satisfied, and up to 70% of crude fiber is digested. There is a relationship between the chemical composition and nutritional value of the forage substrate, the number of rumen microorganisms, and productivity performance [1, 2]. Substrates rich in nitrogen, protein, fat, nitrogen-free extractive substances significantly stimulate growth and reproduction of microorganisms. The acetic fermentation and pH values of 6.6-6.9 are optimal for the reproduction of rumen microorganisms. conditions include propionic fermentation and pH of 6.2-6.5 are less favorable. In the latter case, the greatest additional load to neutralize pH of remen content lies on the salivary glands [3, 4]. In practice, ergotropics, mushroom cultures, modifiers, antioxidants, enzymes, and other feed additives with various biological properties are used to provide optimal environment for vital activity of rumen microorganisms and digestion [5-7]. Natural biologically active substances are preferred as an alternative to antibiotics [8-10].

Dihydroquercetin (DHQ) from Dahurian larch (*Larix dahurica* Turez), can regulate metabolic processes, positively affects the functions of internal organs, is involved cell protection from pathologies. DHQ is widely used in medicine, food industry, etc. [11]. At the request of the European Commission (EC), a group of dietary and nutritional scientists have demonstrated the safety of using Dahurian larch extract rich in dihydroquercetin (taxifolin) as a food ingredient in accordance with Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Dihydroquercetin was used in the form of a feed additive Ecostimul 2 (JSC Ametis, Russia), containing up to 88% dihydroquercetin and about 10% of associated bioflavonoids [12].

An organic iodine (OI)-based feed additive (FA) Prost (LLC InBioTech, Russia) is a mixture of complete proteins of milk serum, which contain 2.5% of covalently bound I. Due to the covalent bond with proteins, bioiodine is highly stable, e.g. it can withstand heating up to 300 °C, is resistant to light and upon long-term storage. Iodine is a component of thyroid hormones in the form of iodinated derivatives of L-tyrosine residues. The physiological effect of thyroid hormones is associated with regulation of the rate of cell respiration, a direct effect on the absorption of oxygen by mitochondria and other cellular compartments, an increase in oxidative reactions and basic metabolism. Thyroid hormones have a significant effect on the activity of enzymes and the gene apparatus of cells, on morphogenesis and reproductive function [13, 14]. Feeding organic iodine to small ruminants contributed to a significant increase in live weight and average daily gain, which may due to high concentration of thyroxine [13]. Iodine organification occurs in the thyroid gland, in the mammary and salivary glands, in other tissues and organs. About 90% of the iodine contained in animal diets is accumulated in the thyroid gland as iodides. The latter are oxidized to iodine with the participation of hydrogen peroxide, which is catalyzed by thyroid peroxidase. Preliminary studies have shown that the combined feeding of antioxidants with organic iodine promoted, on the one hand, an increase in the activity of glutathione peroxidase, correcting the imbalance of the oxidative and antioxidant systems, and on the other hand, the regulation of thyroid function [14, 15].

Calcium carbonate micellate (M-Ca) is a complex suspension containing calcium carbonate as the main component and serving as one of the regulators of its metabolism in the body. When M-Ca colloidal solution enters the gastrointestinal tract, it is neutralized with hydrochloric acid with the formation of calcium ions (remember, only its ionized form contributes to the replenishment of calcium deficiency), as well as reactive oxygen species (ROS). ROS are produced in small amounts in the aquatic environment, their concentration does not go beyond the physiological norm. ROS promote production of free electrons. The presence of M-Ca in water initiates electronic activation, which corrects disorders caused by various pathological factors. This normalizes oxidative phosphorylation and improves cellular antioxidant status [16, 17]. As a result, previously weakened functions of organs and tissues are restored, including those responsible for bone remodeling and regeneration of damaged bones, destructive processes in bone tissue slow down, the level of osteoclastogenesis regulators changes towards their balance, epithelial tissue regeneration is stimulated, and the peripheral microcirculation is improved [18-20].

Microalgae Spirulina (*Spirulina platensis*) contains absolutely all substances that are necessary for humans and animals for normal life [21, 22] and also has a wide spectrum of biological activity, e.g. stimulates metabolism, strengthens the skeleton, normalizes the condition of the skin, hair and mucous membranes, neutralizes toxins, improves digestion, ultimately contributing to an increase in the productivity and resistance of animals to various diseases [23, 24]. This product has been shown to serve as a valuable feed for many animal species. Feeding spirulina improves the quantitative and qualitative composition of lactic bacteria in the intestine, maintains higher levels of blood hemoglobin and erythrocytes, increases growth rate and animal fertility, and betters the quality of food products. Researcher associate the positive effect of spirulina with a rich set of biologically active substances and a unique protein composition [25, 26].

