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Influence of Antioxidant and Adsorbent on the Processes of Digestive and Intermediate Metabolism in Lacting Cows During Denitrification

Marina G. Kokaeva,
North-Caucasian Mining and Metallurgical Institute (State Technological University), 362021, Russian Federation, Vladikavkaz, 44 Nikolaev Street.
Rustem B. Temiraev,
North-Caucasian Mining and Metallurgical Institute (State Technological University),362021, Russian Federation, Vladikavkaz, 44 Nikolaev Street.
Eduard V. Beslaneev,
Kabardino-Balkarian State Agrarian University by V.M. Kokov, 360030, Russian Federation, Nalchik, 1V Lenin Ave.
Susanna K. Cherchesova,
North-Ossetian State University by K.L. Khetagurov, 362025, Russian Federation, Vladikavkaz, 46 Vatutin Street.
Zalina A. Kubatieva (Gutieva),
Gorsky State Agrarian University, 362040, Russian Federation, Vladikavkaz, Kirov Street, 37.
Soslan G. Kozyrev,
Gorsky State Agrarian University, 362040, Russian Federation, Vladikavkaz, 37 Kirov Street.

Abstract.

To intensify the digestive and intermediate metabolism in lactating cows consuming diets that contain nitrates, particular importance is paid to feeding adsorbents and antioxidants. **The research aims** to study the effect of the adsorbent toxy-nil and antioxidant vitamin C on the digestive metabolism of lactating cows, the diets of which contain a subtoxic dose of nitrates. **Methods.** To achieve this aim, scientific and economic experiment was conducted with black-pied cows. By the analogue scale 30 selected calves were divided into three groups of 10 animals each. The obtained data is processed statistically using information program Microsoft Excel. **The research results.** It was found that the using vitamin C at a dose of 0,04% of the dry weight, together with preparation toxy-nil at a dose of 2,0 g/kg of concentrates in lactating cows' diets with the subtoxic dose of nitrates helped to improve the intermediate metabolism by optimizing the rumen digestion mechanism with involving nitrate nitrogen in the synthesis of high-quality microbial protein. Combined addition of vitamin C and preparation toxy-nil most favored the reproduction of infusoria and cellulolytic activity of cows' rumen fluid. We managed to reliably (P> 0,95) reduce the nitrates concentration by 47,4% and nitrites-53,9% in the preventriculi content of cows in the 2nd test group versus the control counterparts. In the cows' blood of the 2nd test group the concentration of erythrocytes and hemoglobin versus the control was significantly (P> 0,95) higher by 2,29 · 10^{12}/l and 20,9 g/l but the methemoglobin concentration — by 87,75% less. Combined additions of preparations promoted to increase the content of ammonia by 29,3% (P> 0,95) in the blood, while reducing nitrates and nitrites — 2,47 (P> 0,95) and 3,15 (P> 0,95) times than in the control.

Key words: cows, nitrates and nitrites, adsorbent, antioxidant, digestive metabolism, morphological and biochemical composition of blood.

Relevance of the topic. The increased level of nitrate nitrogen in various natural components decreases the biological value of food products on the one hand and via them negatively affects the humans and animals on the other. The nitrates toxic effect is related to its reduction to nitrites, which oxidize iron in the hemoglobin molecule converting it from bivalent to trivalent form. The produced methemoglobin is unable to perform reversible oxygen-binding that leads to hypoxia [1, 2, 3].

One of the ways to reduce the risk of nitrate-nitrite poisoning of dairy cattle and the migration of these toxicants to dairy products is the addition of adsorptive preparations into lactating cows' diets, which binding nitrate and nitrite ions in the gastrointestinal tract carry them out of the body [4, 5, 6].

Another trend is their feeding with biologically active additives, primarily antioxidants, in order to stimulate the development of those populations in the rumen microflora that are able to synthesize nitrate reductases, reducing nitrates to nitrites, and the latter to ammonia, and to synthesize from the latter high-quality microbial protein. Such preparations include vitamin C (ascorbic acid) [7, 8].

To intensify the digestive metabolism in lactating cows consuming diets that contain nitrates and nitrites particular importance is paid to increasing in its composition denitrifying components such as vitamin C.

The research aims to study the effect of adsorbent toxy-nil and antioxidant vitamin C on the digestive metabolism in lactating cows, whose diets contain the subtoxic nitrates dose.

Methods of research. To achieve the aim in the conditions of the collective farm "Ukraina" in Mozdok District of RNO-Alania we conducted the scientific and economic experiment with black-pied cows according to

the design given in Table 1. By the analogue scale 30 selected calves were divided into three groups of 10 animals each.

Feeding of test cows was carried out with diets balanced in accordance with detailed standards. The nitrates and nitrites concentration in feeds samples and rumen content was determined according to the generally accepted method.

In the course of the experiment, studies of rumen digestion were conducted according to the generally accepted method. By the standard method, in the middle of lactation, a physiological exchange experiment was carried out with two groups of cows, for which 3 animals from the control group and 3 – from the test one were selected.

The results of the studies were processed by the method of Student's variation statistics.

The research results. It was found that none of the feeds included into the test cows' diets had the excess of the maximum permissible concentration (MPC) in nitrates and nitrite content. On this basis to assess the denitrification properties of the antioxidant and adsorbent into the diets of the test animals (in view of the nitrate content in feeds) was included sodium nitrate at the rate to have their level subtoxic — no more than 0,03 g/kg of cows' live weight [9, 10].

The factor of the nutrition ecology affects not only the quantitative and qualitative component of the rumen microflora, but also the enzymatic activity of the rumen content (Table 2).

It has been found that the combined feeding of ascorbic acid and the preparation toxy-nil containing organic acids (acetic, citric and oxalic) and chelators provided versus the control counterparts the highest growth in the proventriculi of cows in the 2nd test group the number of Flavobacterium vitarumen by 21,9% (P> 0,95) and total proteinase activity - by 3,6% (P> 0,95).

Combined use of vitamin C and toxy-nil in diets with the subtoxic nitrates dose most favored the infusoria reproduction and cellulolytic activity of rumen fluid in cows of the 2nd test group, significantly (P> 0.95) outrun their control coounterparts on these indexes by 18,1 and 3,7% respectively.

The reaction of the rumen content is constantly maintained within pH 6,5-7,4 and is displaced towards acidity during the most intensive feed fermentation. Organic acids (acetic, citric, etc.) in the preparation toxy-nil in combination with ascorbic acid promoted in comparison with the control the reliable (P> 0,95) pH medium shift of cows' rumen fluid in the 2nd test group by 6,7% (Table 3).

Table 1 – Design of scientific and economic experiment

Groups	Number of animals	Main diet (MD)	Additive doses		
			Sodium nitrate, g/kg of live weight	Toxy-nil g/kg of concentrates	Vitamin C, % of dry weight
Control	10	MD	0,03	-	-
1 test	10	MD	0,03	2,0	-
2 test	10	MD	0,03	-	0,04

Table 2 — Level of some microorganisms in the rumen content and their enzymatic activity.

Index	Group		
	control	1 test	2 test
Infuzoria, thous/mln	321 ± 2,8	360 ± 2,5*	379 ± 2,3*
Flavobacterium vitarumen, thous/mln	114 ± 2,0	129 ± 1,7*	139 ± 2,1*
Cellulolytic activity,%	14,38 ± 0,37	17,90 ± 0,41*	18,08 ± 0,44*
Proteolytic activity,%	42,43 ± 0,48	45,47 ± 0,50*	46,00 ± 0,49*

* P > 0,95, n = 3

Table 3 - Level of VFA, nitrogenous substances and pH medium in the rumen content