Shungite is a natural mineral, unusual in origin, the structure of its carbon and the structure of the rocks themselves [27, 28]. The shungite carbon which constitutes 30% of the mineral is a multilayer globule about 10 nm in size. This structure is very active in oxidation-reduction reactions, and possesses sorption and catalytic properties. Oxides of macro- and microelements (more than 20 in total) make up 70% of the mineral and are mainly silicon compounds. The mineral part of shungite has adsorption, binding, buffering and ion-exchange properties. Silicon oxides contribute to an increase in the absorption of macro- and microelements, affect natural resistance of animals, the metabolism of vitamins and biologically active substances. Fullerenes in the composition of shungite increase the resistance of cell membranes to damaging factors, have antioxidant and radioprotective properties due to the suppression of excess formation of free radicals, and have a positive effect on the energy systems of the body [29, 30]. The antioxidant properties of fullerenes are due to inactivation of reactive oxygen species, in particular, hydroxyl radicals (via their binding to double bonds which are abundant in fullerenes), and also to accumulation in mitochondria and a decrease in the transmembrane potential [31]. The study of the biological effects of shungite and its derivatives in biomedicine and veterinary medicine remains relevant [32, 33]. Studies on farm animals and poultry have established a high efficiency of shungite, which does not have cumulative, allergenic, embryotoxic, teratogenic effects, does not irritate mucous membranes and has no negative effects on the functional activity of the liver [32, 34].

Thus, numerous positive effects of the described feed factors are known and have found practical application. However, the current understanding of how they act on the enzymatic and microbiological systems of the rumen is limited.

This study is the first to disclose effect of these natural feed components on the sheep rumen enzymes and microbiota that improve feed conversion and ensure metabolic and clinical health of animals.

The work aimed to assess the impact of bioactive feed additives with different properties on sheep digestion and rumen microbiota.

Materials and methods. Dietary trials were performed in the conditions of the physiological yard, the Ernst Federal Science Center for Animal Husbandry, with Romanov sheep (*Ovis aries*). In 2014, 12 animals aged 4-5 months were assigned to four dietary treatments: a basal diet without supplementation (BD, the control animals), BD + shungite (0.3 % dry matter), BD + shungite (0.9 % dry matter), and BD + shungite (1.5 % dry matter). BD consisted of 2 kg of feed mixture and 0.35 kg of concentrates, with a total nutritional value of 9 MJ OE (0.9 ECE), natural shungite (Zazhoginskoe deposit, Republic of Kareliya, LLC NPK Carbon-shungite, Russia) dosage was 0.3, 0.9 and 1.5% BD dry matter.

In 2018-2019, six fistulated sheep aged 2 years were fed according to two schemes. The first scheme of dietary treatment was as follows: BD for equalization, BD (control), BD + dihydroquercetin (DHQ) (100 mg $\cdot$ head^{-1} $\cdot$ day^{-1}) + organic iodine (OI) (1.05 mg $\cdot$ head^{-1} $\cdot$ day^{-1}), BD for equalization, and BD + DHQ (100 mg $\cdot$ head^{-1} $\cdot$ day^{-1}), each period lasted 14 days. In the second scheme, calcium carbonate micellate (M-Ca) injected into the rumen through a fistula (50 μl $\cdot$ head^{-1} $\cdot$ day^{-1}) was used instead of DHQ + OI, and spirulina (1.25 g $\cdot$ head^{-1} $\cdot$ day^{-1}) instead of DHQ. BD consisted of 1.5 kg of hay and 0.4 kg of concentrates (total nutritional value 13.2 MJ OE = 1.32 ECU) containing 180 g of protein, 40 g of fat, 380 g of fiber. Bioactive substrates (10 g $\cdot$ head^{-1} $\cdot$ day^{-1}) were applied as mixtures. The mixture for BD + DHQ + OI contained filler (dry crushed seedlings) and feed additives Ecostimul-2 (DHQ 80%, JSC Ametis, Russia) + Prost (iodine 7 mg/g, LLC InBioTech, Russia), for BD + DHQ, the mixture contained filler (dry crushed seedlings) and feed additive Ecostimul-2. M-Ca was applied as a stock solution (ZAO Petrokhim, Russia), spirulina (OOO Biosolar MSU, Russia) was added as dry powder mixed with 23.75 g filler.

In all tests, at the end of each period, 3 hours after feeding, rumen contents were sampled to assess pH (a device Akvilon 420, AO Akvilon, Russia), redox potential (ORP) (an ORP meter ORP-2069, YIERYI, Chine), oxidization by reaction with thiobarbituric acid, total amount of volatile fatty acids (VFA) by steam distillation in a Markham apparatus, concentration of ammonia nitrogen by Conway microdiffusion technique, amylolytic activity photometrically (a spectrophotometer KFK-3-01, ZOMZ, Russia), the biomass of ruminal protozoa and bacteria by differential centrifugation (J2-21, Beckman Coulter GmbH, Germany), and the microbial profiles in surface and submerged cultures using 10-fold dilution series (growth and differential media, FBUN SSC PMB, Obolensk, Russia; HiMedia Laboratories Pvt. Ltd, India) to estimate microbial counts (CFU/g).

Biometric processing was performed by analysis of variance (ANOVA) method (STATISTICA 10, StatSoft, Inc., USA). The arithmetic mean values (*M*), standard error of the mean (±SEM) were calculated, and Student's *t*-test was used to assess significance levels (p).

Results. The intensity and peculiarities of rumen digestion in ruminants, and, consequently, the efficiency of feed use, are closely related to the composition of the aquatic fraction of the chyme, which is determined by pH and ORP and depends on the structure and nutritional value of the diet, the quality of drinking water, and the intensity of secretion in the salivary glands, as well as by the state of microbiocenosis. With an increase in the ORP of water, its bioenergetic, metabolic and immunostimulating properties improve, which favors the development of microorganisms [16, 17].

Dietary DHQ, fed separately and together with organic iodine, had a significant effect on the physicochemical properties of rumen chyme, enzymatic and microbiological processes in sheep. In the control period, the pH of the chyme was 5.69±0.135 at an oxidation rate of 0.146±0.049 U eq. DHQ + OI fed during the first test period increased pH value of the chyme to 5.85±0.078 together with a significant 2.8-fold increase in its oxidation ($p < 0.01$). Under the effect of DHQ, the pH of the chyme reached 6.41±0.053 with a 1.80- and 5.08-fold decrease in oxidation compared to the control and the first test period ($p < 0.05$) (Table 1).

ORP values of the chyme showed that dietary OI intensifies oxidation while DHQ, on the contrary, intensified the reduction processes (see Table 1). This is consistent with the data on the oxidation and pH of the chyme and illustrates characteristic oxidizing properties of iodine and the reducing properties of dihydroquercetin as an antioxidant.

1. Biochemical and microbiological indicators of rumen chyme in Romanov sheep (*Ovis aries*) fed dietary dihydroquercetin (DHQ) **and organic iodine** (OI) (*n* = 6, *M*±SEM, experimental animal yard of Ernst Federal Science Center for Animal Husbandry, 2018-2019)

Indicator	Period		
	control (BD)	1 (BD + DHQ + OI)	2 (BD + DHQ)
pH	5.69±0.135	5.85±0.078	6.41±0.053**/ϵϵ
Oxidation, U eq.	0.15±0.049	0.41±0.032**	0.08±0.003ϵϵ
ORP, mV	–272±15.9	+107±10.27***	–404±7.78***/ϵϵϵ
VFA, mmol/100 ml	11.84±0.325	11.07±0.226	9.61±0.613*
Ammonia, mg%	17.42±2.777	20.31±1.171	13.23±1.892ϵϵ
Ammonia/VFA	1.47	1.83	1.37
Amylolytic activity, U/ml	17.52±0.249	17.72±0.324	16.25±0.621
Microorganisms, g/100 ml:			
total	0.89±0.059	1.02±0.064	0.91±0.040
including			
infusoria, g/100 ml	0.36±0.040	0.45±0.031	0.26±0.024*/ϵϵ
infusoria, %	40.3	43.6	28.6
bacteria, g/100 ml	0.53±0.024	0.57±0.053	0.65±0.033*
bacteria, %	59.7	56.4	71.4