Index	Group		
	control	1 test	2 test
pH medium	7,03 ± 0,11	6,71 ± 0,10*	6,56 ± 0,17*
VFA, mmol/100 ml	11,07 ± 0,18	11,79 ± 0,11*	12,05 ± 0,15*
therefrom, %: acetic	62,88 ± 0,63	64,71 ± 0,67*	65,11 ± 0,71*
propionic	19,38 ± 0,31	19,40 ± 0,34	19,46 ± 0,32
butanoic	12,51 ± 0,21	10,79 ± 0,23*	10,34 ± 0,21*
Nitrates, mmol/l	0,19 ± 0,0011	0,13 ± 0,0012*	0,10± 0,0011*
Nitrites, mmol/l	0,039 ± 0,0004	0,025±0,0003*	0,018±0,0003*
Ammonia, mmol/l	14,00 ± 0,18	15,99 ± 0,14*	16,44 ± 0,19*

*P > 0,95, n = 3

Table 4 - Morphological blood composition of test animals

Index	Group		
	control	1 test	2 test
Hemoglobin, g/l	98,0 ± 2,0	116,0 ± 1,9*	118,9 ± 1,6*
Methemoglobin,%	7,89 ± 0,22	3,71 ± 0,16*	1,44 ± 0,19*
Erythrocytes, 10^{12}/l	5,66 ± 0,14	6,93 ± 0,13*	7,95 ± 0,16*
Leukocytes, 10^{9}/l	10,14 ± 1,52	9,99 ± 1,44	10,21 ± 1,63

* P > 0,95, n = 3

Table 5 - Biochemical composition of cows' blood.

Index	Group		
	control	1 test	2 test
Sugar, mmol/l	2,60 ± 0,08	3,20 ± 0,06*	3,41 ± 0,10*
Total protein, g/l	71,6 ± 1,05	76,01 ± 1,01*	77,3 ± 1,14*
Nitrates, mg/kg	12,03 ± 0,22	6,83 ± 0,26*	4,87 ± 0,33*
Nitrites, mg/kg	0,41 ± 0,002	0,24 ± 0,002*	0,13 ± 0,003*
Ammonia, mmol/l	4,19 ± 0,10	5,14 ± 0,11*	5,42 ± 0,08*
Acetone, mmol/l	0,50 ± 0,006	0,26 ± 0,005*	0,15 ± 0,008*
Vitamin A, mmol/l	0,14 ± 0,001	0,39 ± 0,001*	0,49 ± 0,002*
Vitamin C, mmol/l	1,30 ± 0,005	2,32 ± 0,004*	2,74 ± 0,007*
Calcium, mmol/l	10,13 ± 0,52	10,21 ± 0,44	10,31 ± 0,55
Phosphorus, mmol/l	5,05 ± 0,17	5,55 ± 0,12*	5,68 ± 0,14*

* P > 0,95, n = 3

Due to its more intensive microorganisms fermentation the cows in the 2nd test group had in their proventriculi the highest molar content of volatile fatty acids (VFA), significantly (P> 0,95) outrun the control on this index by 8,85%.

Additives of ascorbic acid and toxy-nil reliably increased relative to the control the rumen fluid concentration of the acetic acid by 2,23% and simultaneously reduced the level of butanoic acid by 2,17% (P> 0,95) in cows of the 2nd test group.

Additions of vitamin C, which has denitrifying properties, and sorbent toxy-nil into diets containing the subtoxic nitrates dose due to activation of nitrate and nitrite reductase release by rumen microflora allowed the cows of the 2nd test group versus the control counterparts to reliably (P> 0,95) decrease in the proventriculus content the nitrates concentration by 47,4% and nitrite - by 53,9%.

The decrease in these xenobiotics level under the influence of nitrate and nitrite-reducing enzymes was accompanied by simultaneous increase of the ammonia concentration in the rumen content. Therefore, relative to the control animals of the 2nd test group had reliably (P> 0,95) 17,4% higher ammonia level in their proventriculi content.