N o t e. BD — basal diet. ORP — redox potential, VFA — volatile fatty acids. For design of trials and description of treatments, see *Materials and methods*.
*, **, *** Differences between the treatment and the control are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0,001$, respectively.
ϵ, ϵϵ, ϵϵϵ Differences between the treatment 2 and the treatment 1 are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

The amylolytic activity of chyme is determined by microorganisms, for which an acidic environment (pH of 5.4-6.2) is most favorable. In our studies, the pH of the chyme in the control and upon adding DHQ + OI were close, which determined a similar amylolytic activity (see Table 1). An increase in the pH of the chyme when using only DHQ led to a decrease in amylolytic activity to 16.25±0.621 U/ml. The more acidic environment of the chyme is also favorable for the vital activity of microorganisms fermenting sugars to lactic, acetic, propionic and butyric acids, which are almost completely absorbed in the proventriculus. In this regard, the amount of volatile fatty acids (VFA) in the chyme of sheep in the control and under DHQ + OI was similar, 11.84±0.325 and 11.07±0.226 mmol/100 ml, respectively. The dietary antioxidant DHQ used separately increased pH of the chyme, which could affect microbial profile and fermentation, resulting in an 18.83% decrease of the chyme VFA level ($p < 0.05$) (see Table 1).

Significant differences between treatment occurred in the chyme levels of ammonia, infusoria and bacteria, which was associated with the specific properties of the used bioactive substances, DHQ and OI. The chyme ammonia concentration increased by 16.6% during period 1 and decreased by 24.1% ($p < 0.01$) during period 2 compared to the control, which may be due to the intensity of its use in microbiological protein synthesis. This is also confirmed by changes in microbial profile of the chyme. The total number of microorganisms in the control period was 0.89±0.059 g/100 ml, with a 14.6% increase during period 1 and a 2.2% increase during period 2. The species composition also changed. E.g., the ratio of infusoria to bacteria counts was 40.3:59.7 % in the control period, 43.6:56.4% during period 1, and 28.6:71.4% during period 2. That is, the number of infusoria increased by 25.0% and bacteria by 7.5% during period 1, in contrast to period 2, when the abundance of infusoria decreased by 27.8% ($p < 0.05$), and bacteria increased by 22.6% ($p < 0.05$) (see Table 1) as compared to the control.

DHQ with OI also significantly influenced the composition of the rumen microbiota. Under their combined action, the number of lactic bacteria in the rumen content increased 10 times, under the action of DHQ 11.5 times. In period 1 the abundance lactic bacteria increased 10 times compared to the control (Table 2). Mesophilic aerobic and facultatively anaerobic microorganisms are a significant part of gut microbiota. Dihydroquercetin, together with iodine and separately, had a specific effect on the quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), *Escherichia coli*, *Candida* fungi, molds and yeasts in the sheep proventriculus. QMAFAnM increased 4-fold during period 1 and 21-fold during period 2 as compared to the control. Candida fungi, molds and yeasts decreased 3.6 times and increased 1.8 times, for periods 1 and 2, respectively. As compared to the control. When comparing period 2 with period 1, these microorganisms were 6.4 times more abundant (see Table 2.). Note that *Bacillus* spp. was found while *Clostridia* and lactose-negative *E. coli* were not detected in all periods (see Table 2).

2. Members of rumen microbiocenosis (КОЕ/мл) **in Romanov sheep (*Ovis aries*) fed dietary dihydroquercetin** (DHQ) **and organic iodine** (OI) (n = 6, M±SEM, experimental animal yard of Ernst Federal Science Center for Animal Husbandry, 2018-2019)

Indicator	Period		
	control (BD)	1 (BD + DHQ + OI)	2 (BD + DHQ)
Lactic bacteria	$5.6\times10^3\pm2.90\times10^3$	$5.6\times10^4\pm2.20\times10^4$	$6.4\times10^4\pm2.70\times10^3$
Spore forming microorganisms:			
Bacillus spp.	Revealed	Revealed	Revealed
Clostridia	Not found	Not found	Not found
QMAFAnM	$1.2\times10^3\pm1.10\times10^2$	$4.9\times10^3\pm1.12\times10^3$	$2.6\times10^4\pm8.75\times10^3$
Escherichia coli:			
lactose-positive	$6.8\times10^3\pm5.98\times10^3$	$0.8\times10^1\pm0.74\times10^1$	$5.3\times10^2\pm1.42\times10^2$
lactose-negative	Not found	Not found	Not found
Genus *Candida*, molds and yeasts	$2.7\times10^2\pm5.98\times10^1$	$7.5\times10^1\pm0.74\times10^1$	$4.8\times10^2\pm1.42\times10^2$

Note. BD — basal diet. QMAFAnM — mesophilic aerobic and facultative anaerobic microorganisms. For design of trials and description of treatments, see *Materials and methods*.

The differences between DHQ + OI and DHQ in the eliminating effect are most likely associated with the antiseptic properties of iodine and, then, with the DHQ selectiveness towards various microbial strains. Dihydroquercetin has strong bactericidal properties that inhibit putrefactive processes, which gives reason to consider it a natural analogue of antibiotics [9].

M-Ca and dietary spirulina also showed positive trends. The acid-base balance, enzymatic activity and chyme microbiota changed. Under the influence of M-Ca and spirulina, the chyme pH increased by 3.6 and 9.5%, respectively, with a 2.4 and 19.5% decrease in its oxidation combined with a decrease in the reduction potential (Table 3). M-Ca in the chyme favored VFA production and amylolytic activity, with a 6.6% and 1.7% increase, respectively, and decrease the ammonia concertation by 26.3% and the ammonia/VFA index to 1.46, which indicates a better use of ammonia by the microbiota.

3. Biochemical and microbiological indicators of rumen chyme in Romanov sheep (*Ovis aries*) upon application of calcium carbonate micellate (M-Ca) **and dietary spirulina** (*n* = 6, *M*±SEM, experimental animal yard of Ernst Federal Science Center for Animal Husbandry, 2018-2019)

Indicator	Period		
	control (BD)	1 (BD + M-Ca)	2 (BD + spirulina)
pH	6.31±0.107	6.54±0.137	6.91±0.111
Oxidation, U eq.	0.41±0.050	0.40±0.018	0.33±0.009
ORP, mV	–282±10.7	–237±13.1	–261±14.9
VFA, mmol/100 ml	8.59±0.489	9.16±0.355	7.03±0.197
Ammonia, mg%	18.19±0.552	13.40±1.047	17.57±1.608
Ammonia/VFA	2.11	1.46	2.49
Amylolytic activity, U/ml	16.70±0.448	16.99±0.160	17.72±0.483
Microorganisms, g/100 ml:			
total	1.12±0.083	1.14±0.147	0.96±0.113
including			
infusoria, g/100 ml	0.58±0.053	0.58±0.096	0.47±0.060
infusoria, %	51.3	50.9	48.2
bacteria, g/100 ml	0.55±0.064	0.56±0.096	0. 50±0.055
bacteria, %	48.7	49.1	51.8

N o t e. BD — basal diet. ORP — redox potential, VFA — volatile fatty acids. For design of trials and description of treatments, see *Materials and methods*.

Dietary spirulina decreased the VFA concentration by 18.2%, with a slight increase in amylolytic activity and a decrease in the ammonia level compared to the control period, which affected the state of the microbiota (see Table 3). The total number of microorganisms in the chyme decreased compared to the control period by 14.3%, including infusoria by 19.0%, bacteria by 9.1%. This effect of spirulina on rumen digestion is possibly associated with insufficient energy in the diet. Thus, in dairy Black-and-White cows, the dietary premix containing 2 g of spirulina when added at a dose of 20 g per head per day improved physiological and microbiological processes in the rumen [26]. In this, there was a 32.95% vs. 9.4% increase in VFA production, and 37.7% vs. 11.29% increase in infusoria counts at the beginning and by the end of the trial, respectively. The QMAFanM increased by 5.2%, abundance of lactobacillus by 6.9% compared to the control, which indicates the probiotic properties of spirulina.