Violation of the blood respiratory function caused by the converting bivalent iron hemoglobin to trivalent with methemoglobin formation when nitrate and nitrite intoxication is accompanied for highly productive cows by significant shifts in the morphological blood composition (Table 4).

Due to high denitrifying properties of ascorbic acid and preparation sorbent the cows of the 2nd test group versus the control had in their blood significantly (P> 0,95) higher concentration of erythrocytes and hemoglobin by $2{,}29 \cdot 10^{12}$/l and 20,9 g/l, respectively. Owing to this, the animals of the 2nd test group had the increase in the level of their blood respiratory function due to the significant decrease (P> 0,95) versus the control counterparts in the erythrocyte saturation with methemoglobin by 87,75%.

Use of vitamin C and preparation toxy-nil for denitrification favourably affected the biochemical parameters of blood serum in animals of test groups, as can be seen from Table 5.

The results of our research have shown that due to the high denitrifying effect of combined using ascorbic acid and sorbent in the blood serum of cows in the 2nd test group versus the control was the significant (P> 0,95) increase in the sugar content by 31,1%. Moreover, the sugar content in the animals' blood of the 2nd test group was within the physiological norm.

It was found that under the conditions of subtoxic nitrate load on the body, the combined feeding of ascorbic acid and sorbent relative to the control counterparts allowed the cows of the 2nd test groups reliably (P> 0,95) to increase the vitamin C retention and vitamin A synthesis from feeds carotene in the liver that allowed enriching blood respectively 2,1 and 3,5 times.

The ammonia – final metabolite to reduce nitrates and nitrites was inversely proportional to these toxicants in the blood, although the content of ammonia and xenobiotics in the test cows' milk, on the contrary, was directly dependent. Combined additives of ascorbic acid and preparation toxy-nil helped to increase in cows' proventriculi of the 2nd test group the production of nitrate-reducing enzymes, therefore the ammonia content in their blood was 29,3% (P> 0,95) more but nitrates and nitrites, on the contrary, 2,47 (P> 0,95) and 3,15 (P> 0,95) times less than in the control.

Discussion of the research results.

To assess the denitrification properties of the antioxidant and adsorbent, sodium nitrate was included into the test animals' diets (in view of the nitrate content in feeds) in order to have their subtoxic level no more than 0,03 g/kg of cows' live weight.

Additives of ascorbic acid and toxy-nil allowed in the cows of the 2nd test group versus the control to improve their digestive metabolism. The increase in the number of vitamin-forming bacteria caused the increase in test animals' proventriculi the number of their phagocytes-infusoria, which are characterized by the high level of cellulases release. The increase in cellulase activity of the rumen content under the influence of vitamin C and the preparation toxy-nil contributed to more completely destroy β-glucoside bonds of feeds cellulose with glucose formation.

To increase the rate of reducing nitrates into nitrites and the latter into ammonia (which is subsequently successfully used by proteolytic microorganisms for the protein synthesis of the own body), it is advantageous to include vitamin C in combination with toxy-nil into highly productive cows' diets containing the subtoxic nitrates dose. Therefore, combined use of ascorbic acid and toxy-nil contributed in cows' proventriculi of the 2nd test group to increase nitrate-reducing enzymes, thus the ammonia content in their blood was 29,3% (P> 0,95) higher but nitrates and nitrites, on the contrary, 2,47 (P> 0,95) and 3,15 (P> 0,95) times less than in the control.

Conclusion.

Vitamin C at a dose of 0,04% of the dry matter together with toxy-nil at a dose of 2,0 g/kg of concentrates included into lactating cows' diets that contain the subtoxic dose of nitrates promoted the improvement of the intermediate metabolism by optimizing the rumen digestion mechanism involving nitrate nitrogen into the synthesis of high-quality microbial protein.

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