4. Biochemical and microbiological indicators of rumen chyme in Romanov sheep (*Ovis aries*) fed dietary shungite (*n* = 3, experimental animal yard of Ernst Federal Science Center for Animal Husbandry, 2014)

Показатель	Группа			
	контроль	опыт (шунгит, % сухого вещества)		
		0,3	0,9	1,5
pH	5.7±0.39	5.7±0.06	5.9±0.09	5.9±0.33
VFA, mmol/100 ml	9.2±0.43	11.1±0.95	11.7±0.36*	11.3±0.97
Ammonia, mg%	24.7±0.46	20.1±0.96*	21.6±0.53*	22.6±1.38
Ammonia/VFA	2.67	1.81	1.85	1.99
Amylolytic activity, U/ml	22.6±1.38	18.6±0.41	18.8±0.40	18.1±1.40
Microorganisms, g/100 ml:				
total	1.35±0.100	2.70±0.280**	1.99±0.540*	1.69±0.170
including				
infusoria, g/100 ml	0.93±0.090	2.03±0.110**	1.57±0.500**	0.92±0.040
infusoria, %	68.8	75.2	78.9	54.4
bacteria, g/100 ml	0.42±0.060	0.67±0.190	0.42±0.080	0.77±0.170
bacteria, %	31.2	24.8	21.1	45.6

N o t e. BD — basal diet. VFA — volatile fatty acids. For design of trials and description of treatments, see *Materials and methods*.

*, ** Differences between the treatment and the control are statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

The effectiveness of bioactive feed additives depends not only on their functional properties, but also on the dosage. We examined the effect of dietary shungite (0.3, 0.9 and 1.5% of dry matter) on enzymatic and microbiological processes in sheep rumen. The dose of 0.3% did not affect pH of the chyme. When the dose increased 3 and 5 times, a slight increase in pH occurred, by 3.5% compared to the control period (Table 4). Dietary shungite increased the VFA level in chyme by 20.6, 27.2 ($p < 0.05$) and 22.8% and reduced the ammonia concentration by 18.6 ($p < 0.05$), 12.6 ($p < 0.05$) and 8.5% compared to the control group. As a result, the ammonia/VFA index decreased significantly for the doses of shungite to 1.81, 1.85 and 1.99, respectively, while in the control group it was 2.67. All dosages of shungite had equal effect on the amylolytic activity of chyme, which decreased by 16.8-19.9%. Changes in chyme fermentation, as influenced by shungite, were adequately reflected in the microbiota.

Rumen acidity is known to be one of the most variable factors that can affect microbiota and VFA production. Bacteria capable of digesting fiber are most active in the pH range of 6.2-6.8. For bacteria that hydrolyze starch, a more acidic environment with a pH of 5.4-6.2 is favorable. The number of protozoa can be significantly reduced at pH 5.5. Dietary Shungite had a positive effect on the abundance of rumen microbiota. The greatest 2-fold increase occurred at a dosage of 0.3% ($p < 0.01$). With increasing dosage, the effect decreased. Thus, 0.9 and 1.5% shungite increased the number of rumen microorganisms only by 47.4 ($p < 0.05$) and 25.2%, respectively. There was also a dose-dependent effect of shungite on the microbiota composition. The total microbial mass increased mainly due to infusoria. In sheep fed 0.3 and 0.9% shungite, the infusoria increased significantly, more than 2-fold ($p < 0.01$) and by 68.8% ($p < 0.01$), respectively. The infusoria to bacteria proportion was 75.2% vs. 24.8% for 0.3% shungite, 78.9% vs. 21.1% for 0.9% shungite, and 54.4% vs. 45.6% for 1.5% shungite.

The productivity performance in ruminants depends on the rumen digestion of a diet the structure and nutritional value of which varies significantly. This directly affects the fermentation processes and the formation of microbiocenosis. The key factor that favors rumen digestion is an optimal environment for the reproduction of microorganisms, which is characteristic of the acetic fermentation (pH 6.6-6.9). Among the studied feed additives of natural origin, DHQ and spirulina turned out to be the most effective. When they added, the pH of the chyme increased by 12.6 and 9.5%, respectively, while other feed additives were less effective.

Other studies have examined the digestive effects of low rumen pH, including the profiles of available VFAs [35]. The sensitivity of cows to subacute acidosis was studied at a highly concentrated diet, consisting of 35% coarse and 65% concentrated feed [34], as well as on fistulated animals using various feed additives [36]. The acidity in the rumen was studied using a transducer implanted into the reticulum. As a result, a wide range of pH was established, from 5.05 to 6.98, which indicates adaptation and tolerance of cows to acidosis.

M.A.M. Abdullah et al. [37] evaluated the effect of acacia pods when added to sheep feed at 1.5 and 3.0%. These changes in the diets did not affect the rumen pH, but the protozoa abundance in the rumen decreased. Production and absorption of VFAs has been reported to be directly related to the pH of the chyme. The low chyme pH slowed down lipolysis and hydrolysis of fatty acids, which was associated with a decrease in the amount of bacterial DNA [38, 39]. As to VFA production in out feed trials, shungite at all dosages and M-Ca were the most effective and increased the chyme VFA levels by 20.6, 27.2 ($p < 0.05$), 22.8 and 6.6%, respectively. The effect of DHQ + OI, DHQ and spirulina on the

VFA production was negative, with a decrease to 93.5, 81.2 and 81.8% of the control level. All the studied feed additives, except for DHQ + OI, led to a decrease in the chyme ammonia, with the largest decrease of 24.1% ($p < 0.05$) and 26.3% under the influence of DHQ and M-Ca. Shungite also reduced the chyme ammonia concentration to 81.3, 87.45 and 91.5% for 0.3, 0.9 and 1.5% shungite, respectively, compared to the control period.

Ghaffari et al. [40] reported similar results when replacing alfalfa hay in the diet of sheep with by-products. The concentration of ammonia decreased with an increase in the portion of pistachio husks which replaced hay. However, no differences in rumen pH were observed in the sheep [40]. Waste from the processing of the date palm crop (9, 18, and 27% DM) decreased the ammonium nitrogen concentration in the rumen and enhanced synthesis of microbial protein, but the diet composition did not affect the rumen pH [41]. In our trials, dietary DHQ + OI increased the ammonia level in the chyme by 16.5% compared to the control period, which may be associated with an increase in oxidative processes and a decrease in the VFA production under the influence of iodine.

The effects of the studied feed additives on the chyme amylolytic activity also differed. An increase by 6.1, 1.7 and 1.1% occurred upon the use of spirulina, M-Ca and DHQ + OI, respectively. Under the influence of shungite, the amylolytic activity significantly decreased, by 17.7, 16.8 and 19.9% of the control activity at a dosage of 0.3, 0.9 and 1.5%, respectively. Under the action of DHQ, the decrease was minimal, by 7.3%.

Enzymatic processes and their changes directly affected the microbiota. Shungite caused the greatest increase in the chyme microbial mass, but the effect was dose-depended. Dietary shungite increased the total number of microorganisms increased 2 times ($p <0.01$), by 47.4% ($p < 0.05$) and 25.2% compared to the control, mainly due to infusoria, as a result, the infusoria to bacteria ratio changed. With an increase in dietary shungite dosage, the total number of microorganisms decreased and the percentage ratio of infusoria and bacteria changed. DHQ + OI, DHQ and M-Ca influenced the increase in microbial mass in the chyme to a much lesser extent than shungite, the indicators increased by 14.6, 2.2 and 1.8%, respectively, compared to the control period. Under the influence of spirulina, the total number of microorganisms decreased by 14.3% due to both infusoria and bacteria.

In analyzing effects of the studied feed additive, their positive relationship with the ammonia/VFA index was traced, which characterizes the feed conversion rate into microbial protein. The amount of glucogenic fermentation products and the formation of microbial protein largely depend on the rate of fermentation. Slow fermentation of substrates reduces the formation of these products, increases the proportion of propionic acid, and decreases the levels of acetic and butyric acids [42, 43).

There are also publications about modulation of rumen microflora in sheep and young cattle by biologically active substances [44-47]. The use of dietary phytobiotics and mineral components promoted enzymatic and microbial processes in the rumen. Among the feed products, seaweeds which are used as prebiotics to increase the productivity and productive health of animals deserve special attention. Dietary algae can improve the intestinal ecology in animals [48].

So, a comparative study of feed additives of natural origin, the dihydroquercetin, organic iodine, calcium carbonate micellate, spirulina and shungite showed their specific effect on enzymatic and microbiological processes in the rumen of sheep. Dihydroquercetin increases pH and reduction potential in the rumen, stimulates bacterial growth. Organic iodine increases pH and oxidative potential of the chyme and promotes bacterial growth. M-Ca increases pH and decreased the reduction potential of the chyme. Spirulina raises the chyme pH values. The 0.3 and 0.9% shungite more effectively promotes production of volatile fatty acids and chyme biomass compared to 1.5% shungite. Our findings can be used in feeding sheep in order to modulate functionality of diets. As in choosing feed additives, one should consider their effect on metabolic processes and animal immunity, dihydroquercetin and shungite seem to be the most promising.

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